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November 4, 2002

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 Imperial Sensus, LLC has determined Frutafit®, an inulin product derived from the root of the chicory plant, is "generally recognized as safe" ("GRAS") through scientific procedures. Frutafit® is intended for use as a bulking agent in a variety of foods in which it serves as a source of reduced energy carbohydrate for uses as a sugar replacer, humectant, binder, fat-replacer and/or texture modifier. Accordingly, Frutafit® 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 Imperial Sensus, LLC 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., Principal, 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.
Principal

cc: V. H. Frankos
R. S. Slesinski
B. Tungland

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GENERALLY RECOGNIZED AS SAFE (GRAS) NOTIFICATION FOR FRUTAFIT®

Prepared for

Imperial-Sensus, LLC
Sugar Land, TX

Prepared by

ENVIRON International Corporation
Arlington, Virginia

November 4, 2002

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I. GRAS EXEMPTION CLAIM

A. Name and Address of Notifier

Imperial-Sensus LLC
P.O. Box 9
Sugar Land, TX 77487-0009

Contact: Mr. Bryan Tungland

Telephone:
Telefax:

B. Name of GRAS Substance

The substance that is the subject of this Generally Recognized as Safe (GRAS) notification is Frutafit.® It is the trade name used by Imperial Sensus, LLC for inulin derived from the root of the chicory plant, *Cichorium intybus*. Inulin is a naturally- occurring polysaccharide that belongs to a class of carbohydrates known as fructans. Inulin is composed of a chain of fructose units joined by beta 2-1 glycosidic linkages usually connected to a single, terminal glucose molecule. The number of fructose units in Frutafit® (termed degree of polymerization "DP") is in a range of 2 to >60 with a modal DP of ≥ 9 fructose units.

C. Intended Use

Frutafit® is intended for use as a bulking agent in foods in which it serves as a source of reduced energy carbohydrate for uses as a sugar replacer, humectant, binder, fat-replacer and/or texture modifier. Bulking agents are not digested fully in the small intestine and therefore can pass unchanged into the large intestine where they are available to serve as a selective substrate for fermentation by nonpathogenic bacteria in the colon. Inulin acts as a selective substrate for beneficial bacteria in the colon, most notably lactobacilli and bifidobacteria.

Frutafit® is proposed for addition to various foods and at levels specified in Table 1.

Table 1. Proposed Food Use Categories and Use Levels of Frutafit®	
Food Category	**Maximum Use Level of Frutafit (g per 100 g food)**
Baby foods: all types of baby foods and beverages, including ready-to-serve and dry baby foods (excluding infant formula)	0.25g/serving[a]
Baked goods, lite cakes: fat free/reduced fat/sugar/calorie baked goods including cakes, brownies, and pastries	5
Baked goods, lite cookies: fat free/reduced fat/sugar/calorie cookies	8
Bars: all types, including breakfast bars, granola bars, energy bars, and diet/meal replacement bars	10
Beverages, fermented milks: kefir, buttermilk, yogurt drinks	2
Beverages, functional: meal replacement beverages and meal supplement beverages, including ready-to-drink beverages and dry beverage mixes[b]	5
Beverages, juices and juice drinks: fruit juices and drinks, including ades, cocktails, cider, nectar, and smoothies, vegetable juices, flavored waters, soy drinks, gelatin drinks, and lightly carbonated beverages, including ready-to-drink beverages and dry beverage mixes[b] (excluding citrus juices and highly carbonated beverages)	1.5
Beverages, milk-based: dairy-based beverages, including ready-to-drink beverages and dry beverage mixes[b]	1
Biscuits, reduced fat: fat free/reduced fat biscuits	6
Breads, conventional: conventional yeast breads, rolls, and buns	0.5
Breads, specialty: specialty types such as breads reduced in calories or fat and/or containing added fiber or added calcium	6
Candy, hard dietetic	15
Candy, soft dietetic	5
Condiments: catsup and mustard	5
Cream cheese, reduced fat: fat free/reduced fat cream cheese	5
French fry coatings: coatings on French fries	1.7[c]
Frozen dairy desserts, lite: fat free/reduced fat/sugar/calorie ice creams and dairy-based frozen desserts, including novelties and frozen yogurt	8
Icings/glazes, lite: fat free/reduced fat/sugar icings and glazes	5
Jams and jellies, lite: reduced sugar/calorie jams and jellies	2
Meat products: processed meats, including frankfurters, sausages, bratwurst, beef patties, chicken patties, loaves, pates, and deli meats	4
Mousse, reduced fat	3
Pancake syrup, lite	2
Pasta fillings: fillings used in pasta, such as tortellini, ravioli and manicotti fillings	5
Pasta, fresh: fresh pasta, such as spaghetti, fettuccini, linguini, tortellini, ravioli, or lasagna (excluding noodles)	4
Pasta, precooked macaroni	4

Table 1. Proposed Food Use Categories and Use Levels of Frutafit®	
Food Category	**Maximum Use Level of Frutafit (g per 100 g food)**
Pizza crust	5
Potatoes, mashed: prepared or in frozen meals (excluding dry mix types)	3
Pretzels, soft	5
Processed cheese, reduced fat: fat free/reduced fat processed cheese and cheese products	5
Pudding mix: regular and reduced sugar/calorie pudding mix	7
RTE breakfast cereals· all types of ready-to-eat (RTE) breakfast cereals	5 g/serving[a]
Salad dressings, lite: fat free/reduced fat/calorie dressings, including mayonnaise, salad dressings and mayonnaise-type dressings	5
Sauces and gravies: entrée, dipping and condiment sauces such as Alfredo, BBQ, cheese, clam, Hollandaise, pasta, pizza, soy, sweet & sour and white sauces, salsa, and gravies, including prepared sauces and dry sauce mixes[a] (excluding tomato sauce and paste)	2
Snack chips, reduced fat: fat free/reduced fat snacks, including chips and extruded snacks	3
Snack crackers: savory snack, sandwich, and whole grain crackers (excluding plain crackers such as saltines, matzo crackers or oyster crackers)	4
Soups, dry	3
Spreads, reduced fat: fat free/reduced fat margarines and margarine-like spreads	10
Surimi surimi, imitation crab, and reconstructed seafood	3
Toppings, dessert: toppings used on desserts (excluding whipped toppings)	2
Tortillas, reduced fat	3
Vegetarian patties/crumbles	2
Whipped toppings, lite: fat free/reduced fat/sugar non-dairy whipped cream toppings	6
Yogurt, reduced fat: fat free/reduced fat refrigerator-type yogurts	3

(a) Serving sizes correspond to Reference Amounts Customarily Consumed per Eating Occasion; 21 CFR 101 12

(b) Maximum use levels correspond to g Frutafit per 100 g prepared beverage or sauce.

(c) Maximum use level per 100 g coated French fry (as consumed)

Note Unless indicated otherwise, all food categories include both regular and lite versions of all food products

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D. Basis for GRAS Determination

This GRAS determination for Frutafit® is based upon scientific procedures in accordance with section 201(s) (21 U.S.C. §321(s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C.§301 et. seq.) ("the Act"), is set forth at 21 CFR 170.30, which states:

- 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. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food.
- General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.
- General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies that may be corroborated by unpublished studies and other data and information.

Once Frutafit® 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.

1. Safety of Frutafit® Inulin for its Proposed Use

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 are 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). The ADI represents the maximum amount of the additive that can be safely consumed on a daily basis for a lifetime. The FDA has specified that an ADI is usually established by application of a safety factor of at least 100 to the highest NOAEL identified in the most sensitive animal species studied. FDA also considers evidence that might justify use of a different safety factor (21 CFR §170.22). 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.

For a food ingredient or macro-additive such as Frutafit®, the FDA Red Book II 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, an acceptable level of intake, hereafter referred to as the Acceptable Intake Level (AIL), for the macro-additive, Frutafit®, was derived by means other than the traditional safety factor approach. This approach is described in the following section.

2. Acceptable Intake Level (AIL) of Frutafit®

An extensive database, consisting of animal and/or human exposure and safety data is available on inulin and related for derivation of an acceptable daily intake (ADI). Purified, branded inulin is sold and consumed in a wide range of food products in Europe; total 1994 European sales volume is estimated at around 1000 metric tons. It is added to dairy products (yogurt, ice-cream, spreads), bakery products and pasta, meat, jams and jellies, and special purpose foods (baby foods, slimming foods, clinical foods, special health foods) to replace fat and sugar. Furthermore, inulin is virtually unabsorbed from the gastrointestinal tract and is not hydrolyzed by human digestive enzymes. The metabolism of inulin and its actions on the gastrointestinal tract as a consequence of its non-digestibility can be considered evidence that it acts in a manner similar to other non-digestible food components such as dietary fiber.

The safety of inulin has been demonstrated in animal studies. Studies addressing the metabolism of inulin have shown that it is resistant to digestive enzymes; consequently, inulin reaches the colon where it can be fermented by the microflora. The fermentation processes provide energy for bacterial proliferation, gases (H_2, CO_2, CH_2) and small organic acids such as acetate, propionate, butyrate and L-lactate, which provide metabolic energy for the host. Inulin is preferentially utilized by bifidobacteria and thereby produces a modification in the colonic microflora. An increase in fecal weight excretion is related to the increased number of bacteria resulting from the fermentation.

Human data are preferred over animal data for establishing the threshold intake associated with substances that produce disruption of gastrointestinal tract function. The extensive amount of human exposure and tolerance data allows derivation of an AIL (Acceptable Intake Level) for the purposes of this section without the use of safety factors typically applied to animal or poorer quality human data. A safety factor approach using animal data for establishment of an ADI is not warranted. This is fully consistent with FDA's regulation dealing with safety factors (21 CFR 170.22), which gives FDA the scientific flexibility to conclude that no safety factor is indeed necessary.

Human tolerance to inulin has been thoroughly evaluated in historical and contemporary diets and in clinical studies employing bolus, short-term, and long-term exposures. Overall, the regular consumption of up to 40 grams of inulin (i.e., Frutafit®) per day by healthy adults appears to result in no significant adverse effects when consumed in divided doses over the course of a day. The AIL of 40 grams for Frutafit® is a conservative estimate of Frutafit® tolerance because studies have suggested that up to 70 grams of inulin per day, consumed as a regular part of the diet, may be well tolerated. The safety and tolerance of ingestion of β 2-1 fructans by infants is documented in a Japanese nationwide survey of 20,742 infants ingesting formula containing 0.32 g FOS/100 ml. This level of intake results in an estimated mean and 90th percentile consumption of 3.0 and 4.2 grams FOS/day.

3. Estimated Average Daily Intake (EDI) of Frutafit® from Proposed Uses

ENVIRON used food intake data reported in the United States Department of Agriculture's 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) and its 1998 Supplemental Children's Survey (USDA 2000) to calculate the estimated daily intake (EDI) of Frutafit® and inulin that would result from the proposed uses in specific foods and beverages. Estimates of inulin intake resulting from the proposed uses were calculated by multiplying the estimated Frutafit® intake by 90 percent, as inulin comprises 90 percent of Frutafit® by weight. All estimates were calculated from 2-day average intakes by individuals who consumed one or more foods from the proposed use categories at least once during the recall period.

Frutafit® and inulin intake by the U.S. population of non-breastfeeding infants under 1 year of age from all GRAS proposed use categories by infants were calculated with a mean of 2.6 and 2.3 g per user per day, respectively. The estimated 90th percentile intakes of Frutafit® and inulin from the proposed uses are 6.4 and 5.7 g per user per day, respectively. Approximately 80 percent of infants under one year of age consume foods proposed for fortification with Frutafit.

Non-breastfeeding infants 1 year of age are estimated to consume an average of 8.4 g Frutafit® and 7.6 g inulin per day per user from the fortified products. The 90th percentile intakes of Frutafit® and inulin are estimated to be 15.2 and 13.7 g per user per day, respectively. Almost all infant one year of age consume one or more of the foods and beverages included in the list of proposed GRAS uses in a 2-day period.

The estimated 2-day average mean intake of Frutafit® and inulin by the U.S. population ages 2 years and older from all GRAS proposed use categories are 11.3 and 10.1 g per user per day, respectively, and the estimated 90th percentile intakes of Frutafit® and inulin from the proposed uses are 21.3 and 19.2 g per user per day, respectively. Results of the estimates of exposure from all proposed use categories combined indicate that nearly all individuals in the population ages 2 years and older consume one or more of the foods and beverages included in the list of proposed GRAS uses in a 2-day period. The proposed use categories that represent the most frequently consumed proposed uses in the population ages 2 years and older are breads (conventional), sauces and gravies, meat products, ready-to-eat breakfast cereals, juice and juice drink beverages, and condiments.

4. Safety Evaluation Following Chronic and Acute Consumption of Frutafit®

Evaluation of the safety of Frutafit®, incorporated into foods as a bulking agent, is accomplished through a review of the extensive database on the safety of inulin, the production process, gastrointestinal fate, animal studies, human exposure, and a comparison of the AIL to the estimated daily intake (EDI) of Frutafit®. If the EDI is less than the AIL then the use can be assumed to be safe.

The AIL is derived from clinical trials of tolerance to Frutafit®, as well as information derived from clinical data on the closely-related β2-1 fructans oligofructose and FOS. Results of these studies indicate that ingestion of up to 40 grams inulin/day, equivalent to 0.67 grams inulin/kg/day, based on an adult body weight of 60 kg, is safe and well tolerated. The EDI for estimated 90th percentile intakes of Frutafit® the U.S. population ages 2 years and older from all GRAS proposed use categories of Frutafit® is 41.5% of the AIL (16.6 g/day) and is, therefore, considered to be safe. Any adverse effects that occur are expected to be gastrointestinal in nature and are not expected to adversely affect the health of the individual.

5. General Recognition of Safety of Frutafit®

The safety of Frutafit® has been reviewed previously by the FDA (FDA, FMF 000613; Rulis 1999) without objection, as part of the submission to U.S.D.A., Food Safety and Inspection Service for use of Frutafit® as a binder, emulsifier, stabilizer and texturizer in processed meat products (ENVIRON 1997). In addition, the FDA Center for Veterinary Medicine reviewed the safety of Frutafit® without objection for inclusion by the Association of Feed Control Officials as an additive to the food of poultry, ruminants, non-ruminants and companion animals (ENVIRON 2001). Fructo-oligosaccharide, a shorter chain length β 2-1 linked fructan, was determined to be GRAS without questions from the FDA (GRN 44, FDA 2000).

The use of Frutafit® as a food ingredient has been determined to be GRAS through application of scientific procedures supported by data demonstrating the safe ingestion of inulin as a naturally occurring component in foods. This determination of safety is based upon the substantial body of data demonstrating human exposure to inulin and related β 2-1 fructans, in addition to a critical review of the production process, published animal and human studies and data on inulin, oligofructose and fructooligosaccharide (FOS), as well as other corroborative, unpublished studies, data and information referenced and analyzed in this document.

The determination of the use of Frutafit® as a GRAS food ingredient has been made through an application of scientific procedures through deliberation of Vasilios H. Frankos, Ph.D., Principal, ENVIRON International Corporation, Claire L. Kruger Ph.D., DABT, Principal, ENVIRON International Corporation, and Ronald S. Slesinski Ph.D., DABT, Senior Science Manager, ENVIRON International Corporation. These individuals are qualified by scientific training and experience to evaluate the safety of foods and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document as well as consideration of the potential human exposure to this compound, and have concluded:

> *There is no evidence in the available information on Frutafit® that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected from the proposed food applications. Frutafit® is GRAS for the uses and at the levels proposed by Imperial Sensus, LLC.*

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Other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, Frutafit®, used as a bulking agent in foods and as a humectant, binder, fat-replacer and/or texture modifier at use levels specified in Table 1 is safe, and is GRAS. Because Frutafit® 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 promulgation of a specific food additive regulation by the FDA.

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., Principal, ENVIRON International Corporation, 4350 North Fairfax Drive, Suite 300, Arlington, VA 22203; Telephone: 703-516-2309; facsimile: 703-516-2304.

II. DESCRIPTION OF SUBSTANCE

A. Identity

1. Chemical Composition

Inulin is a naturally-occurring polysaccharide that belongs to a class of carbohydrates known as fructans. Inulin is characterized by the β 2-1 linkages of its fructose chains and usually having only a single terminal glucose molecule. However, the length of these fructose chains is variable and depends on the plant source, time of harvest and the duration and conditions of post-harvest storage. The degree of polymerization ("DP") of inulin can range from 2 to greater than 60.

Of the various naturally-occurring chain length species of polysaccharides, for which information is available in the scientific literature, the most common fractions are referred to as inulin, oligofructose, and fructooligosaccharides (FOS). Oligofructoses of various chain lengths can be obtained from inulin by enzymatic hydrolysis. The term inulin generally refers to polysaccharide chains with a DP of 2 to greater than 60, oligofructose may have a DP range of 2 to 20, and FOS is a mixture of GF2, GF3, and GF4 sugars (i.e., DP of 3 to 5). Because of the potential for confusion over inulin terminology, an attempt is made throughout this report to consistently define the various fractions of inulin referred to in the scientific literature, with references to the terminology used by the original authors as needed. The β 2-1 linkage is responsible for many of the physiological and chemical properties of inulin, oligofructose, and FOS. Therefore, information from animal and human studies on the gastrointestinal fate and systemic effects for all of these β 2-1 fructans is used to evaluate the safety of Frutafit® for its proposed use.

2. Common and Trade Names

Frutafit® is the trade name for the inulin product produced by the Imperial Sensus LLC Company in Sugar Land, TX from chicory roots grown and processed in the Netherlands by Sensus Operations, Roosendaal, The Netherlands.

3. **Structure**

The chemical structure of Frutafit® is shown below:

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B. Production Process

1. **Pesticides**

No fungicides, slimicides, or other biocides are used by Imperial-Sensus, LLC in the production of Frutafit®

2. **Processing Aids and Process Chemicals**

C. Properties and Finished Product Specifications

1. Physical Chemical Properties

Water Solubility:	60 g/L @ 10°C
pH (10% solution):	4.5-7.0
Particle size:	Max. 10% <10 µm
	Modal range 40-80 µm
Density (tapped):	550 ± 100 g/L
Color:	cream/white
Storage stability:	stable, hygroscopic
Average chain length:	≥9 units
Taste:	neutral-slightly sweet

2. Product Specifications

ash content:	≤0.2% (w/w)
dry matter:	≥95% carbohydrates
Composition:	≥90% inulin (w/w)
	≤ 5.8% disaccharides
	≤ 1.0% glucose
	≤ 5.0% fructose
Heavy metals:	≤0.2 ppm (≤ 0.2 mg/kg)*
	*limit of detection
Aflatoxin & Microbiological Contaminants:	Specifications are shown in the following summary:

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Total mesophilic plate count	<2000 CFU/g
Total aerobic plate count	<2000 CFU/g
Staph. aureus count	Absent in 1g
Salmonella spp.	Absent in 25 g
Total coliform count	Absent in 1g
Bacillus cereus	<100 CFU/g
Yeast and mold count	<20 CFU/g
Aflatoxin (B_1, B_2, G_1, G_2)	<0.0001 mg/kg

3. Pesticide Contaminants

Pesticides listed in Attachment 1 were undectable in Frutafit® at the respective detection limits for each pesticide (which differ slightly depending on the type of analytical method).

4. Chain Length Distribution of Frutafit®

High-performance anion exchange chromatography with a pulsed electrochemical detector working in pulsed amperometric detection mode (HPAEC-PAD analysis) was used to characterize the chain length distribution of Frutafit®. The range of chain lengths was in a range that is characteristic for inulin from DP 2 to greater than 60, and the modal chain length was greater than or equal to a DP of 9. Small side-peaks or shoulders seen on principal peaks may be due to oligofructose molecules (Fn; without the terminal glucose moiety) of the same DP as the corresponding inulin molecule carrying a terminal glucose moiety (GFn-1). The analysis of Frutafit® chain length indicates that it is consistent with that of other inulins consumed by humans.

D. Analytical Methods

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The official AOAC method for determination of inulin that specifically quantifies the fructan portion of the dietary fiber in foods, food and food products is published as AOAC method 997.08. This same method for determination of inulin content is published by the European Ministry of Agriculture, Food and Fisheries (MAFF). Although not published

by MAFF, a second specific official AOAC method that may serve as an alternative analytical procedure for determination of inulin content in food is published as AOAC method 999.03: "Total Fructan in Foodstuffs by Enzymatic/Spectrophotometric Method." Prosky and Hoebregs (1999) recently reviewed the range of analytical methods used to determine inulin in food as a part of efforts by international organizations such as the International Life Sciences Institute and the AOAC to standardize definitions and analytical methods for dealing with complex carbohydrates.

III. NATURAL OCCURRENCE AND EXPOSURE TO INULIN

A. Food Sources of Inulin

Similar to starch in corn, wheat, or potato, inulin is the energy-reserve in an estimated 36,000 fruits and plants consumed as food world-wide (Raffinerie Tirlemontoise 1993; Van Loo 1995). Inulin is found in the roots, stems, leaves, and seeds of a wide range of edible plants and fruits as summarized in Table 1. Inulin is a found in many plants including the *Liliaceae*, *Graminae* (grass) and *Compositae* (sunflower/daisy) families. There are many examples of plants consumed as foodstuffs that contain inulin and a fraction of inulin defined as oligofructose. Several inulin-laden foods, specifically chicory, dahlia, Jerusalem artichokes, murnong, and yacon, have been used either as dietary staples or as sustenance crops in times of hardship. A variety of crops containing inulin comprise substantial portions of the animal and human diets.

Chicory is indigenous to Europe and has been cultivated on a significant scale since the 16th century. Both chicory roots and greens (known as "Belgian endive") have been consumed. Post-World War II populations in England and Germany used the roasted root of the chicory plant as either an extender 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 1992; and Meijer, Mathijssen, and Borm 1993).

Wild and cultivated versions of the camas root, and the dahlia, Jerusalem artichoke, and yacon tubers that have relatively high inulin contents have been consumed for centuries by native populations in North, Central, and South America (Shoemaker 1927; Wyse and Wilfahrt 1982; Whitley 1985; and Raffinerie Tirlemontoise 1992). Noted for their storage life and in-ground ability to withstand the damaging effects of frost, these plants commonly served as staple foods during both the winter months and in times of drought. The dahlia plant was also used for its noted medicinal capabilities as a diuretic, diaphoretic, and against colics and flatulence (Whitley 1985). Dahlia tubers are still a popular food in some parts of Mexico, and a beverage produced from roasted dahlia juice, Dacopa, is currently sold in health food stores in the U.S. (Whitley 1985). Consumption of the yacon tuber also continues and a South Pacific variety of the yacon tuber has recently been introduced to Japan from New Zealand and is growing in popularity (Asami et al. 1989). 000022

The Jerusalem artichoke tuber was introduced into parts of Europe in 1612 and was cultivated as a staple agricultural crop, primarily in The Netherlands, France and the Mediterranean region, before it was superseded by the potato in the middle of the 18th century (Wyse and Wilfahrt 1982; Kosaric et al. 1985). The Jerusalem artichoke's historical use in the diet as an adequate potato substitute has caused it to be referred to as wild potato,

horse potato, and diabetic potato. Using contemporary 90th percentile consumption values for white potatoes to mimic the historical consumption of Jerusalem artichokes, it can be estimated that 158 grams per day of Jerusalem artichokes were consumed (Pao et al. 1982). Thus, based on values of inulin content ranging from 16 to 20 percent, approximately 25 to 32 grams per day of inulin was consumed by these populations.

Murnong, also consumed in large amounts, has been referred to historically as a preferred food of the Australian aborigines. Murnong is reported by Gott (1984) to have been consumed in amounts estimated to be greater than two kilograms *per sitting*. Using values of inulin content ranging from 8 to 13 percent, the intake of inulin from murnong consumption can be conservatively calculated to be approximately 160 to 260 grams per day. Other varieties of tubers known to be food plants of Australian aborigines, including two species from the Asteraceae (*Microseris lanceolata*, and *Microseris aff. Lanceolata*), contain inulin (Van Hee 1982).

A large variety of food crops containing inulin are commonplace in contemporary Western diets. These foods include artichokes, asparagus, garlic, leeks, onions, and several important cereal grains, including wheat, rye, and barley (Fuchs 1991; Van Loo 1995; Moshfegh et al. 1999). Although the inulin content of most of these foods is low -- relative to that of chicory, dahlia, Jerusalem artichokes, murnong, and yacon -- the high levels of consumption of these foods result in substantial inulin intake. For example, in onions, the inulin content can range up to 7.5% by weight. Moshfegh et al. (1999) recently reviewed the exposure to inulin and oligofructose in the American diet and concluded that, although there were significant differences in the intake of these substances between regions of the country, season, income status, and race and national origin, the actual differences were small and major contributors in the diet were wheat and onions that provided approximately 95% of the dietary exposure. American diets were found to provide an average of 2.6 g of and 2.5 g. of inulin and oligofructose/day and teenage boys and adult males had average intakes of 3.5 g inulin/day.

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B. Inulin Content in Edible Plants

The fraction of the 36,000 inulin-containing plants historically used as food contain inulin concentrations ranging from less than 1 percent of the wet weight (i.e., many cereal grains) to greater than 20 percent of the wet weight (i.e., Jerusalem artichokes and other cultivated or wild varieties of tubers), depending on the harvest- and storage-time factors mentioned above. To date, analyses of the inulin content in the large majority of these foods have not included detailed studies of their chain length distributions, however, a review of what is known about the chain lengths of inulin from numerous sources indicates that the chain length distribution and mode for Frutafit® is consistent with that of other inulins consumed by humans (Winton and Winton 1935; Rutherford and Weston 1968; Edelman

and Jefford 1968 as cited in Incoll and Bonnett 1993; Fleming and GrootWassink 1979; Oku, Tōkunaga, and Hosoya 1984; Nilsson and Björck 1988; Incoll, Bonnett, and Gott 1989; Suzuki and Cucliffe 1989; Grühn 1994 and Van Loo et al. 1995). Moshfegh et al. (1999) noted that approximately 95% of the ingestion of an average of 2.6 g inulin and oligofructose/day in the American diet was attributable to wheat and onions.

A summary of the inulin content in a range of edible plants is presented in Table 2.

C. Commercial Sources

Most of the inulin or fructooligosaccharide products that are commercially available include:

D. Background Intake of Inulin from Food Sources

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Humans have been historically exposed to inulin or its hydrolysis product oligofructose in a variety of foods, including several kinds of tubers that served as staple crops. Inulin is present in a number of foods that are currently eaten on a daily basis throughout the world (Table 2). In the United States, the most commonly consumed foods containing inulin include bananas, garlic, onions, tomatoes and several of the cereal grains. Daily intake of inulin and oligofructose in the U.S. and Europe has been estimated at up to 10g/day (Coussement 1999) with recent estimates of inulin intakes of 2.6 g/day by Moshfegh et al. (1999).

Average daily exposure of the U.S. population to inulin through consumption of these foods was obtained from the EPA's Dietary Risk Evaluation System (DRES) database. The DRES consumption estimates are based on data from the 1977-78 Nationwide Individual Food Consumption Survey conducted by the U.S. Department of Agriculture (USDA). This questionnaire-style study involved 30,770 people, intentionally weighted towards the young

and underprivileged subpopulations, who specified the types and amounts of food they had each consumed over the three days prior to the survey. The consumption estimates used for this analysis were for the average U.S. population residing in the 48 contiguous states and represented average daily food consumption corrected for seasonal variations.

To calculate dietary exposure to inulin, ENVIRON combined the DRES consumption estimates with food-specific inulin concentrations found in the scientific literature. Because inulin concentrations are commonly stated as ranges, calculations of both the lower and upper bound concentrations were performed. The resulting values represent lower and upper estimates of total inulin exposure from the average U.S. diet. The daily consumption estimates of foods containing inulin, the lower and upper estimates of inulin in these foods, and the resulting inulin intake for the average U.S. diet are listed in Table 2.

Table 2. Inulin Content of Edible Plants				
Plant	**Edible Part**	**% Inulin[1]**	**Remarks**	**Reference**
Artichoke	leaf/heart	3 - 10		Raffinerie Tirlemontoise 1992
		3 - 10		Okey and Williams 1920, as cited in Roberfroid, Gibson, and Delzenne 1993
		2.5		Okey and Williams 1920
Asparagus	root/ tuber	1 - 30		Raffinerie Tirlemontoise 1993
		2 - 3		Fiala and Jolivet 1982, as cited in Roberfroid, Gibson, and Delzenne 1993
		detected[2]		Madan 1972, as cited in Incoll and Bonnett 1993
	stem	not detected[2]		Pressman et al. 1989, as cited in Incoll and Bonnett 1993
Asphodel	leaf/stem/tuber	detected[2]		Schlubach and Lendzian 1937, as cited in Incoll and Bonnett 1993
Banana	fruit	0.3 - 0.7		Raffinerie Tirlemontoise 1993
		0.3 - 0.7		Asami et al. 1989, as cited in Roberfroid, Gibson, and Delzenne 1993
Barley	cereal	0.5 - 1.5		Raffinerie Tirlemontoise 1993
		detected[2]		Belval 1924 and MacLeod and Preece 1954, as cited in Incoll and Bonnett 1993
Burdock	root	3.5 - 4.0		Raffinerie Tirlemontoise 1992
		detected[2]		Rundqvist 1909, as cited in Incoll and Bonnett 1993

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Table 2. Inulin Content of Edible Plants				
Plant	**Edible Part**	**% Inulin[1]**	**Remarks**	**Reference**
Camas	bulb	12 - 22		Raffinerie Tirlemontoise 1992
		detected[2]		Yanovsky and Kingsbury 1938, as cited in Incoll and Bonnett 1993
Chicory	root	15 -20		Raffinerie Tirlemontoise 1993
		15 - 20		Douglas and Poll 1986, as cited in Roberfroid, Gibson, and Delzenne 1993
	root/chicon	detected[2]		Rutherford and Phillips 1975, as cited in Incoll and Bonnett 1993
Coffee Chicory Powder	extract	20 - 60		Raffinerie Tirlemontoise 1993
Comfrey	leaf	detected[2]		Pollock 1986. as cited in Incoll and Bonnett 1993
Dahlia	tuber	90[3]		Raffinerie Tirlemontoise 1993
		9 - 13		Harrison 1953
		10	From *Dahlia pinnata*	Whitley 1985
Dahlia-inulin dried juice extract	--	80	Described as inulin and inulides	CNP 1986
Dandelion	leaf	12 - 15		Raffinerie Tirlemontoise 1992
		12 - 15		Yanovsky and Kingsbury 1938, as cited in Roberfroid, Gibson, and Delzenne 1993
	root	detected[2]		Pollock 1986, as cited in Incoll and Bonnett 1993
		detected[2]		Medcalf and Cheung 1971, as cited in Incoll and Bonnett 1993

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Table 2. Inulin Content of Edible Plants				
Plant	**Edible Part**	**% Inulin[1]**	**Remarks**	**Reference**
Durum Wheat	cereal	detected		Raffinerie Tirlemontoise 1992
		detected[2]		Medcalf and Cheung 1971, as cited in Incoll and Bonnett 1993
Elecampane	root	detected		Rose 1804, as cited in Incoll and Bonnett 1993
Garlic	bulb	9 - 16		Raffinerie Tirlemontoise 1992
		9-16		Darbyshire and Henry 1981, as cited in Roberfroid, Gibson, and Delzenne 1993
		detected[2]		Darbyshire and Henry 1981 and Pollock 1986, as cited in Incoll and Bonnett 1993
Gau sun	stem	detected[2]		Chan and Thrower 1980, as cited in Incoll and Bonnett 1993
Jerusalem artichoke	tuber	16 - 20		Raffinerie Tirlemontoise 1992
		15 - 20[2]		John 1992
		detected[2]		Edelman and Jefford 1968, as cited in Incoll and Bonnett 1993
		12	Fresh weight	de Bruyne et al. 1947

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Table 2. Inulin Content of Edible Plants				
Plant	**Edible Part**	**% Inulin[1]**	**Remarks**	**Reference**
Jerusalem artichoke (cont'd)	leaf/bulb	Up to 20	Described as poly-saccharides usable as sweetening agents	Budavari, O'Neil, and Smith 1989
		16 - 20		Six references, as cited in Roberfroid, Gibson, and Delzenne 1993
		55 - 65	Inulin content depends on season	Whistler and Smart 1973
Leek	bulb	3 - 10		Raffinerie Tirlemontoise 1992
		3 - 10		Bacon 1959, as cited in Roberfroid, Gibson, and Delzenne 1993
		10 - 15[2]		John 1992
	leaf/bulb	detected[2]		Darbyshire and Henry 1981, as cited in Incoll and Bonnett 1993
Meadow cabbage	root	detected[2]		Quillet and Bourdu 1952, as cited in Incoll and Bonnett 1993
Murnong	root	8 - 13		Raffinerie Tirlemontoise 1992
		~ 8	Tubers analyzed at breaking of dormancy	Gott 1983
		detected[2]		Incoll et al. 1989, as cited in Incoll and Bonnett 1993

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Table 2. Inulin Content of Edible Plants				
Plant	**Edible Part**	**% Inulin[1]**	**Remarks**	**Reference**
Oats	cereal	detected[2]		Belval 1924 and MacLeod and Preece 1954, as cited in Incoll and Bonnett 1993
Onion	bulb	2 - 6		Raffinerie Tirlemontoise 1993
		2 - 6[2]		John 1992
		1.1 - 7.5		Suzuki and Cucliffe 1989, as cited in Roberfroid, Gibson, and Delzenne 1993
		detected[2]		Darbyshire and Henry 1981 and Pollock 1986, as cited in Incoll and Bonnett 1993
Palm Lily	tuber	detected[2]		Boggs and Smith 1956 and Ekstrand and Johanson 1887, as cited in Incoll and Bonnett 1993
Rampion	root	detected[2]		Bacon 1959, as cited in Incoll and Bonnett 1993
Rye	cereal	1 - 4		Raffinerie Tirlemontoise 1992
		0.5 - 1		Asami et al. 1989, as cited in Roberfroid, Gibson, and Delzenne 1993
		detected[2]		Belval 1924 and MacLeod and Preece 1954, as cited in Incoll and Bonnett 1993
Salsify	root	4 - 11		Raffinerie Tirlemontoise 1992
		detected[2]		Rundqvist 1909, as cited in Incoll and Bonnett 1993
		4 - 11		Van Hee 1982, as cited in Roberfroid, Gibson, and Delzenne 1993
		~11[3]		Van Hee 1982

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Table 2. Inulin Content of Edible Plants				
Plant	**Edible Part**	**% Inulin[1]**	**Remarks**	**Reference**
Tomato		0.15 [4]		Spiegel et al. 1994
Yacon	root/tuber	3 - 19		Raffinerie Tirlemontoise 1993
		1.35[3]	Excludes GF_2 to GF_9	Asami et al. 1989
		detected[2]		Ohyama et al. 1990, as cited in Incoll and Bonnett 1993
Wheat	cereal	1 - 4		Spiegel et al. 1994
		1 - 4		
		detected[2]		Belval 1924 and MacLeod and Preece 1954, as cited in Incoll and Bonnett 1993

[1] Wet weight basis (unless otherwise indicated)
[2] Specified as "fructan" or "polyfructan" content
[3] Dry weight basis
[4] Specified as "FOS"

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E. Proposed Uses and Estimated Daily Intake of Frutafit®

1. Proposed Uses of Frutafit®

Imperial Sensus proposes use of Frutafit® in a variety of foods and beverages. The proposed use categories and the maximum proposed use levels of Frutafit® per category are presented in Table 1 in Chapter 1 and in Attachment 2. Attachment 2 also includes a description of the intended function of Frutafit® within each proposed use category.

2. Estimated Daily Intake of Frutafit® and Inulin from Proposed Uses

a. Food Consumption Data

Using food intake data reported in the United States Department of Agriculture's 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) and its 1998 Supplemental Children's Survey (USDA 2000), ENVIRON calculated the estimated daily intake (EDI) of Frutafit® and inulin provided by Frutafit® that would result from the proposed uses in food and beverages. The CSFII provides the most current multiple-day dietary recall food consumption data available for the American population.

The CSFII was conducted between January 1994 and January 1997 with non-institutionalized individuals in the United States. In each of the three survey years, data were collected from a nationally representative sample of individuals of all ages. The CSFII 1998 survey was a survey of children ages 0 through 9 years, which was supplemental to the CSFII 1994-96. It used the same sample design as the CSFII 1994-96 and was intended to be merged with CSFII 1994-96 to increase the sample size for children. The merged surveys are designated as CSFII 1994-96, 1998. In the CSFII 1994-96, 1998, dietary intakes were collected through in-person interviews using 24-hour recalls on two nonconsecutive days approximately one week apart. A total of 21,662 individuals provided data for the first day; of those individuals, 20,607 provided data for a second day. The food record for each individual includes the gram weight and nutrient data for all foods consumed during the day of the recall.

The survey database includes a list of more than 9,000 unique food codes that were consumed by survey respondents. ENVIRON identified food codes representative of the proposed uses from the list of CSFII 1994-96, 1998 food codes and from the CSFII recipe files (USDA 2000).

b. Intake Estimates from Food Consumption Data

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Estimates of 2-day average intakes of Frutafit® from the proposed uses of Frutafit® were calculated from the food code list and the survey database of diet recalls. Estimates of inulin intake resulting from the proposed uses were calculated by multiplying the estimated Frutafit® intake by 90 percent, as inulin comprises 90 -

percent of Frutafit® by weight. All estimates were calculated from 2-day average intakes by individuals who consumed one or more foods from the proposed use categories at least once during the recall period. The estimates were generated with USDA sampling weights to adjust for differences in representation of subpopulations.

Table 3 presents estimates of Frutafit® and inulin intake by the U.S. population of non-breastfeeding infants under 1 year of age; estimates are presented for each proposed use category, and for all proposed use categories combined. The 2-day average Frutafit® and inulin intakes by this population from all GRAS proposed use categories combined are estimated to be 2.6 and 2.3 g per user per day, respectively. The estimated 90th percentile intakes of Frutafit® and inulin from the proposed uses are 6.4 and 5.7 g per user per day, respectively. Approximately 80 percent of infants under one year of age consume foods proposed for fortification with Frutafit®. The most widely consumed food category proposed for fortification with Frutafit® in this age group is baby foods; nearly three-fourths of infants under the age of one year consumed one or more of these products during the 2 days of diet recall. Approximately 20 percent of all infants consume foods from the juice and juice drink beverages category, the breads (conventional) category, or the sauces and gravies category.

Non-breastfeeding infants 1 year of age are estimated to consume an average of 8.4 g Frutafit® and 7.6 g inulin per day per user from the fortified products. The 90th percentile intakes of Frutafit® and inulin are estimated to be 15.2 and 13.7 g per user per day, respectively. Almost all infants one year of age consume one or more of the foods and beverages included in the list of proposed GRAS uses in a 2-day period. Among infants one year of age, the most commonly consumed food categories proposed for fortification with Frutafit® are breads (conventional), juice and juice drink beverages, sauces and gravies, meat products and ready-to-eat (RTE) breakfast cereals.

The estimates of Frutafit® and inulin intake by the U.S. population ages 2 years and older are presented in Table 5. The estimated 2-day average mean intakes of Frutafit® and inulin by the U.S. population ages 2 years and older from all GRAS proposed use categories are 11.3 and 10.1 g per user per day, respectively, and the estimated 90th percentile intakes of Frutafit® and inulin from the proposed uses are 21.3 and 19.2 g per user per day, respectively. Results of the estimates of exposure from all proposed use categories combined indicate that nearly all individuals in the population ages 2 years and older consume one or more of the foods and beverages included in the list of proposed GRAS uses in a 2-day period. The proposed use categories that represent the most frequently consumed proposed uses in the population ages 2 years and older are breads (conventional), sauces and gravies, meat

products, ready-to-eat breakfast cereals, juice and juice drink beverages, and condiments.

It is important to note that all estimates are likely overestimates of potential intake of Frutafit® and the inulin provided by this product from foods proposed for supplementation with Frutafit®, as the estimates assume that all foods in the proposed use categories are supplemented with Frutafit® at the maximum proposed use level. Additionally, in some food categories, it was necessary to use a broader category of representative foods than defined by the proposed use category. For example, the CSFII 1994-96, 1998 does not include food codes for low fat tortillas, therefore regular tortillas were used to derive estimates of intake from this food category, and it is reasonable to assume that regular tortillas are more widely consumed by the U.S. population than low fat varieties.

Estimates of per capita consumption of inulin in Western Europe are somewhat higher than that of the U.S. Inulin intake in Belgium is estimated to be between 5 and 8 grams per person per day, while similar estimates for the Spanish population are between 7 and 12 grams per person per day. For the entire Western European community, inulin intake is estimated to range from 2 to 12 grams per day (John 1992; Van Loo 1995). In addition, purified branded inulin is sold and consumed in a wide range of food products in Europe; total 1994 European sales volume is estimated at around 1000 metric tons. It is added to dairy products (yogurt, ice-cream, spreads), bakery products and pasta, meat, jams and jellies, and special purpose foods (baby foods, slimming foods, clinical foods, special health foods) to replace fat and sugar.

Per capita or average estimates of inulin consumption, which include both consumers and non-consumers of inulin-containing foods, are somewhat misleading, however, because they may vastly underestimate the true inulin consumption of large portions of the population. The conservative nature of these consumption values is exemplified by the lower and upper bound estimates of inulin consumption from a single serving of specific dishes, such as French onion soup or a salsify dish. A single serving of French onion or onion-leek soup, made from 300 grams of onions and leeks, is estimated to result in the intake of 6 to 20 grams of inulin, while a single serving of a salsify dish, made with 300 grams of salsify, is estimated to result in the intake of 4 to 12 grams of inulin (Raffinerie Tirlemontoise 1993; Van Loo 1995).

Table 3. Estimated Daily Intake of Frutafit® and Inulin from Proposed Uses by U.S. Infants Under 1 Year of Age

	Users		2-Day Average Frutafit Intake (g/d)		2-Day Average Inulin Intake [a] (g/d)	
Food Category	N	%	Mean	90th Percentile	Mean	90th Percentile
ALL CATEGORIES COMBINED	849	80.3	2.6	6.4	2.3	5.7
Baby foods	776	73.1	0.9	1.9	0.8	1.7
Baked goods, lite cakes	1	0.1	0.7	0.7	0.6	0.6
Baked goods, lite cookies	1	0.1	3.5	3.5	3.2	3.2
Bars	4	0.5	1.4	3.3	1.2	2.9
Beverages, fermented milks	0	-----	-----	-----	-----	-----
Beverages, functional	1	< 0.05	29.1	29.1	26.2	26.2
Beverages, juices and juice drinks	237	22.9	2.5	4.7	2.3	4.2
Beverages, milk-based	7	0.4	0.8	1.9	0.8	1.7
Biscuits, reduced fat	1	0.1	0.2	0.2	0.1	0.1
Breads, conventional	195	18.0	0.1	0.2	0.1	0.2
Breads, specialty	3	0.3	0.4	0.9	0.4	0.8
Candy, hard dietetic	0	-----	-----	-----	-----	-----
Candy, soft dietetic	0	-----	-----	-----	-----	-----
Condiments	12	1.3	0.1	0.3	0.1	0.3
Cream cheese, reduced fat	0	-----	-----	-----	-----	-----
French fry coatings	58	5.8	0.3	0.4	0.2	0.4
Frozen dairy desserts, lite	4	0.3	2.5	3.5	2.3	3.2
Icings/glazes, lite	0	-----	-----	-----	-----	-----
Jams and jellies, lite	1	0.1	0.1	0.1	0.1	0.1
Meat products	107	11.4	0.9	2.3	0.9	2.1
Mousse, reduced fat [b]	0	-----	-----	-----	-----	-----
Pancake syrup, lite	3	0.3	0.1	0.1	0.1	0.1
Pasta fillings	8	0.7	0.4	0.7	0.4	0.6
Pasta, fresh [c]	48	4.7	0.7	1.4	0.6	1.3
Pasta, precooked macaroni [c]	46	4.2	1.1	2.0	1.0	1.8
Pizza crust	24	2.7	0.4	0.6	0.4	0.5
Potatoes, mashed	63	6.5	1.3	3.2	1.2	2.8
Pretzels, soft	1	0.1	1.4	1.4	1.3	1.3
Processed cheese, reduced fat	1	0.1	0.5	0.5	0.4	0.4
Pudding mix	10	1.2	0.5	0.8	0.5	0.7
RTE breakfast cereals	128	12.9	1.5	3.1	1.4	2.8
Salad dressings, lite	0	-----	-----	-----	-----	-----
Sauces and gravies	189	18.1	0.7	1.7	0.6	1.5
Snack chips, reduced fat	0	-----	-----	-----	-----	-----
Snack crackers	77	6.9	0.3	0.6	0.2	0.6
Soups, dry	25	2.0	0.4	1.0	0.4	0.9
Spreads, reduced fat	41	4.0	0.3	0.7	0.2	0.6
Surimi	0	-----	-----	-----	-----	-----
Toppings, dessert	2	0.3	0.1	0.1	0.1	0.1

Table 3. Estimated Daily Intake of Frutafit® and Inulin from Proposed Uses by U.S. Infants Under 1 Year of Age

	Users		2-Day Average Frutafit Intake (g/d)		2-Day Average Inulin Intake [a] (g/d)	
Food Category	**N**	**%**	**Mean**	**90th Percentile**	**Mean**	**90th Percentile**
Tortillas, reduced fat [b]	26	2.3	0.2	0.4	0.2	0.4
Vegetarian patties/crumbles	1	0.1	0.4	0.4	0.3	0.3
Whipped toppings, lite	1	0.1	1.3	1.3	1.2	1.2
Yogurt, reduced fat	36	3.9	2.7	5.5	2.4	5.0

Data source: USDA Continuing Survey of Food Intakes by Individuals (CSFII), 1994-96, 1998. Breastfeeding infants and children were excluded from the analysis. Estimates are based on food consumption reported by individuals who provided two 24-hour diet recalls and maximum Frutafit use levels specified in Table 1.

(a) Frutafit is % inulin (by weight).

(b) No food codes for reduced fat forms of this food category are in the 1994-96, 98 CSFII; estimates are based on consumption of full fat versions.

(c) No food codes for fresh pasta or precooked macaroni are in the 1994-96, 98 CSFII; estimates are based on consumption of all pasta and macaroni.

Note: Unless indicated otherwise, all food categories include both regular and lite versions of all food products.

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Table 4. Estimated Daily Intake of Frutafit® and Inulin from Proposed Uses by U.S. Infants 1 Year of Age

	Users		2-Day Average Frutafit Intake (g/d)		2-Day Average Inulin Intake [a] (g/d)	
Food Category	N	%	Mean	90th Percentile	Mean	90th Percentile
ALL CATEGORIES COMBINED	967	99.6	8.4	15.2	7.6	13.7
Baby foods	262	26.7	0.7	1.5	0.6	1.3
Baked goods, lite cakes	9	0.7	0.6	1.3	0.5	1.2
Baked goods, lite cookies	15	1.6	1.0	1.4	0.9	1.3
Bars	47	5.4	2.0	2.8	1.8	2.6
Beverages, fermented milks	0	-----	-----	-----	-----	-----
Beverages, functional	4	0.4	7.6	14.0	6.8	12.6
Beverages, juices and juice drinks	727	75.3	4.0	7.9	3.6	7.1
Beverages, milk-based	65	6.1	1.5	3.8	1.4	3.4
Biscuits, reduced fat	1	0.1	0.9	0.9	0.9	0.9
Breads, conventional	760	78.0	0.1	0.3	0.1	0.3
Breads, specialty	14	1.3	0.6	1.0	0.6	0.9
Candy, hard dietetic	0	-----	-----	-----	-----	-----
Candy, soft dietetic	0	-----	-----	-----	-----	-----
Condiments	240	24.2	0.3	0.7	0.2	0.6
Cream cheese, reduced fat	5	0.4	0.2	0.4	0.1	0.3
French fry coatings	253	25.8	0.4	0.9	0.4	0.8
Frozen dairy desserts, lite	35	2.9	3.4	7.0	3.1	6.3
Icings/glazes, lite	1	0.1	< 0.05	< 0.05	< 0.05	< 0.05
Jams and jellies, lite	10	0.9	0.1	0.2	0.1	0.2
Meat products	623	62.5	1.2	2.7	1.1	2.4
Mousse, reduced fat [b]	0	-----	-----	-----	-----	-----
Pancake syrup, lite	26	3.2	0.2	0.5	0.2	0.4
Pasta fillings	46	4.7	0.6	1.1	0.5	1.0
Pasta, fresh [c]	213	22.0	0.8	1.5	0.7	1.3
Pasta, precooked macaroni [c]	152	16.2	1.5	3.4	1.4	3.1
Pizza crust	160	17.5	0.7	1.2	0.6	1.1
Potatoes, mashed	128	12.6	1.4	3.2	1.3	2.8
Pretzels, soft	8	0.7	1.5	2.5	1.3	2.3
Processed cheese, reduced fat	7	0.7	0.4	0.7	0.4	0.6
Pudding mix	30	3.0	0.6	1.0	0.5	0.9
RTE breakfast cereals	598	62.1	2.7	5.5	2.4	5.0
Salad dressings, lite	21	2.0	0.2	0.4	0.2	0.4
Sauces and gravies	686	71.4	1.0	2.2	0.9	2.0
Snack chips, reduced fat	9	1.0	0.2	0.3	0.2	0.3
Snack crackers	245	26.5	0.4	1.0	0.4	0.9
Soups, dry	72	7.7	0.5	0.8	0.4	0.8
Spreads, reduced fat	213	21.5	0.3	0.6	0.3	0.5
Surimi	1	0.1	0.1	0.1	0.1	0.1
Toppings, dessert	7	0.7	0.1	0.4	0.1	0.3
Tortillas, reduced fat [b]	103	11.1	0.4	0.7	0.4	0.6
Vegetarian patties/crumbles	3	0.3	0.4	0.6	0.3	0.5

Table 4. Estimated Daily Intake of Frutafit® and Inulin from Proposed Uses by U.S. Infants 1 Year of Age

	Users		2-Day Average Frutafit Intake (g/d)		2-Day Average Inulin Intake [a] (g/d)	
Food Category	N	%	Mean	90th Percentile	Mean	90th Percentile
Whipped toppings, lite	0	-----	-----	-----	-----	-----
Yogurt, reduced fat	114	11.8	2.6	5.1	2.4	4.6

Data source: USDA Continuing Survey of Food Intakes by Individuals (CSFII), 1994-96, 1998. Breastfeeding infants and children were excluded from the analysis. Estimates are based on food consumption reported by individuals who provided two 24-hour diet recalls and maximum Frutafit use levels specified in Table 1.

(a) Frutafit is [redacted]% inulin (by weight).

(b) No food codes for reduced fat forms of this food category are in the 1994-96, 98 CSFII; estimates are based on consumption of full fat versions.

(c) No food codes for fresh pasta or precooked macaroni are in the 1994-96, 98 CSFII; estimates are based on consumption of all pasta and macaroni.

Note: Unless indicated otherwise, all food categories include both regular and lite versions of all food products.

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Table 5. Estimated Daily Intake of Frutafit® and Inulin from Proposed Uses by the U.S. Population Ages 2 Years and Older

	Users		2-Day Average Frutafit Intake (g/d)		2-Day Average Inulin Intake [a] (g/d)	
Food Category	N	%	Mean	90th Percentile	Mean	90th Percentile
ALL CATEGORIES COMBINED	18033	99.8	11.3	21.3	10.1	19.2
Baby foods	93	0.2	0.3	0.7	0.3	0.6
Baked goods, lite cakes	250	1.6	1.9	3.2	1.7	2.9
Baked goods, lite cookies	365	2.3	2.0	3.8	1.8	3.4
Bars	748	4.0	2.7	4.6	2.5	4.1
Beverages, fermented milks	98	0.5	3.9	7.3	3.5	6.6
Beverages, functional	227	1.7	13.8	25.8	12.4	23.2
Beverages, juices and juice drinks	9146	39.9	4.7	9.4	4.3	8.4
Beverages, milk-based	2140	9.3	1.8	3.2	1.7	2.9
Biscuits, reduced fat	6	< 0.05	1.5	2.5	1.4	2.3
Breads, conventional	16565	91.9	0.3	0.6	0.3	0.5
Breads, specialty	578	3.6	2.2	4.2	2.0	3.8
Candy, hard dietetic	14	0.1	0.4	0.7	0.3	0.6
Candy, soft dietetic	5	< 0.05	0.4	1.3	0.4	1.1
Condiments	7153	39.0	0.5	1.2	0.5	1.0
Cream cheese, reduced fat	265	1.8	0.6	1.4	0.5	1.3
French fry coatings	4721	25.5	0.9	1.5	0.8	1.3
Frozen dairy desserts, lite	1294	7.1	7.6	14.9	6.8	13.4
Icings/glazes, lite	81	0.5	0.5	0.9	0.4	0.8
Jams and jellies, lite	141	0.7	0.2	0.4	0.2	0.4
Meat products	13274	72.1	2.2	4.6	2.0	4.1
Mousse, reduced fat [b]	13	0.1	1.3	1.9	1.1	1.7
Pancake syrup, lite	438	2.0	0.5	1.1	0.4	1.0
Pasta fillings	422	2.0	1.8	3.8	1.6	3.4
Pasta, fresh [c]	3375	18.6	2.0	4.2	1.8	3.8
Pasta, precooked macaroni [c]	2133	10.9	3.0	6.0	2.7	5.4
Pizza crust	3572	20.0	2.1	4.4	1.9	3.9
Potatoes, mashed	2517	13.6	2.9	6.3	2.6	5.7
Pretzels, soft	155	0.9	3.8	7.2	3.4	6.4
Processed cheese, reduced fat	340	2.2	0.8	1.6	0.8	1.4
Pudding mix	549	2.7	0.8	1.5	0.7	1.4
RTE breakfast cereals	9049	40.8	5.5	10.1	4.9	9.1
Salad dressings, lite	1827	12.2	0.8	1.7	0.7	1.6
Sauces and gravies	13266	73.9	1.5	3.3	1.3	3.0
Snack chips, reduced fat	350	2.0	0.5	0.9	0.5	0.8
Snack crackers	2550	11.7	0.8	1.6	0.7	1.4
Soups, dry	1199	5.9	0.6	1.1	0.5	1.0
Spreads, reduced fat	4569	24.1	0.7	1.5	0.7	1.4
Surimi	72	0.6	0.7	1.7	0.6	1.5
Toppings, dessert	301	1.8	0.5	1.1	0.5	1.0

Table 5. Estimated Daily Intake of Frutafit® and Inulin from Proposed Uses by the U.S. Population Ages 2 Years and Older

	Users		2-Day Average Frutafit Intake (g/d)		2-Day Average Inulin Intake [a] (g/d)	
Food Category	N	%	Mean	90th Percentile	Mean	90th Percentile
Tortillas, reduced fat [b]	2405	13.3	1.3	2.6	1.2	2.3
Vegetarian patties/crumbles	329	2.1	0.2	0.7	0.2	0.6
Whipped toppings, lite	101	0.7	0.6	1.7	0.5	1.5
Yogurt, reduced fat	1191	6.0	3.4	6.4	3.1	5.8

Data source: USDA Continuing Survey of Food Intakes by Individuals (CSFII), 1994-96, 1998. Breastfeeding infants and children were excluded from the analysis. Estimates are based on food consumption reported by individuals who provided two 24-hour diet recalls and maximum Frutafit use levels specified in Table 1.

(a) Frutafit is [redacted]% inulin (by weight).

(b) No food codes for reduced fat forms of this food category are in the 1994-96, 98 CSFII; estimates are based on consumption of full fat versions.

(c) No food codes for fresh pasta or precooked macaroni are in the 1994-96, 98 CSFII; estimates are based on consumption of all pasta and macaroni.

Note: Unless indicated otherwise, all food categories include both regular and lite versions of all food products.

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IV. INTENDED TECHNICAL EFFECT

Inulin possesses several beneficial characteristics when consumed as an ingredient in food products. Because inulin is a reduced-calorie carbohydrate with a slightly sweet taste, Frutafit® can be used to replace fat and sugar as a "bulking agent" in a wide variety of food products with a resultant reduction in the energy content of the food. In addition, as a result of its β 2-1 linkage, inulin is not hydrolyzed or absorbed from the human intestinal tract and can, serve as a source of fermentable carbohydrate in the diet. Fermentation by the nonpathogenic colonic bacteria, most notably, bifidobacteria and lactobacilli, results in the formation of bioavailable, short-chain fatty acids (SCFAs). Preferential utilization of inulin by these nonpathogenic organisms alters the colonic flora in favor of a normal healthy composition. Taking into account the bioavailability of the SCFAs produced by the colonic microflora fermentation, 1 gram of Frutafit® provides 1 to 1.5 kcal of energy in comparison to 4 kcal/gram of other carbohydrates. The reduction in calories is predicted based on the lack of digestion in the small intestine

A. Bulking Agent

Bulking agents are defined as any reduced-calorie or non-calorie carbohydrate. Bulking agents are not generally digested fully in the small intestine, and consequently, their major energy contribution is through bacterial fermentation in the colon. Hosoya et al. (1988) evaluated the caloric value of inulin (defined by the authors as FOS; 1F-(β-fructofuranosyl)n-1-sucrose in which n varies from 2 to 4) in humans using a radiorespirometry method after ingestion of 6.1 grams [^{14}C]FOS. The results of these studies showed that the [^{14}C]FOS was fermented by intestinal bacteria into $^{14}CO_2$ and [^{14}C]volatile fatty acids that are absorbed and utilized to yield respiratory $^{14}CO_2$. Calculation of the caloric value of FOS was determined through a quantification of the amount of the [^{14}C]volatile fatty acids that are absorbed from the colon and the results showed that the energy of 1.0 g of FOS (i.e., inulin) was 1.5 kcal/gram.

Ranhotra et al. (1993) evaluated four bulking agents, including inulin (defined by the authors as oligofructose; Raftiline®, with DP of 2 to 50) for usable energy based on the efficiency of conversion of gross food energy to net energy (carcass energy). Young rats fed oligofructose-containing diets over 3 weeks had increased body weight relative to similar groups fed diets containing similar amounts of silica (nutritionally inert). Body composition data and weight gains in rats fed oligofructose were compared to efficiency of conversion in similar groups fed equivalent amounts of starch in the diet (3.67 calories/gram) to result in an estimate of 1.48 kcal/gram for oligofructose (i.e., inulin).

Amstrong (1969) cited by Roberfroid et al. (1993) reported similar caloric values for a fructosyl unit of oligofructose ranging from 1 to 1.5 kcal/gram following comparison of relative efficiency of utilization of energy of short chain fatty acids when compared to glucose or fructose.

Roberfroid (1999) reviewed the caloric values determined for inulin and oligofructose as part of the international conference on Nutritional and Health Benefits of Inulin and Oligofructose, Bethesda, MD in 1998. The caloric value determined for inulin was noted to depend on both the degree of colonic fermentation and the biochemical assumptions of the model used. The recommendation was made that nutrition labeling should consider that inulin and oligofructose should be given a caloric value of 1.5 kcal/g, which is approximately 65 to 75% less than a fully digested hexose such as glucose, fructose, etc. (i.e., 3.9 kcal/g).

B. Role of Inulin in Maintaining The Optimal Balance of Colonic Microflora

Inulin and related β 1-2 linked fructans are not generally digested in the small intestine, and consequently, their major energy contribution is through bacterial fermentation in the large intestine (Ranhotra et al. 1993). Once liberated by oligosaccharide hydrolysis, β d-fructose serves as a substrate for the distinct metabolic pathway by bifidobacteria in the colon that can oxidize these carbohydrates. Data from Roberfroid et al. (1993) and others cited in this publication indicate that in terms of carbon units, colonic fermentation of 1 mol hexosyl equivalent from oligofructose produces 40 percent short-chain fatty acids (SCFA), 15 percent lactate, and 5 percent CO_2, and up to 40 percent bacterial biomass, mainly bifidobacteria.

The experimental evidence that demonstrates that inulin and related oligofructose and FOS are fermented by the colonic microflora, alter the colonic flora in favor of a healthier composition, and are preferentially utilized by the bifidobacteria, is demonstrated in both *in vitro* and *in vivo* studies. Bacteria such as *Bifidobacterium* and *Lactobacillus* are considered to be beneficial to health whereas *Salmonella* spp., Listeria, shigellas, campylobacters, enteropathogenic *Escherichia coli* and clostridia are harmful to health (Gibson and Roberfroid 1995; Tomomatsu 1994; Gibson et al. 1994). Through the process of fermentation, colonic bacteria influence gut physiology as well as having other systemic effects. For example, colonic bacteria produce short chain fatty acids (SCFA) as a result of carbohydrate and protein fermentation that can then be salvaged by the host for energy from SCFA absorption. Following absorption, SCFA are metabolically utilized by various tissues: butyrate by the colonic epithelium, propionate, L-lactate and acetate (partly) by the liver and acetate (partly) by muscle and other peripheral tissues (Gibson and Roberfroid 1995). Inulin has been shown to serve as a preferential substrate for growth of bifidobacteria.

The fact that inulin is fermented by the colonic microflora and, in particular, preferentially utilized by the bifidobacteria, is demonstrated in both *in vitro* and clinical studies summarized below and in Table 6.

1. ***In Vitro* Evidence**

Wang and Gibson (1993) measured the *in vitro* fermentability of oligofructose (defined by the authors as a fraction of inulin with a DP below 20), inulin and a range of reference carbohydrates by measuring bacterial end-product formation in batch culture. The production of SCFA and gas indicated that these substrates were utilized by mixed populations of gut bacteria. Bacterial growth data showed that oligofructose and inulin were preferentially utilized by the *Bifidobacterium* while potentially pathogenic species such as *E. coli* and *Clostridium* were maintained at relatively low levels.

Gibson and Wang (1994a) investigated batch culture growth of eight species of bifidobacteria using short chain, linear oligofructose with a DP of 4, inulin with a DP of 10, or branched chain oligofructose was a DP of 13 (supplied by Raffinerie Tirlemontoise) compared to glucose. Highest specific growth rates of bifidobacteria were obtained in media which contained oligofructose as the sole source of carbon and energy, in comparison to glucose. Most of the bifidobacteria tested were able to grow well with the inulins tested, apart from B. *bifidum* which grew relatively slowly. In particular, *B. infantis, B. pseudolongum,* and *B. angulatum* growth was enhanced with inulins compared to glucose. Generally, the preferred growth substrate for the bifidobacteria was short chain linear oligofructose, followed by inulin.

Chemostat cultures of human fecal bacteria were used to determine the ability of bifidobacteria to utilize oligofructose (M_r 580) and inulin (M_r 1440) (extracted from chicory, supplied by Raffinerie Tirlemontoise) as a substrate (Gibson and Wang 1994b). The study demonstrated that in chemostat cultures, oligofructose and inulin may specifically stimulate populations of bifidobacteria. This effect was enhanced when conditions were imposed, such as higher carbon source availability, high bacterial growth rate and an acidic environment, to resemble those that occur in the proximal colon.

Continuous measurement of the growth of bifidobacteria using various inulin preparations (including Neosugar® and inulin from Jerusalem artichokes and Dahlia tubers) was determined using evacuated culture tubes (Yamazaki and Dilawri 1990). Inulin (described by the authors as FOS; DP of 2 to >5) from the Jerusalem artichoke supported good growth of *B. infantis, B. adolescentis,* and *B. longum.* Dahlia tuber inulin (with a greater percentage of long chain lengths, DP > 5, than inulin from the Jerusalem artichoke) was utilized more slowly by *B. longum* than *B. infantis* or *B. adolescentis*, indicating that *B. longum* was deficient in the enzymes for hydrolyzing high molecular weight inulin.

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Inulin isolated from a commercial preparation (defined by the authors as FOS; pure GF_n oligosaccharides and a mixture of two oligosaccharides, F_3 and GF_2, with a DP of 3) was fermented *in vitro* by bifidobacteria, including *B. adolescentis* (Van Laere et al. 1995). The production of fructose and glucose by the fermentation indicates the presence of a β-fructofuranosidase which hydrolyses the GF_n oligomers as well as the F_n oligomers. To investigate whether production of hydrolytic enzymes can be induced by culturing

bifidobacteria on different carbohydrates, *B. adolescentis* was grown on glucose, galactose, maltose, melibiose and transgalactooligosaccharides (TOS). β-fructofuranosidase activity was observed in the enzyme-extract after growth on glucose, TOS and maltose. Incubation of FOS with the enzyme-extract of cells cultured on maltose resulted in complete degradation to fructose and glucose; degradation to glucose, fructose and sucrose was obtained from extracts of cells cultured on TOS and glucose. The level of this enzyme in the extracts was dependent upon the type of carbohydrate the cells were grown on.

Fecal slurries were prepared from samples obtained from healthy volunteers and individual carbohydrates were added as growth substrates for colonic bacteria (Billingham et al. 1995). To confirm active fermentation in the systems, SCFA production was continuously monitored. Comparisons between the growth of gut bacteria utilizing pectin, fructose, polydextrose, starch, oligofructose (DP not defined by authors) and inulin indicate that bifidobacteria prefer oligofructose and inulin as substrates. Results of *in vitro* studies on bifidogenic effects of inulin are summarized in Table 3.

2. *In Vivo* Evidence

Many studies conducted in both animals and humans have demonstrated the selective utilization of inulin, oligofructose and FOS by bifidobacteria. Results of *in vivo* studies on bifidogenic effects of inulin are summarized in Table 6.

a. Animals

In a study using heteroxenic rats, obtained by inoculating germ free rats with a suspension of human feces from a methane producer, animals were fed control diets or diets containing 40 grams per kg of body weight of gluco- or fructo- or galactooligosaccharides (Andrieux et al. 1991). After a 5 week adaptation period, bacteriological counts, bacterial enzyme activities, and concentrations of fermentation products were measured. Rats fed the control diet kept the major characteristic of the human donor in terms of bacterial population, enzyme activities and metabolite profile. Galacto-oligosaccharides and FOS (not specifically defined by the authors) were the preferred growth substrates for bifidobacteria which increased in number by 2 log values compared with control. Counts of other bacteria were not affected by the different oligosaccharides. Ingestion of galacto-oligosaccharides and FOS specifically induced β-galactosidase or fructo-hydrolase activities. In addition, a decrease of pH of intestinal contents and increase of SCFA production was obtained. 000044

It is not yet clear whether the predominance of bifidobacteria is a result of their ability to ferment inulin faster than most competitive species, or whether the drop in pH, due to fermentation of inulin, creates a more favorable environment for bifidobacteria (Hartemink et al. 1993). The fact that bifidobacteria grow faster on inulin than on glucose indicates that they may possess a specific mechanism for either the uptake or

fermentation of inulin. Bifidobacteria may accumulate inulin intracellularly by means of a specific uptake mechanism (Hartemink et al. 1993). Finally, bifidobacteria ferment hexoses by a different mechanism that most other species; this process may be a more efficient one for degrading inulin than other pathways (Hartemink et al. 1993; Modler et al. 1990).

b. Human Subjects

The selective fermentation of inulin by bifidobacteria has also been confirmed *in vivo* in human clinical studies. Both elderly persons as well as diabetics ingesting FOS (defined by the authors as Neosugar® in the elderly study) have demonstrated large changes in intestinal flora, including increased numbers of bifidobacteria and lactobacilli; the numbers of Enterobacteriaceae and clostridia, however, decreased rapidly (Hartemink et al. 1993). Bacteriological analysis was completed on fecal samples collected from subjects given 5 grams of oligofructose (DP<9), three times per day (15 grams/day) for two weeks (Gibson and Roberfroid 1995). Ingestion of the oligofructose significantly increased the proportion of bifidobacteria from 6 to 22 percent while bacteroides, clostridia and fusobacteria were decreased from 25 to 4 percent, 1 to 0.2 percent and 4 to 0.4 percent, respectively.

Another human volunteer trial assessed the bifidogenic effect of inulin or oligofructose (Raftilose®) given at a level of 15 grams/day for a 45 day feeding period (Gibson et al. 1995). Fecal bacterial composition was evaluated in these subjects in comparison to sucrose (15 grams/day) as a control. Inulin or oligofructose, used as a replacement for sucrose in the diet, caused a marked increase in bifidobacteria while bacteroides, fusobacteria and clostridia all decreased. Inulin or oligofructose appeared to specifically favor beneficial bacteria that were indigenous in the gastrointestinal tract (Gibson 1999).

Clinical studies of Neosugar® (FOS with a DP of 3 to 5) demonstrate that it also has a beneficial effect on the bifidobacteria population of the intestine. Neosugar® (8 grams/day) was administered daily for two weeks to 23 senile inpatients ranging from 50 to 90 years of age (Hidaka et al. 1986). The number of bifidobacteria increased significantly from 4 to 7 days after Neosugar® administration. There was a significant negative correlation between average count of bifidobacteria and the occurrence of *Clostridium perfringens*; it is possible that bifidobacteria may suppress the growth of *Clostridium perfringens* by producing acetic and lactic acids. Similar findings were observed in a study of ten adult subjects given 4 grams of Neosugar® in the diet each day for 14 days (Williams et al. 1994). Increased populations of bifidobacteria were noted; there was a decrease in enteric bacteria.

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Mitsuoka et al. (1987) fed a diet containing 15 grams/day of Neosugar® G (35 percent glucose and fructose, 10 percent sucrose and 55 percent GF_2, GF_3, and GF_4) to 23 elderly patients (50 to 90 years old) in hospitals for 2 weeks. During the ingestion

of Neosugar® G, the numbers of bifidobacteria in the stools increased about 10 times compared before ingestion and the frequency of occurrence of bifidobacteria was increased from 87 percent to 100 percent. After final ingestion, the numbers of bifidobacteria decreased. Neosugar® (1, 2, or 4 grams per day over 4 to 12 weeks) was administered to 30 patients suffering from hyperlipemia, diabetes, high blood pressure and peripheral arterial occlusion (Mitsuoka et al. 1986). A significant increase in bifidobacteria was seen in the group of patients given 4 grams/day; the increase appeared to be dose related. A group of 13 diabetic patients administered 8 to 10 grams of Neosugar® daily for 4 weeks were evaluated for effects on intestinal microflora (Sanno 1986). Fecal samples obtained from five patients were examined for intestinal microflora, and it was found that the number of bifidobacteria had increased remarkably, while the count of *Clostridium* had decreased sharply.

Rao (1999) concluded that a majority of human studies with inulin and its hydrolysis product oligofructose have shown that beneficial effects were seen on populations of intestinal microflora, particularly bifidobacteria, using relatively high dietary concentrations of 8 to 40 g/day. When results from multiple studies were combined and evaluated for determination of a dose-response relationship, a good correlation was not found with regard to dose of inulin used and log increases in counts of bifidobacteria. The stimulation of population growth of bifidobacteria by inulin appeared to be primarily dependent upon the number of bacteria present initially rather than the dose level of inulin employed. Thus, relatively low concentrations of inulin may be effective in stimulating bifidobacteria and high doses are not required to achieve beneficial effects on levels of endogenous bifidobacteria.

A recent review of the effect of inulin and oligofructose on intestinal function in humans (Jenkins et al. 1999) concluded that there is strong evidence that both materials promote growth of bifidobacteria at the expense of other anaerobes and increase fecal bulk and fecal nitrogen elimination.

C. Conclusions

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In conclusion, colonic microflora play an important role in human health. Bifidobacteria and lactobacilli have been recognized as beneficial; they have been attributed various health promoting functions such as the production of SCFA which acidify the gut contents, synthesis of B group vitamins, immunostimulation and inhibitory effects on the growth of potential pathogens such as bacterioids, clostridia or coliforms. Inulin has been shown both *in vitro* and *in vivo* to enhance to ability of bifidobacteria and lactobacilli to grow. It is not yet clear whether the predominance of bifidobacteria and lactobacilli enhanced by inulin is a result of their ability to ferment inulin faster than most competitive species, or whether the drop in pH, due to fermentation of inulin, intake of inulin creates a more favorable environment (Hartemink et al. 1993).

Table 6. Studies on the Bifidogenic Effects of Inulin				
Study Type	**Substrate**	**Study Design**	**Effect**	**Reference**
In vitro	Oligofructose (DP < 20)	Bacterial end-product formation in batch culture	SCFA and gas production; oligofructose and inulin preferentially utilized by Bifidobacteria	Wang and Gibson (1993)
In vitro	Oligofructose (DP 4), inulin (DP 10), oligofructose (DP 13)	Batch culture growth	Preferred growth substrate for bifidobacteria is short chain linear oligofructose, followed by inulin	Gibson and Wang (1994a)
In vitro	Oligofructose (Mw 580), inulin (Mw 1440)	Chemostat cultures of human fecal bacteria	Oligofructose specifically stimulated bifidobacteria compared with inulin or sucrose	Gibson and Wang (1994b)
In vitro	FOS from Jerusalem artichoke and Dahlia tuber inulin	Continuous growth measurement in evacuated culture tubes	Both supported good growth of bifidobacteria, however, there were strain differences in substrate specificity	Yamazaki and Dilawri (1990)
In vitro	FOS from a commercial preparation	Measurement of glucose and fructose production by fermentation to indicate presence of a β-fructo-furanosidase	FOS was a good substrate for fermentation by bifidobacteria	Van Laere et al. (1995)
In vitro	Oligofructose and inulin	Fecal slurries prepared; carbohydrates added as growth substrates. Measurement of SCFA production	Bifidobacteria prefer oligofructose and inulin to pectin, fructose, polydextrose or starch as a growth substrate	Billingham et al. (1995)

Table 6. Studies on the Bifidogenic Effects of Inulin				
Study Type	**Substrate**	**Study Design**	**Effect**	**Reference**
In vivo	FOS and inulin	Elderly person and diabetics ingesting FOS and inulin	Demonstrate large changes in intestinal flora, including increased numbers of bifidobacteria and lactobacilli	Hartemink et al. (1993)
In vivo	FOS (Raftilose®; defined as DP 3 to 7 with an average DP of 4)	Subjects given 5 grams, three times per day (15 grams/day) for two weeks	Ingestion of FOS significantly increased proportion of bifidobacteria, while bacteroides, clostridia and fusobacteria were decreased	Gibson and Roberfroid (1995)
In vivo	FOS (Raftilose®; defined as DP 3 to 7 with an average DP of 4)	Subjects given 15 grams/day for 45 days	Ingestion of FOS produced a marked increase in bifidobacteria while bacteroides, fusobacteria and clostridia all decreased	Gibson et al. (1995)
In vivo	Neosugar	Subjects (senile inpatients ranging from 50 to 90 years of age) given 8 grams/day for two weeks	Number of bifidobacteria increased significantly from 4 to 7 days after Neosugar® administration; *C. perfringens* was decreased	Hidaka et al. (1986)

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Table 6. Studies on the Bifidogenic Effects of Inulin				
Study Type	**Substrate**	**Study Design**	**Effect**	**Reference**
In vivo	Neosugar®	Subjects (adult) given 4 grams per day for 14 days	Increase in number of bifidobacteria and lactobacilli noted	Williams et al. (1994)
In vivo	Neosugar®	Subjects (patients suffering from hyperlipemia, diabetes, high blood pressure and peripheral arterial occlusion) given 1, 2, or 4 grams/day for 4 to 12 weeks	Dose related increase in bifidobacteria	Mitsuoka et al. (1986)
In vivo	Neosugar®	Diabetic patients given 8 to 10 grams per day for 4 weeks	Number of bifidobacteria increased, *Clostridium* decreased	Sanno (1986)

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V. REVIEW OF SAFETY DATA

A. Metabolism and Physiological Properties of Inulin in the Gastrointestinal Tract

Inulin, oligofructose and FOS belong to a class of carbohydrates known as fructans that consist of linear chains of β 2-1 linked D-fructofuranose units with a terminal glucose moiety, and often branched through β 2-6 linkages. These bonds are resistant to human digestive enzymes; consequently, inulin reaches the colon where it can be fermented by the microflora. The fermentation processes not only provide energy for the bacterial proliferation, but they also produce gases (H_2, CO_2, CH_4), which are not of metabolic value to the host, and small organic acids such as acetate, propionate, butyrate and L-lactate (short-chain fatty acids, SCFA). The SCFA (except for butyrate) are largely absorbed through the intestinal wall, reach the portal circulation and enter the liver where they are utilized. Part of the acetate (25 to 50 percent) is transported via the systemic circulation to the peripheral tissues, predominantly muscle. Thus, the bacterial fermentation of SCFA provides the host with energy (Roberfroid et al. 1993).

Malabsorption of fermentable substrates, such as inulin, results in hydrogen (H_2) production by the colonic flora. This H_2 is absorbed and excreted in expired air and breath. Studies in humans measuring breath H_2 release indirectly demonstrate that inulin reaches the colon and is subsequently fermented by the microflora.

During fermentation by the colonic microflora, inulin is metabolized to SCFA (Tokunaga, Oku, and Hosoya 1986, 1989; Wang and Gibson 1993; Oku 1986; Roberfroid, Gibson, and Delzenne 1993). The stoichiometry of this metabolic conversion, as measured both *in vitro* using a fecal flora and *in vivo* in the cecum of inulin (defined by the authors as a FOS fraction with a DP of 8) fed rats has been defined as follows (Roberfroid et al. 1993): 1 mol fructosyl unit in FOS produces about 1 mol SCFA (0.9 mol acetate, 0.12 mol propionate and 0.06 mol butyrate) and 0.3 mol L(+)-lactate. In terms of C-atoms, the overall balance is 40 percent SCFA, 15 percent L(+)-lactate, 5 percent CO_2 , and about 40 percent bacterial mass. The significance of the production of the SCFA is in their resorption through the colonic epithelium into the portal blood, thus becoming a source of energy and systemic effects for the host. Butyrate is metabolized by the colonocytes. Propionate and L-lactate are completely metabolized in the liver, propionate being transformed into methylmalonyl-SCoA and then succinyl-CoA and L(+)-lactate being a precursor in gluconeogenesis. Acetate is only partly metabolized in the liver; the remaining fraction is metabolized in peripheral tissues, mainly muscle (Roberfroid et al. 1993). There are several physiological consequences of microbial

fermentation. A decrease of the pH in the colon and feces occurs because of production of SCFA by the colonic microflora (Roberfroid et al. 1993). This decrease in pH is thought to be a result of fermentation by colonic bacteria, particularly bifidobacteria, which produce both acetic and lactic acid. The production of these acids and subsequent reduction in intestinal pH may restrict or prohibit the growth of pathogenic bacteria (Modler et al. 1990).

Recent reviews on dietary fructans and nutritional and health implications of inulin have summarized the vast literature on the beneficial effects of these substances in the gastrointestinal tract (Roberfroid 1993; Roberfroid and Delzenne 1998; Grizard and Barthomeuf 1999; and Boeckner et al. 2001).

1. *In Vitro* and *In Vivo* Animal Studies

The *in vitro* studies used homogenates of rat or human intestinal mucosa or purified enzymes and demonstrated that inulin in relatively resistant to various hydrolytic enzymes. Oku et al. (1984) investigated the *in vitro* digestion of inulin (Neosugar®) using rat pancreatic homogenates and small intestinal mucosa homogenates. They found that both GF_2 and GF_3 were not hydrolyzed by the pancreatic homogenate. The GF2 and GF_3 hydrolyzing activities of the intestinal mucosa homogenate enzymes were negligible compared with the digestion of maltose and sucrose by maltase and sucrase, respectively. Digestion of inulin by rat small intestine was measured in isolated everted intestinal sac preparations using measurement of Na^+ dependent active transport of glucose (Tsuji et al. 1986). The results indicated that inulin hydrolysis is negligible. Because the fructofuranosidic linkages in inulin are known to be hydrolyzed under mild acidic conditions, there is the possibility of hydrolysis by gastric juice. Nilsson and Bjorck (1988) demonstrated very slow hydrolysis of cereal fructans (FOS; five different fractions: trisaccharides, tetrasaccharides, pentasaccharides, and two fractions with average DPs of 9) by human gastric juice and by a homogenate of the rat intestinal mucosa. The difference in pH greatly influences the velocity of hydrolysis. Previous studies by Nilsson et al. (1988) of fructan breakdown by human gastric juice have shown that after 1 hour at 37^0 C., the degree of hydrolysis was 10 to 15 percent at pH 1.05 but only 1 percent at pH 2.25. Graham and Aman (1986) reported an increase in low molecular weight sugars following feeding of fructans from Jerusalem artichoke to pigs due to hydrolysis of fructans in the stomach. The pH and the extent of acid hydrolysis of inulin in the stomach *in vivo* may depend on several factors such as the buffering capacity of the ingested food or the rate of gastric emptying. Therefore, although acid hydrolysis may occur, the extent is unknown; the upper limit of hydrolysis of inulin suggested from *in vitro* work is 20 to 30 percent.

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In vivo studies performed in animals have demonstrated that oligofructose, inulin and FOS are practically non-digestible in the small intestine; however, a small amount of acid hydrolysis to fructose and glucose may take place in the stomach. In rats, dietary

consumption of these products leads to an increased fecal excretion of short-chain fatty acids (SCFAs) with a resulting decrease in fecal pH (Roberfroid 1993; Oku, Tokunaga, and Hosoya 1984; Oku 1986; Nilsson and Björck 1988). In addition, dietary inulin (Neosugar®) (10 percent and 20 percent in the diet for 6 to 8 weeks) in rats produces an increase in cecum and colon weight. Stool weight is also significantly increased in a dose-dependent manner and is accompanied by an increased excretion of fecal sterols and bile acids (Tokunaga, Oku, and Hosoya 1986; Oku, Tokunaga, and Hosoya 1984; Roberfroid 1993).

Digestibility of inulin, present in Jerusalem artichoke (JA) tubers added at a level of 12% of the diet on a dry-weight basis, was evaluated in two, 7-month old cannulated pigs (Graham and Aman 1986). Results showed that inulin is partially degraded in the stomach and that shorter-chain polymers are more highly degraded than longer polymers. Degradation was considered to result from the combined action of acid hydrolysis, enzyme activity and digestion by bacteria. Rossi et al. (1997; 1998) evaluated inulin digestion in ileal and fecal samples in 5, 8 week-old cannulated piglets weaned at 28 days of age and fed a diet containing 10% inulin. Fermentation and hindgut parameters were studied in another group of 20 piglets given control diet or diet with 10% inulin and sacrificed at 5 or 9 weeks post-weaning. Inulin digestion was low in the small intestine (7.5% ± 11.4%) but complete in the feces. Following inulin fermentation, higher (nonsignificant) total levels of SCFAs with significant increases in n-valerate were found.

2. Studies in Humans

Studies performed in ileostomy patients showed that inulin (DP >2) isolated from Jerusalem artichoke in practically indigestible in the small intestine of humans (Knudsen and Hessov 1995). The recovery of inulin in ileal effluent was 87 percent at both the low (10 grams inulin product) and the high (30 grams inulin product) intake levels. This confirmed results from human (Rumessen et al. 1990) and rat (Nilsson et al. 1988) studies that showed that inulin is virtually indigestible in the small intestine. The significant change in the fructose:glucose ration of inulin from ingestion (4.1) to recovery in ileal effluent (4.5-4.7) and a lower recovery of the glucose residue that of the fructose residue of inulin indicate that the low molecular weight inulin are more sensitive to hydrolysis than the high molecular weight fragments. This is possibly due to hydrolysis by either acids or enzymes and to microbial degradation by the microflora in the small intestine (Knudsen and Hessov 1995).

Stone-Dorshow and Levitt (1987) measured H_2 excretion during human ingestion of inulin (Neosugar®) at a level of 15 grams per day (5 grams, 3 times/day) for 12 days. Breath H_2 after 10 grams of inulin was similar to that of 10 grams of lactulose, suggesting near total malabsorption of the inulin. Breath H_2 was also increased (not statistically significant) by 50 percent after a 12-day period on the inulin. Rumessen et al. (1990)

measured breath H_2 excretion in eight healthy subjects after consumption of 5, 10 or 20 grams of FOS from Jerusalem artichoke. The increase in breath H_2 suggested that FOS was completely fermented.

3. Conclusions

In conclusion, the *in vivo* studies corroborate *in vitro* work and show that systemic effects of inulin include the metabolism of SCFA produced by the colonic microflora, and effects on lipid metabolism. The metabolism of inulin and its actions on the gastrointestinal tract because of its non-digestibility can be considered evidence that it acts in a manner similar to other non-digestible polysaccharides.

B. Physiological Effects in the Gastrointestinal Tract

There are numerous recent reviews on the physiological effects of inulin and related fructans (Roberfroid and Delzenne 1998; Grizard and Barthomeuf 1999; Carabin and Flamm 1999; Flamm et al. 2001; and Boeckner et al. 2001). The following sections briefly summarize studies that address major issues that have been raised regarding these dietary components.

1. Effect on Mineral Absorption

There are numerous studies in animals that demonstrate that inulin and related β 2-fructans enhance absorption of some minerals in the colon and gastrointestinal system. Baba et al. (1996) compared the effects of FOS-containing diets (50 g FOS/kg diet) relative to control diets on absorption of magnesium (Mg) from the hindgut of 28 male Sprague Dawley rats with cannulated ceca that were given Mg-containing or Mg-free diets for 15 days. Animals fed Mg-free diets were administered Mg by infusion into the cecum. FOS supplementation produced statistically significant increases in Mg retention in rats either given Mg in the diet or by cecal infusion.

In 28 male Sprague Dawley rats fed a diet supplemented with 50 mg FOS/g diet Ohta et al. (1995) reported a statistically significant increase in calcium and Mg absorption in the colon and rectum compared to animals fed a control diet. This result was confirmed in later studies in which Ohta et al. (1998) fed three groups of 9 male Sprague Dawley rats for 10 days with a diet containing 100g.kg sucrose, sucrose and FOS each at 50 g/kg diet (5% FOS) or FOS alone at 100 g/kg diet (10% FOS). The rats fed either FOS-containing diet had significantly higher mucosal weights than rats fed the sucrose control diet. Absorption of calcium was significantly higher ($p<0.001$) in the rats fed diets containing either 5% or 10% FOS compared to the controls. A significant positive relationship between the amount of calcium absorption and the amounts of calcium-binding protein (calbindin-D9k) in the cecum and colorectum was found.

Levrat et al. (1991) evaluated the effect of dietary inulin on mineral absorption in 48 male Wistar rats fed diets for 21 days that contained 0, 5, 10 or 20% inulin. The cecal pH values were significantly decreased in direct relationship to dose of inulin and the cecal absorption of calcium and Mg were statistically significantly greater than controls at each inulin dose level. Acidification of the intestinal contents as seen in this study is considered to be due to formation of SCFAs from fermentation of inulin. Reduction of pH values in the intestinal lumen and colon would increase the ionization of calcium compounds and thereby increase solubility and bioavailability for the mucosal cells (Takasaki et al. 2000).

In humans, Coudray et al. (1997) fed 9 healthy men a control diet (18 g fiber) or the same diet fortified with 40 g inulin/day for 28 days and evaluated the effect on mineral absorption. The apparent absorption value (intake minus fecal loss) of calcium in subjects fed the inulin diet was statistically increased relative to subjects receiving the control diet. The absorption of Mg, iron and zinc was not significantly different from control values in the subjects ingesting the inulin diet. Ellegard et al. (1997) evaluated mineral absorption in 5 male and 5 female subjects with ileostomies who were fed diets containing 17 g inulin, 17 g FOS or 7 g sucrose for 3 days in a rotating schedule (4 day rest period between treatments) that allowed each subject to also serve as a control for each comparison. In comparison to retention values seen with sucrose, neither inulin nor FOS produced statistically significant differences in excretion of calcium, Mg, zinc or iron.

Mineral absorption was studied by comparing a control diet or diet supplemented with inulin, FOS or galactooligosaccharides (GOS) was given to 12 healthy young men at 15 g/day for 21 days. Each subject served as his own control according to a randomized, cross-over study design (van den Heuvel et al. 1998). No effect on absorption of iron or calcium in comparison to the control diet was seen with any of the fructans evaluated. Amounts of iron in erythrocytes and calcium excretion into urine were not statistically different from among the different treatments as compared to the control diet.

Abrams and Griffin (2000) evaluated the effect of oligofructose administered as a supplement in orange juice on calcium absorption in adolescent girls. Calcium-fortified orange juice (480ml/day) was supplemented with a total of 8 g oligofructose or a placebo and consumed daily for 3 weeks. No significant differences were seen in the amount of calcium absorbed in comparisons of oligofructose versus placebo. Urinary excretion of calcium was also similar in the test and placebo groups.

Teuri et al. (1999) investigated the effect of inulin on the short-term calcium status of 15 women given cheese containing 210 mg of calcium (control) or the cheese with addition of 15 g inulin over a 2-day period. In a follow up study one-week later, the same subjects were randomized and given a 210 mg calcium supplement for 2 days with or without 15 g of inulin. Neither study showed any significant differences in urinary excretion of calcium in comparison of inulin and control groups. Inulin did not affect the levels of serum phosphate, serum calcium concentrations or parathyroid hormone levels.

In conclusion, dietary supplementation with inulin or related β 2- fructans has been shown to enhance uptake of calcium and Mg in animal studies but these same effects on mineral absorption have not been noted in clinical studies with human subjects.

2. Effect on Nitrogen Balance

Levrat et al. (1993) used Wistar rats to evaluate the effect of dietary supplementation with 15% inulin on nitrogen balance and ammonia production in the large intestine. Net nitrogen balance was not significantly different among the groups as determined in the final 3 days following 21 days adaptation to diets containing 15% casein (control), 45% casein (control) and the same diets containing 15% inulin. During this same period fecal nitrogen excretion was significantly increased in all groups as compared to the animals fed 15% casein but plasma urea nitrogen was significantly affected (decreased) only in the rats in the 15% casein/15% inulin group. Inulin supplementation in the casein diets did not significantly alter net nitrogen balance or retention in the rats but inulin depressed cecal ammonia concentrations in the animals fed the 15% but not 45% casein diets.

Delzenne et al. (1995) reported that rats fed control diets or diets supplemented with 10% Raftilose® (oligofructose) or 10% Raftiline® (inulin) for 50 days had statistically significant increases in both fecal and urinary nitrogen excretion and significant decreases in serum urea values compared to controls. However, there were no significant differences in nitrogen balance values in any of the treated groups.

Diez et al. (1998a) reported that feeding adult beagles a diet supplemented with oligofructose (50 g/kg diet) produced approximately 10% reduction in plasma urea levels compared to controls determined at 6 hr following ingestion. These results were supported by a second study in which oligofructose in dog diets (40 or 80 g/kg) for 6 weeks. After 6 wk, the 80 g/kg oligofructose diet decreased ($P < 0.05$) plasma urea levels as compared to the control (13.6 vs. 15.0 mg/dl). There were no differences among treatments in plasma a-amino-nitrogen concentrations. A second study (Diez et al. 1998b) on effects of inulin on blood urea nitrogen levels did not find a similar reduction in beagle plasma urea concentrations attributable to dietary inulin (70 g/kg) as seen in the study with oligofructose.

Thus, dietary supplementation with fructans may produce moderate changes in nitrogen status. This effect may be considered beneficial because of 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; Roberfroid and Delzenne 1998).

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3. Effect on Colonocytes

Howard et al. (1995) evaluated the effect of dietary FOS on colonic epithelia in neonatal pigs fed a liquid formula or liquid formula supplemented with 3% FOS over a 15 day period. The pigs given the FOS supplement had significant increases both in mucosal cell density ($p<0.01$) and in cellular proliferation ($p<0.05$) in the cecum, proximal and distal colon. Similar increases in proximal and distal colonic mucosal crypt height, leading edge and proliferation zone also increased with FOS consumption.

Five male and female Beagle dogs were fed either a diet of fermentable fibers (beet pulp and oligofructose) or nonfermentable fiber (cellulose) and were evaluated for effects upon intestinal parameters and nutrient absorption. Small intestines of dogs fed the diet containing oligofructose had 28% more surface area and 37% more mucosal mass than seen with cellulose diet (Buddington et al. 1999). The oligofructose group also had intestines that were 35% heavier and had a 95% greater capacity for carrier-mediated glucose uptake than the cellulose group. Differences in uptake of proline and glucose were particularly pronounced in the proximal and middle portions of the colon. These beneficial effects on intestinal ultrastructure may reduce risk of enteric infections or aid in treatment of intestinal diseases involving impaired nutrient absorption.

Reddy et al. (1997) fed Fischer 344 rats diets supplemented with 10% oligofructose, 10% inulin or control diets for a 2-3 week period prior to s.c. injection with azoxymethane, which induces preneoplastic lesions seen as aberrant crypt foci. Compared to the incidence of aberrant crypt foci in animals fed the control diet, both inulin and oligofructose produced significantly reduced numbers of total lesions/colon (inulin $p<0.006$ and oligofructose $p<0.02$). The number of aberrant crypts/focus was also significantly inhibited with inulin ($p<0.0001$-0.02) as well as by oligofructose ($p<0.04$-0.01).

C. Systemic Effects

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1. Glucose Metabolism

The potential for inulin and related fructans to alter glucose utilization has been studied in animal models as well as in humans. In studies with rats, Oku et al. (1984) reported that hydrolysis of sucrose or maltose was not affected in rats fed up to 20 percent FOS for 6 weeks. Yamada et al. (1990) found that there was no increase in plasma fructose, plasma glucose, or plasma insulin levels after rats received up to 1 g of FOS by gavage. Kaufhold et al. (2000) conducted a feeding trial in veal calves to evaluate potential befits of FOS supplementation in the diet for reducing hyperglycemia, glucosuria and insulin resistance during periods of fattening involving high lactose intakes. The post-prandial increase in glucose levels, lactate and growth hormone peak frequencies were all lower in the group receiving FOS than the control. Maximal insulin concentrations

reached post-prandially were significantly higher in the FOS group than in control animals. In dogs, Diez et al. (1998) measured plasma glucose and insulin concentrations in response to supplemental (70 g/kg) inulin, guar gum or sugar beet fiber given in the diet. No effects on these parameters from inulin supplementation were found.

In humans, Drevon and Bornet (1992) studied the effect of FOS administration on glycemia and insulinemia in diabetic subjects and demonstrated an improvement of glucose tolerance. Their results showed that neither healthy nor diabetic subjects that ingested a solution containing a total of 22.5 g of FOS had alterations in postprandial plasma glucose, fructose or insulin. Yamashita et al. (1984) reported, that in a clinical study involving daily intake of 8.0 g of FOS for 14 days by in non-insulin dependent diabetic subjects, significant reduced mean fasting blood glucose levels were seen of up to 15 mg/ dl. Control diabetic subjects who were given 5.0 g/day of sucrose showed no significant reduction in blood glucose.

In studies of sugar tolerance by 24 non-insulin dependent diabetic subjects, Sanno et al. (1984) evaluated the effects of FOS consumption on blood sugar, serum insulin, serum lipids and plasma glucagon in comparisons to 6 healthy control individuals. Subjects were given oral doses of 22.5 g FOS alone or simultaneously with 75 g of glucose. No significant effects of FOS ingestion in diabetic subjects was seen in comparison to control subjects on blood sugar, serum insulin, serum lipids or plasma glucagon when FOS was given alone. The effect of glucose plus FOS on these parameters was the same as with glucose alone. The results of this study indicate that diabetics can consume FOS without affecting their blood sugar levels and that FOS had no effect on insulin responses in the control or diabetic subjects. Sanno (1986) reported that in a group of 13 diabetic patients administered 8 to 10 g of FOS (Neosugar®) daily, there was no significant effect on fasting blood sugar or serum lipids after 4 weeks of administration, but there was a significant reduction after 8 and 16 weeks of administration. Luo et al. 1996 (cited in Carabin and Flamm 1999) reported that 12 healthy males that received 20 g FOS /day for two 4-week periods, separated by a 2-week washout period, had no detectable effects on basal hepatic glucose production or insulin-stimulated glucose metabolism.

An overview of the results in human studies indicates that fructans such as FOS have no acute affects on blood sugar or insulin levels in healthy individuals but that high doses or long-term administration may have beneficial effects in controlling these parameters in diabetics.

2. Effect on Blood Lipid Levels

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a. Animal Studies

Ingestion of inulin has also been associated with effects on lipid metabolism; studies have shown that feeding of inulin to rats causes a significant decrease in

serum triglycerides, blood cholesterol and total lipids (Bhattathiry 1971 and Tokunaga et al. 1986). Effects of fructans on lipid profiles in animals have been reviewed recently by Delzenne and Kok (2001) and Roberfroid and Delzenne (1998) and significant effects on decreasing levels of total cholesterol, triglycerides and low density lipoproteins have been seen in numerous studies summarized by these authors..

Roberfroid et al. (1993) evaluated effects on lipid profiles with rats fed a standard diet containing either 5 percent FOS (defined by the authors as a FOS fraction with a DP of 8), 10 percent FOS, 10 percent inulin for 4 weeks or 20 percent FOS for 5 weeks, a fiber-free diet containing either 5 percent FOS or 10 percent FOS for 5 weeks, or a fiber free diet plus 1 percent cholesterol containing 5 percent FOS. Despite differences in basal diet, the dose of FOS or inulin and the duration of the treatment, serum triglyceride concentrations decreased significantly. Percentages of serum triglycerides as compared with controls in standard diet fed rats were 90, 66, 41, and 40 (5 percent FOS, 10 percent FOS, 10 percent inulin for 4 weeks and 20 percent FOS for 5 weeks, respectively); in fiber-free fed rats were 62, and 27 (5 percent and 10 percent FOS for 5 weeks, respectively); and in fiber-free + 1 percent cholesterol fed rats was 57 (5 percent FOS for 5 weeks).

FOS given to rats for 3 months at 10 percent in the diet was accompanied by a decrease in serum cholesterol and phospholipids (Roberfroid et al. 1993). Inulin included in the diet of adult rats at 25 percent (w/w) for three weeks produced a reduction in total blood lipids and cholesterol of approximately half that of control rats (Bhattathiry 1971). A 6 to 8 week dietary administration of 10 percent or 20 percent Neosugar® to rats produced slight but not statistically significant reductions is serum cholesterol levels in the rats fed 20 percent Neosugar diet. The serum triacylglycerol level was statistically significantly decreased by Neosugar intake; the effect was greater in the 20 percent group than the 10 percent group.

Studies by Diez et al. (1998a) with adult beagle dogs showed that diets supplemented with FOS (50 or 102 g/kg body weight) for 6 weeks of supplementation, both 50 and 102 g/kg dietary FOS groups had ($P > 0.05$) decreased plasma cholesterol levels as compared to the control diet (146 and 144 vs. 157 mg/dl, respectively). In several breeds of hyperlipidemic dogs, dietary supplementation with scFOS (short chain FOS) produced resolution of hyperlipidemia in obese dogs and regression of corneal lipidosis in 4 of 7 affected dogs (Diez et al. 2000). The biochemical basis for the hypolipidemic effect of oligofructose and other fructans in animals was described by Delzenne and Kok (1999) as related to modulation of de novo lipogenesis due to oligofructose modification of gene expression of lipogenic enzymes. This mechanism results in decrease in liver lipid accumulation and

increases the intestinal production of insulinotropic peptides that result from tissue proliferation stimulated by fermentation by-products of the oligofructose.

b. Human Trials

In humans, a daily intake of 8.0 grams of inulin (Neosugar®) for 14 days significantly reduced mean fasting blood glucose levels by 15 mg/dl, mean serum total cholesterol levels by 19 mg/dl and LDL-cholesterol levels by 17 mg/dl in diabetic subjects (n = 18). Control diabetic subjects who were given 5.0 grams/day of sucrose did not show any significant change in these parameters (Yamashita, Kawai, and Itakura 1984). The levels of serum HDL-cholesterol, triglycerides or free fatty acids were not significantly affected by inulin consumption.

A study in chronic renal failure patients administered 6 grams per day of inulin (Neosugar®) for 12 months indicated that serum triacylglycerol levels and serum LDL-cholesterol levels were decreased (significance not stated) (Takahashi et al. 1986).

Causey et al. (2000) reported that 12 moderately hyper-cholesterolemic men evaluated in a double-blind, crossover study had a significant reduction of serum triglycerides of 40 mg/dL (p=0.024) when given 20 g/day of chicory inulin. Total serum and LDL cholesterol levels were slightly (non significantly) reduced and no change in HDL cholesterol was seen. Davidson and Maki (1999) also evaluated effects of dietary inulin on serum lipids of 21 hypercholesterolemic men and women using 3 servings/day of inulin containing foods in comparison to similar foods without inulin (control) as well as the effect of 18 g inulin/day together with a low-fat diet. The double-blind, crossover study was conducted in two 6-week treatment periods separated by a 6-week washout interval. During ingestion of the control diet baseline LDL levels increased significantly while nonsignificant declines were noted during the inulin treatment phase. Differences between the inulin and control phase were statistically significant and indicated that inulin may have blunted the hypercholesterolemic effects seen during ingestion of the control foods.

Williams (1999) reviewed the effects of inulin on lipid parameters in humans and concluded that although inulin has demonstrated convincing lipid-lowering effects in animals, similar effects in humans have produced conflicting results. The reason for this difference was hypothesized to be due to the inhibition of hepatic fatty acid synthesis as the major mechanism for inulin action in animals while this pathway is of minor importance in humans unless a high carbohydrate diet is consumed. Thus, they offered that although inulin may have the same lipid-lowering capacity in humans, conflicting results are due to the background diet as a determinant of demonstrating an effect.

In conclusion, although inulin and related β 2- fructans have shown significant potential to produce reduce blood lipid and cholesterol levels in numerous animal studies although similar effects have been reproduced only in some human clinical trials. Effects of inulin on blood lipid levels in human studies may be confounded by interaction with dietary variables and may only be discernable when a high carbohydrate diet is consumed by the subjects and inhibition of hepatic fatty acid synthesis by inulin or its fermentation byproducts becomes a controlling factor for lipogenesis.

D. Toxicology Studies

Although there are no standard toxicology studies conducted on inulin with a DP range similar to that of Frutafit®, there have been extensive animal tests on inulin to determine both metabolism and tolerance. No untoward effects were seen in these studies when dose levels have ranged from 10 to 25 percent inulin in the diet for 3 to 4 weeks or 5 to 20 percent of FOS (DP≤ 8) in the diet for up to 5 weeks. Results from subchronic and chronic toxicity and carcinogenicity studies in rats (Tokunaga, Oku, and Hosoya 1986; Clevenger et al. 1988) demonstrate that there are no significant adverse effects up to 2,170 mg/kg/day. The no-observed effect level (NOEL) for chronic administration of Neosugar® is 2,170 mg/kg/day. The only effect noted was the occurrence of soft stools or diarrhea after ingestion of large quantities of Neosugar® (more than 5 percent in the diet of rats).

Carabin and Flamm (1999) evaluated the safety of inulin and oligofructose and concluded that results from toxicology tests on inulin-type fructans have not shown evidence of mortality, morbidity, target organ toxicity, reproductive or developmental toxicity, mutagenicity or carcinogenicity. These authors concluded that inulin-type fructans are safe for human consumption under intended conditions of use and that up to 20 g/day of inulin and/or oligofructose is well tolerated.

1. *In Vitro* Studies

An Ames assay with *S. typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100, and an *E. coli* WP2 *uvr*A assay was conducted with Neosugar® (FOS) at concentrations of 50 to 5,000μg/plate (Clevenger et al. 1988). There was no increase in frequency of mutation per plate in any bacterial strain with or without metabolic activation compared to control. Therefore, under the conditions of both of these assays, FOS did not possess mutagenic activity. In a mammalian gene mutation assay, mouse lymphoma L5178Y cells were exposed to 2,000 to 5,000 μg/ml of Neosugar® (Clevenger et al. 1988). FOS did not produce an increase in the mutation frequency either with or without metabolic activation. In an assay of DNA damage assessed by Unscheduled DNA synthesis (UDS), HeLa S3 epithelioid cells were exposed to Neosugar® at concentrations

ranging from 25 to 51,200 μg/ml and the percentage of cells in DNA repair was quantified (Clevenger et al. 1988). In the first of two trials, UDS was significantly increased at one Neosugar® concentration, 1600 μg/ml, without metabolic activation. However, no dose response was evident and no significant increase was observed at any concentration either with or without metabolic activation in a repeat test. In all three *in vitro* assays, Neosugar® did not possess genotoxic potential under the conditions of the tests.

2. Animal Studies

A 6 to 8 week study on dietary administration of 10 percent or 20 percent FOS (Neosugar®) (approximately 8 and 16 g/kg/day, respectively) was conducted in rats to determine the effect of intake on body weight gain, organ weight, serum lipids, fecal excretion and intestinal function. Rats developed diarrhea after starting Neosugar® feeding but this was not seen after 2 to 3 weeks. After feeding rats Neosugar® for 6 weeks, the body weight gain of the group receiving the 20 percent Neosugar® diet was statistically significantly lower compared to the control group; no significant effect on body weight gain was note in animals consuming the 10 percent Neosugar® diet. Daily food intake was similar in control and Neosugar®-fed groups. Both the 10 percent and 20 percent Neosugar® diet produced a statistically significant increase in both wet weight and the ratio of cecum to colon weights. a greater effect was observed in the cecum than in the colon of rats fed 20 percent Neosugar®. The wet weight of the small intestine was statistically significantly increased in rats fed the 20 percent Neosugar® diet but not the 10 percent Neosugar® diet. No significant increases in liver or kidney weight were observed. Fecal wet weight was also statistically significantly increased at 10 percent and 20 percent Neosugar®. Serum cholesterol levels were slightly but not statistically significantly reduced by the 20 percent Neosugar® diet. The serum triacylglycerol level was statistically significantly decreased by Neosugar® intake; the effect was greater in the 20 percent group than the 10 percent group. Fecal excretion of sterol is increased significantly by chronic intake of dietary fiber through the inhibition of intestinal cholesterol and bile acid absorption. A statistically significant increase in excretion of neutral sterols and acidic sterols was seen in animals fed Neosugar® at both dose levels and at 20 percent Neosugar®, respectively. The concentration of volatile fatty acids per gram of wet feces greatly increased in rats fed both dose levels of Neosugar®. The greatest increase was in acetic acid, followed by propionic acid. The increase in butyric acid was the smallest.

The results of this study suggest that Neosugar® is not fully utilized by rats as an energy source and that the undigestible or unabsorbable nature of this FOS produced diarrhea and a bulking effect in the intestinal lumen. The fecal volume is increased and intestinal transit time was shortened, similar to actions of a dietary fiber. No adverse effects on the health status of the rats were observed in this study.

In a chronic/carcinogenicity study in Fischer 344 rats (50/sex/group), inulin (Neosugar®) was administered in diets at concentrations of 0, 8,000, 20,000, or 50,000 ppm (equivalent to approximately 341, 854, and 2,170 mg/kg/day, respectively, for male rats and 419, 1,045, and 2,664 mg/kg/day, respectively, for female rats) for 104 weeks (Clevenger et al. 1988). No dose-related effects were noted on survival, growth, food consumption, feed efficiency, hematology parameters, blood chemistry parameters, organ weights, and nonneoplastic lesions. The incidence of rare and spontaneous tumors was comparable between control and Neosugar® treated groups with the exception of pituitary adenomas in male rats. The increased incidence of this tumor was not considered to be related to Neosugar® treatment, however, because the incidence in all groups was within the historical control range of this spontaneous tumor and there was only an equivocal evidence of a dose-response trend. The Cochran-Armitage trend test indicated a significant trend but logistic regression analysis indicated no dose-response relationship. Nonneoplastic lesions common in aging rats were observed in all groups, including controls, but Neosugar® did not affect severity of lesions compared to controls with the exception of renal protein casts in male rats which were not dose-related and was concluded to lack biological significance and relevance to the safety assessment of Neosugar®. For all other non-neoplastic lesions with differences for control values, a comparison to the historical control incidence showed that the various lesions are common and highly variable in aging Fischer 344 rats. Consideration of the absence of dose-related effects and similar findings in historical control animals led to the conclusion that treatment with Neosugar® did not affect the incidence of nonneoplastic lesions. The increased incidence of this tumor was not considered to be related to Neosugar® treatment because the incidence of this spontaneous tumor in all groups was within the historical control range (mean of 31%, with a range of 17-49%) and there was only equivocal evidence of a dose-response trend (the Cochran-Armitage trend test indicated a significant trend but a logistic regression analysis indicated no dose-response relationship, p=0.51).

In response to a review of the toxicology studies of FOS by the Scientific Committee for Food of the European Union, an additional study was undertaken on behalf of Beghin Meiji Industries (Meiji Seika Kaisha 1982b). The objective of this study was to provide additional information that could be used to interpret findings from the chronic/ carcinogenicity study. The study was designed to address the following two points: 1) FOS increases the weight of the small intestine, cecum and colon, and is fermented by the colonic microflora. Intestinal bacteria are known to play a role in colon carcinogenesis. Therefore, the effects of high dose consumption of FOS on chemically induced colon cancer should be tested by the microadenoma assay to determine if there is a promoting effect of FOS on colon cancer; 2) A significant increase in liver granulation was noted in the chronic feeding study. An experiment examined the subchronic effects of FOS on the liver by measuring various relevant hepatic parameters. In the first experiment, results of the microadenoma

assay indicate that at levels up to 15 percent in the diet of rats, FOS did not significantly modify the number of aberrant crypts and therefore did not act as a promotor of chemically-induced carcinogenesis. In the second experiment, oral administration of 7.5 or 15 percent FOS (approximately 7.2 and 16.7 g/kg/day) in the diet of rats for 13 weeks did not modify the total content of retinol, retinol palmitate and tocopherol as well as glutathione in the rat liver. Therefore, no effect on these hepatic free radical scavengers was seen. The hepatic cytochrome P450 level as well as the cytochrome P450 dependent testosterone metabolism was not modified by FOS administration.

3. Conclusions

Standard toxicology studies have been conducted on FOS, which is a β 1-2 linked fructan with chemical and physiological properties similar to inulin, and available data include a genotoxicity battery, teratology studies and rat subchronic and carcinogenicity assays. Toxicology assays indicate that FOS does not possess genotoxic potential, and there are no significant adverse effects or carcinogenic potential after chronic administration of up to 2,170 mg/kg/day. The gastrointestinal effects noted in animal studies are consistent with the gastrointestinal disruptions caused by high levels of similar non-digestible materials. The absence of toxicological concerns for FOS and related fructans such as inulin is consistent with the same conclusion of safety for inulin and oligofructose by Carabin and Flamm (1999).

E. Assessment of Potential Toxicity Associated with Exposure to Chicory Root

The source of Frutafit® is the chicory root. Therefore, an evaluation of the potential toxicity associated with exposure to the root of the chicory plant, *Cichorium intybus* was performed. The only chemical compounds that were identified from chicory for further evaluation were the sesquiterpene lactones. More specifically, the literature reviewed indicated that a total of twelve sesquiterpene lactones have been isolated from chicory (Seto et al. 1988; van Beek et al. 1990). These compounds are lactucin, lactucopicrin, 8-deoxylactucin, crepidiaside B, cichorioside B, sonchuside A, cichorioside C, and sonchuside C, cichoriolide A, 11(S), 13-dihydrolactucin, 11(S), 13-dihydrolactucopicrin, and 11(S),13-dihydro-8-deoxylactucin. It is established that the bitter taste characteristic of chicory and related species of the Compositae family is caused by the presence of sesquiterpene lactones. A sesquiterpene lactone is a 15-carbon molecule composed of a lactone ring attached to a sesquiterpene. A sesquiterpene contains three isoprene units arranged in a double-ring structure.

1. Toxicity of Chicory

The search of the scientific and medical literature on chicory and its twelve associated sesquiterpene lactones indicated that the only adverse health effects that have been identified with this plant and its roots are associated with occupational dermal and possibly inhalation

exposure. Health findings were associated primarily in food handlers who routinely work with this plant (e.g. green grocers, Friis et al. (1975); market gardeners, Nemery and Demedts (1989); salad makers, Helbling et al. (1997); vegetable wholesalers, Cadot et al. (1996); and farm workers, Malten (1983). According to Warshaw and Zug (1996), the reactions that have been observed are of three types: eczematous contact dermatitis of the hands and forearms, contact urticaria, or seasonal photodermatitis-like eczematous rash of the arms, face and neck. In addition two recent case reports indicated that chicory exposure was associated with: an immediate-type allergy in a green grocer; and occupational asthma in a market gardener.

2. Analysis of Levels of Sesquiterpene Lactones in Inulin Samples

Agrotechnological Research Institute (ATO-DLO) analyzed two inulin samples (IQ and HD) to determine levels of two sesquiterpene lactones: lactucopicrin and lactucin-like compounds (van Amerongen and Berendsen 1998). A third sample (2-2), representing a non-inulin blended animal feed product was also analyzed; this sample is of no relevance to the inulin products under consideration (Peters 1999). To determine the sesquiterpene lactone concentrations, the specific enzyme-linked immunosorbent assays (ELISAs) developed by Peters and van Amerongen (1996) were used. In the two inulin samples, the mean levels of lactucin-like compounds ranged from 1.38-1.41 μg/g dry weight; mean levels of lactucopicrin ranged from 0.48-0.80 μg/g dry weight. These concentrations in inulin samples are at the lower end of the range of concentrations that have been reported in raw chicory samples (e.g. lactucin = 0.108 - 3.464 ppm, lactucoprin = 0.096 - 3.069 ppm in van Beek et al. 1990; lactucin = 5 - 502 ppm, lactucoprin = 7 - 569 ppm in Price et al. 1990; lactucin = 167 - 196 ppm, lactucoprin = 40 - 74 ppm in Peters and van Amerongen, 1996).

3. Conclusions

A review of the scientific literature indicated that the only potential health effects that have been associated with chicory have been infrequently observed in occupationally exposed individuals, who were exposed by the dermal or possibly the inhalation route. In spite of the widespread consumption of chicory as a coffee substitute, development of allergenicity or other effects in consumers in the general population has only been rarely reported. These chicory products are not produced by the same rigorous production methods, practices and quality standards that are used to control dietary fructans such as Frutafit® and other β 2- fructan products enumerated. In addition, exposure to crude chicory by occupationally-exposed individuals may involve exposures to a significantly different spectra of impurities and residuals that differ significantly and affect biological and allergenic potential.

Sesquiterpene lactone levels, suspected to be associated with the potential allergenicity of various members of the Compositae plant family, were found in inulin samples to be present at the lower ends of the reported ranges of concentrations in raw chicory. This suggests that the magnitude of potential exposure that would occur from ingestion of Fruitafit® inulin would be

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far below the levels of occupational exposure to chicory that have only rarely been associated with any adverse health effect.

E. Human Clinical Trials to Determine Tolerance to Inulin

Numerous clinical trials have been conducted on inulin or closely related products such as Raftilose® (oligofructose) or Neosugar® (FOS). A summary list of the results of these clinical studies is presented in Table 7.

The results of these studies allow a conclusion that the consumption of the naturally-occurring, long-chain inulins from foods is tolerated at levels higher than that of Raftilose® or Neosugar. In addition, similar to other dietary fructans, human tolerance to inulin has been demonstrated to be greater when inulin is consumed as part of the regular diet as opposed to via a bolus dose. Even in the case of Neosugar®, which caused adverse effects such as diarrhea when initially consumed of large amounts, greater tolerance was achieved with continued consumption (Oku 1986).

A review of the earliest clinical studies utilizing inulin for the treatment of diabetes was published by Lewis in 1912. As stated in this review, Külz was the first researcher to study the action of inulin in the diet of diabetics. He concluded that in both mild and severe cases of diabetes, the feeding of 50 to 120 grams of inulin resulted in the complete assimilation of inulin, as no inulin was found in the urine or feces (Külz 1874, as reported in Lewis 1912). A lack of inulin in the feces of diabetics fed large doses of inulin over long time periods was reported by Persia in 1905 (Persia 1905, as reported in Lewis 1912). Strauss again reported similar results in the feeding of pure inulin to two diabetics. He found no inulin in the urine of these patients, who were fed inulin in amounts ranging from 40 to 100 grams per day, and observed inulin consumption to be beneficial (Strauss 1911, as reported in Lewis 1912). No adverse side effects were reported by Lewis for any of these studies.

Goudberg (1913) also used diabetics to test inulin utilization. He administered 200 grams of inulin, dissolved in a small amount of hot water, into the stomachs of test subjects using a stomach tube and measured their respiration quotients at specified intervals. Relatively serious diarrhea and strong intestinal gas formation were regularly experienced four to six hours after ingestion of inulin.

Inulin was fed to a patient suffering from levulosuria. A bolus dose of 80 grams of inulin was administered in a single meal, and although no inulin was found in the urine or feces, the patient observed strong intestinal gas formation during the period following the meal (Neubauer 1905, as reported in Lewis 1912). A marked formation of intestinal gas was also reported in two experiments performed by Lewis. In the first experiment, a healthy individual was placed on a cellulose-free diet and fed 40 grams of inulin over a single day's lunch and dinner meals. The subject experienced constipation, which was attributed to the lack of cellulose in the diet, and the formation of intestinal gas. In the second experiment, the same individual was placed on a regular diet and fed 60 grams of inulin over a single day's lunch and dinner meals. Again, intestinal gas formation was experienced, but no other adverse effects were noted (Lewis 1912).

In a test of the digestibility of the Jerusalem artichoke, performed by the Food (War) Committee of the Royal Society of England, three subjects were fed a standard diet containing one pound of potatoes for five days, followed by a standard diet containing one pound of Jerusalem artichokes for five days. No adverse side effects were discussed, although it was stated that most people could not eat much more than this amount (approximately 70 to 90 grams of inulin) without the occurrence of flatulence (Hopkins 1918, as reported in Shoemaker 1927).

Six diabetic patients received between 37 and 81 grams of baked Jerusalem artichokes (approximately 6 to 16 grams of inulin) in a bolus dose. In a subsequent experiment, three normal subjects and two diabetic patients were fed between 60 and 105 grams of inulin (1.5 g/kg body weight) in a bolus dose. No adverse effects were reported (Root and Baker 1925). Root and Baker (1925) also observed that 25 diabetic patients consumed Jerusalem artichokes in addition to their regular diets (or in substitution for five percent of their vegetables) for periods of several days to 15 months without incident. An average of 130 grams of Jerusalem artichokes (approximately 20 to 25 grams of inulin) was consumed either daily or on alternate days by these patients.

Carpenter and Root (1928) studied the effects of feeding Jerusalem artichokes to a patient with diabetes mellitus. The patient, who had consumed large amounts of Jerusalem artichokes as a regular part of his diet for years (including nearly one kilogram of freshly dried artichokes per day for several months prior to the study), was fed an average of 198 grams of dried Jerusalem artichokes per day for six of the seven days he was under observation and 500 grams of baked potato on the other day of the study. No urinary sugar was reported and blood sugar levels were reduced after consumption of the Jerusalem artichoke diet. No adverse effects were reported (Carpenter and Root 1928). [Note: Fresh Jerusalem artichoke tubers contain 16 to 20 percent wet weight of inulin and are approximately 80 percent water. The inulin content of dried Jerusalem artichokes is considerably higher. Conservatively assuming that dried Jerusalem artichokes contain 50 percent inulin, the inulin intake for the patient in this study was nearly 500 grams per day in the months preceding the study and approximately 100 grams per day during the study.

A diabetic patient was fed a daily ration of 30 grams of inulin for three days and 22 grams of inulin on the fourth experimental day. No increase in either blood sugar or urine sugar was observed, and no adverse effects were reported (Wise and Heyl 1931).

Six healthy subjects were fed diets containing 1000 grams per day of Jerusalem artichoke (containing approximately 160 to 200 grams per day of inulin) for one week. All subjects suffered from some degree of intestinal discomfort and considerable gas formation (Cremer and Lang 1950).

Beringer and Wenger (1955) performed a series of inulin experiments on both healthy subjects and diabetics. In their first experiments, healthy subjects were fed 10 grams of inulin, and little inulin was observed in their urine or feces and no increase in blood sugar was detected. In another experiment, diabetics were fed 15 grams of inulin and blood sugar levels were reduced. A subsequent experiment involved the feeding of a diabetic up to 200 grams of a mixture of inulin and soya or inulin and flour, with no excretion of inulin reported and only small increases in blood

sugars detected. No adverse effects were reported in any of these experiments (Beringer and Wenger 1955).

Gibson et al. (1995) evaluated gastrointestinal tolerance of 15 g/day of Raftilose® in 8 healthy volunteers with a mean age of 33.8. Flatulence and mild abdominal pain were the only symptoms reported with the conclusion that 15 g/day was well tolerated by the subjects.

Based on the clinical studies reviewed, no adverse effects have been associated with the repeated consumption of inulin in amounts up to approximately 70 grams per day. At consumption levels above 70 grams of inulin per day, intestinal gas formation, diarrhea, and other gastrointestinal disorders associated with high fiber intake were shown to occur. The subject of the studies by Lewis (1912) did experience intestinal gas formation at lower consumption levels (i.e., 40 and 60 grams per day), although it is probable that the single-day dosing regimen utilized by Lewis did not allow the digestive system of the subject to adjust to the bolus dose of inulin fiber.

The maximum dose of Neosugar® demonstrated not to cause diarrhea, termed the maximum tolerated dose, was demonstrated to be approximately 21 to 24 grams per day (Takahashi et al. 1986; Hata and Nakajima 1985). Lower daily doses of 15 grams of Neosugar® per day did result in flatulence and other mild gastrointestinal effects (Stone-Dorshow and Levitt 1987). However, as discussed previously, the results of the Neosugar® studies for determining human tolerance to inulin (i.e., Frutafit) is limited by the molecular weight of this fraction (DP 3 to 5).

Thus, it can be concluded that the regular consumption 40 to 70 grams of inulin per day by healthy adults appears to result in no significant adverse effects, especially when the consumption is in divided doses over the course of the day. This estimation of tolerance corresponds directly to observations in recent review articles of inulin consumption in which it was stated that

[d]igestibility studies carried out on adults have shown that amounts of up to 40 grams of inulin daily in various food preparations do not lead to any undesired side-effects (Feldheim 1993, as cited in Grühn 1994).

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Table 7. Summary of Clinical Studies Used to Derive Human Tolerance to Inulin (Raftilose® and Neosugar®)			
Study	**Subject(s)**	**Route, Dose & Duration**	**Results & Effects**
Külz (1874), as reported in Lewis (1912)	adult diabetics	50 to 120 grams of inulin (presumed bolus dose)	No inulin in feces or urine; no adverse effects reported
Persia (1905), as reported in Lewis (1912)	adult diabetics	"Large" doses of inulin over "long" time periods	No inulin in feces; no adverse effects reported
Neubauer (1905), as reported in Lewis (1912)	adult patient with levulosuria	80 grams of inulin in a bolus dose administered in a meal	No inulin in feces or urine; strong intestinal gas formation
Strauss (1911), as reported in Lewis (1912)	2 adult diabetics	40 to 100 grams of inulin per day (study period unspecified)	No inulin in urine; no adverse effects reported
Lewis (1912)	healthy adult	40 grams of inulin in a single-day dose administered with meals (cellulose-free diet)	Diarrhea experienced and attributed to cellulose-free diet; strong intestinal gas formation
	healthy adult	60 grams of inulin in a single-day dose administered with meals (normal diet)	Strong intestinal gas formation
Goudberg (1913)	adult diabetics	200 grams of dissolved inulin in a bolus dose administered via a stomach tube	Relatively serious diarrhea and strong intestinal gas formation

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Table 7—Summary of Clinical Studies Used to Derive Human Tolerance to Inulin (Raftilose® and Neosugar®)			
Study	**Subject(s)**	**Route, Dose & Duration**	**Results & Effects**
Hopkins (1918), as reported in Shoemaker (1927)	3 healthy adults	~70 to 90 grams of inulin per day for 5 days via consumption of Jerusalem artichokes	No adverse effects reported, although flatulence was predicted to occur at higher consumption levels
Root and Baker (1925)	6 diabetic adults	~6 to 16 grams of inulin in a bolus dose via consumption of Jerusalem artichokes	No adverse effects reported
	2 diabetic adults	60 to 105 grams of inulin (1.5 g/kg body weight) in a bolus dose	No adverse effects reported
	25 diabetic adults	~20 to 25 grams of inulin per day of consumption on average for several days to 15 months via everyday or alternate day consumption of Jerusalem artichokes	No adverse effects reported
Carpenter and Root (1928)	adult diabetic adapted to inulin consumption	~100 grams of inulin per day (conservative estimate) for six of seven days via the consumption of dried Jerusalem artichokes	No adverse effects reported
Wise and Heyl (1931)	diabetic adult	30 grams of inulin per day for 3 days and 22 grams of inulin on the 4th day	No increase in blood sugar or urine sugar; no adverse effects reported

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Table 7. Summary of Clinical Studies Used to Derive Human Tolerance to Inulin (Raftilose® and Neosugar®)			
Study	**Subject(s)**	**Route, Dose & Duration**	**Results & Effects**
Cremer and Lang (1950)	6 healthy adults	~160 to 200 grams of inulin per day for 1 week via the consumption of Jerusalem artichokes	All suffered from intestinal discomfort and strong intestinal gas formation
Beringer and Wenger (1955)	healthy adults	10 grams of inulin in a bolus dose	No inulin in blood or urine; no adverse effects reported
	diabetic adults	15 grams of inulin in a bolus dose	Inulin and blood sugar levels were reduced; no adverse effects reported
	1 diabetic adult	up to 200 grams of a mixture of inulin and soya or inulin and flour in a bolus doses	No inulin in blood or urine; small increases in blood sugar detected; no adverse effects reported
Yamashita et al. (1984)	18 non-insulin dependent diabetics	8 g Neosugar® daily as single dose for 14 days	No GI or other intolerance reported.
Hata and Nakajima (1985)	85 healthy volunteers (51 men, 34 women)	For men, increasing doses from 12 to 50 g Neosugar® (FOS) and for women, 10 to 41 g FOS administered as a single dose.	In men and women, Neosugar® was well tolerated at levels up to 17 and 14 g; respectively. First signs of diarrhea (9% incidence) as noted in men at 25 g Neosugar® and in women at 26 g.
Hidaka et al. (1986)	Healthy adult subjects	25 g Neosugar® as single dose	No GI or other intolerance reported in any of the studies
	23 senile patients ages 50-90 years old	8 g Neosugar® /day for 2 weeks	
	21 senile patients ages 54 to 88 years old	1,2, and 4g Neosugar/day	
	Healthy adults	8g Neosugar® /day for 2 months	

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Table 7—Summary of Clinical Studies Used to Derive Human Tolerance to Inulin (Raftilose® and Neosugar®)			
Study	**Subject(s)**	**Route, Dose & Duration**	**Results & Effects**
Takahashi et al. (1986)	9 adults with chronic renal failure	6 grams of Neosugar per day administered in diet for 1 year	No adverse effects reported
Stone-Dorshaw and Levitt (1987)	15 healthy volunteers aged 21-65 years	Day 1: all subjects given 10 g Neosugar® Days 2-13: 10 subjects given 5g Neosugar® /meal compared to 5 sucrose controls Day 14: all subjects given 10 g Neosugar®	Gaseous symptoms, such as abdominal discomfort, flatulence and bloating were more prevalent in volunteers receiving Neosugar® than in sucrose group. All symptoms were rated as absent to mild. No difference in severity for diarrhea, nausea, and headaches for treated vs. control groups.
Garleb et al. (1994)	27 male university students	Double-blind study of FOS at 5 g/liter and 10 g/liter with control group. A formula containing FOS was the sole source of nutrition for 14 days with a total consumption of 15 or 31 g/day FOS.	Tolerance was good and there was no adverse effect on serum chemistries. Incidence of complaints was low (<5% of patient days) and no complaints of severe discomfort were reported. Diarrhea was comparable among treatments but subjects consuming the high dose FOS complained slightly more about diarrhea (14% of patient days) than for control or low dose FOS (10% of patient days). Flatus was reported at a greater frequency in the high dose group with reports of adaptation after approx. 4 days.
Gibson et al. 1995	8 healthy volunteers	15 g/day Raftilose®	Well tolerated with transient complaints of flatulence and abdominal distension
Kleesen et al. 1997	10 elderly patients with constipation problems	20 g/day for 7 days followed by 40 g/day for an additional 12 days	Inulin administration increased stool frequency in 8/10 patients. Only mild-moderate flatulence was reported that did not cause discomfort. No nausea or headaches were reported.

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Table 7. Summary of Clinical Studies Used to Derive Human Tolerance to Inulin (Raftilose® and Neosugar®)			
Study	**Subject(s)**	**Route, Dose & Duration**	**Results & Effects**
Pedersen et al. 1997	64 healthy women (age 20-36 yrs)	14 g inulin/day for two 4 week periods (randomized cross-over design without a washout period)	Symptoms reported included rumbling in the stomach and gut, stomach and gut cramps bloatedness and flatulence. Mean values ranged from 0.3 to 1.2 (score of 1 was a weak effect).
Molis et al. (1996)	6 healthy volunteers (3 women; 3 men)	20g FOS per day in three doses following meals for 11 days.	Subjects reported no adverse gastrointestinal side effects.
Davidson et al. 1998	21 men and women	18 g inulin/day. Randomized, double blind crossover design with 2 6-week treatment periods and a 6 week washout period	Gastrointestinal discomfort was attributable to increased flatulence, abdominal cramping, bloating, and changes in the frequency and consistency of bowel movements

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VI. GRAS Safety Evaluation

Evaluation of the safety of Frutafit®, incorporated into foods as a bulking or bifidogenic agent, was accomplished through a review of the extensive database on the safety of inulin and related β 2-1 fructans oligofructose and FOS. This review included the production process, gastrointestinal fate, animal studies and human exposure, and a comparison of the current acceptable intake level (AIL) to the estimated daily intake (EDI) of Frutafit®. If the EDI is less than the AIL, then the use can be assumed to be safe.

The AIL is derived from clinical trials of tolerance to inulin, as well as information derived from clinical data on both oligofructose and FOS. Results of these studies indicate that ingestion of up to 40 grams inulin/day, equivalent to 0.67 grams/kg/day, based on an adult body weight of 60 kg, is safe and well tolerated. Any adverse effects that occur are expected to be gastrointestinal in nature and are not expected to endanger the health of the individual. The AIL of 40 grams for Frutafit® is a conservative estimate of Frutafit® tolerance because studies have suggested that up to 70 grams of inulin per day, consumed as a regular part of the diet, may be well tolerated.

The safety and tolerance of oligofructose ingestion by infants is documented in a Japanese nationwide survey of 20,742 infants ingesting formula containing 0.32 g FOS/100 ml (Japanese Infant Formula Survey 1993). This results in an estimated mean and 90th percentile consumption of 3.0 and 4.2 grams FOS/day. The EDI of inulin from all of the proposed uses of Frutafit® for infants below 1 yr of age were calculated by ENVIRON as 2.3 and 5.7 as the mean and 90th percentile, respectively.

In conclusion, the EDI from consumption of proposed food products is below the AIL of 40 grams/day. Safety of the use of Frutafit® in infant foods is supported by documentation of a history of safe use of FOS in Japanese infant formula at a similar level of addition.

It can thus be concluded, based on both pre-1958 use and application of scientific procedures, that the use of Frutafit® as a bulking agent for the food uses and at the levels specified herein, is GRAS.

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ATTACHMENT 1

Pesticides Included in the Residue Analyses of Fruitafit®

Aldicarb	Dieldrin	Methiocarb
Aldicarb sulfone	Diquat *	Methoxychlor
Aldrin	Endrin	Methyl parathion
AMPA-	Ethion	Mirex
(glyphosate metabolite)	Ethyl parathion	Oxamyl
Asulam *	Fluazifop-p-butyl *	Paraquat *
Atrazine	Gluphosinate-	Pirimicarb *
Bendiocarb	ammonium *	Pirimiphos-methyl
Carbaryl	Glyphosate *	Propazine
Carbetamide *	HCB	Propoxur
Carbofuran	alpha-HCH	Propyzamide *
Chlorpropham *	beta-HCH	Ronnel
Chlorpyrifos	delta-HCH	Sethoxydim *
Cyanazine	HCH (lindane)	Trithion
4,4-DDE	Heptachlor epoxide	
4,4-DDT	3-Hydroxy-carbofuran	
Diazinon	Malathion	

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*an asterisk denotes those pesticides used on chicory in The Netherlands where the crop source for manufacturing Frutafit® is grown.

ATTACHMENT 2

Proposed Uses of Frutafit® and Intended Function		
Food Category	**Maximum Use Level of Frutafit (g per 100 g food)**	**Intended Function of Frutafit**
Baby foods: all types of baby foods and beverages, including ready-to-serve and dry baby foods (excluding infant formula)	0.25g/serving [a]	Bulking agent, texture modification
Baked goods, lite cakes: fat free/reduced fat/sugar/calorie baked goods including cakes, brownies, and pastries	5	Bulking agent, moisture control, fat reduction
Baked goods, lite cookies: fat free/reduced fat/sugar/calorie cookies	8	Bulking agent, moisture control, fat reduction
Bars: all types, including breakfast bars, granola bars, energy bars, and diet/meal replacement bars	10	Bulking agent, moisture control
Beverages, fermented milks: kefir, buttermilk, yogurt drinks	2	Bulking agent, texture modification
Beverages, functional: meal replacement beverages and meal supplement beverages, including ready-to-drink beverages and dry beverage mixes[b]	5	Bulking agent, texture modification
Beverages, juices and drinks: fruit juices and drinks, including ades, cocktails, cider, nectar, and smoothies, vegetable juices, flavored waters, soy drinks, gelatin drinks, and lightly carbonated beverages, including ready-to-drink beverages and dry beverage mixes[b] (excluding citrus juices and highly carbonated beverages)	1.5	Bulking agent, texture modification
Beverages, milk-based: dairy-based beverages, including ready-to-drink beverages and dry beverage mixes[b]	1	Bulking agent, texture modification
Biscuits, reduced fat: fat free/reduced fat biscuits	6	Bulking agent, fat reduction, moisture control
Breads, conventional: conventional yeast breads, rolls, and buns	0.5	Bulking agent/humectant
Breads, specialty: specialty types such as breads reduced in calories or fat and/or containing added fiber or added calcium	6	Bulking agent/humectant
Candy, hard dietetic	15	Bulking agent
Candy, soft dietetic	5	Bulking agent
Condiments: catsup and mustard	5	Bulking agent, moisture control, texture control
Cream cheese, reduced fat: fat free/reduced fat cream cheese	5	Bulking agent, binder, fat replacer
French fry coatings: coatings on French fries	1.7 [c]	Bulking agent, moisture control, fat reduction
Frozen dairy desserts, lite: fat free/reduced fat/sugar/calorie ice creams and dairy-based frozen desserts, including novelties and frozen yogurt	8	Fat replacer, bulking agent

Proposed Uses of Frutafit® and Intended Function		
Food Category	**Maximum Use Level of Frutafit (g per 100 g food)**	**Intended Function of Frutafit**
Icings/glazes, lite: fat free/reduced fat/sugar icings and glazes	5	Bulking agent
Jams and jellies, lite: reduced sugar/calorie jams and jellies	2	Bulking agent
Meat products: processed meats, including frankfurters, sausages, bratwurst, beef patties, chicken patties, loaves, pates, and deli meats	4	Binder, bulking agent
Mousse, reduced fat[d]	3	Bulking agent, fat reduction
Pancake syrup, lite	2	
Pasta fillings: fillings used in pasta, such as tortellini, ravioli and manicotti fillings	5	Binder, fat replacer, texture modification
Pasta, fresh(e): fresh pasta, such as spaghetti, fettuccini, linguini, tortellini, ravioli, or lasagna (excluding noodles)	4	Bulking agent, texture modification, moisture control
Pasta, precooked macaroni[e]	4	Bulking agent, moisture control
Pizza crust	5	Bulking agent, moisture control
Potatoes, mashed: prepared or in frozen meals (excluding dry mix types)	3	Bulking agent, texture modification, moisture control
Pretzels, soft	5	Bulking agent, humectant
Processed cheese, reduced fat: fat free/reduced fat processed cheese and cheese products	5	Fat replacer, binder
Pudding mix: regular and reduced sugar/calorie pudding mix	7	Bulking agent, texture modification
RTE breakfast cereals: all types of ready-to-eat (RTE) breakfast cereals	5 g/serving [a]	Bulking agent, moisture control
Salad dressings, lite: fat free/reduced fat/calorie dressings, including mayonnaise, salad dressings and mayonnaise-type dressings	5	Bulking agent, flavor enhancement, binder, texture modification, fat reduction
Sauces and gravies: entrée, dipping and condiment sauces such as Alfred, BBQ, cheese, clam, Hollandaise, pasta, pizza, soy, sweet & sour and white sauces, salsa, and gravies, including prepared sauces and dry sauce mixes[a] (excluding tomato sauce and paste)	2	Bulking agent, moisture control, texture modification
Snack chips, reduced fat: fat free/reduced fat snacks, including chips and extruded snacks	3	
Snack crackers: savory snack, sandwich, and whole grain crackers (excluding plain crackers such as saltines, matzo crackers or oyster crackers)	4	Bulking agent, moisture control
Soups, dry	3	Bulking agent, energy reduction, texture modification
Spreads, reduced fat: fat free/reduced fat margarines and margarine-like spreads	10	Bulking agent, fat reduction
Surimi: surimi, imitation crab, and reconstructed seafood	3	Bulking agent, binder

Proposed Uses of Frutafit® and Intended Function		
Food Category	**Maximum Use Level of Frutafit (g per 100 g food)**	**Intended Function of Frutafit**
Toppings, dessert: toppings used on desserts (excluding whipped toppings)	2	Bulking agent
Tortillas, reduced fat[d]	3	Bulking agent, moisture control
Vegetarian patties/crumbles	2	Bulking agent, binder
Whipped toppings, lite: fat free/reduced fat/sugar non-dairy whipped cream toppings	6	Bulking agent, fat reduction
Yogurt, reduced fat: fat free/reduced fat refrigerator-type yogurts	3	Bulking agent, fat replacer

a) Serving sizes correspond to Reference Amounts Customarily Consumed per Eating Occasion; 21 CFR 101.12

(b) Maximum use levels correspond to g Frutafit per 100 g prepared beverage or sauce.

(c) Maximum use level per 100 g coated French fry (as consumed)

(d) No food codes for reduced fat forms of this food category are in the 1994-96, 98 CSFII; estimates are based on consumption of full fat versions

(e) No food codes for fresh pasta or precooked macaroni are in the 1994-96, 98 CSFII; estimates are based on consumption of all pasta and macaroni

Note: Unless indicated otherwise, all food categories include both regular and lite versions of all food products.

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End Submission