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Evaluation of Safety of Inulin and Oligofructose as Dietary Fiber

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In the United States, most individuals consume far less dietary fiber than the daily value (DV) set at 25 g. The average daily consumption for inulin and oligofructose is estimated to be between 1 and 4 g in this country, with a higher intake of 3 to 11 g seen in Europe. Inulin and oligofructose are soluble, fermentable dietary fibers, of low net caloric value having many of the possible health benefits attributed to fiber. Such fiber consists of poly- and oligomers of fructose joined by $\beta(2 \rightarrow 1)$ fructosyl–fructose bonds. This class of fiber has been studied in a series of standard toxicological test systems. The studies have demonstrated that inulin-type fructans, when administered in the diet at high levels, do not result in mortality, morbidity, target organ toxicity, reproductive or developmental toxicity, or carcinogenicity. Several *in vitro* studies have also shown the absence of mutagenic or genotoxic potential. The only basis for limiting use of such fiber in the human diet relates to gastrointestinal tolerance. A series of clinical studies has been reported which shows that up to 20 g/day of inulin and/or oligofructose is well tolerated. As foods marketed in the United States bear labels stating both the quantity per serving size and the corresponding percentage of the daily value (% DV) of fiber, consumers can make appropriate choices and decisions about daily consumption without exceeding individual tolerance.

Key Words: **inulin; oligofructose; dietary fiber; tolerance; safety; labeling.**

INTRODUCTION

The Nutrition Labeling and Education Act of 1990 declared dietary fiber a nutrient, for nutrition labeling purposes. This general term covers a wide variety of substances with different physical properties and diversified physiological effects. Dietary fiber has been defined as "a mixture of plant polysaccharides and lignin that cannot be digested by endogenous secretions or by brush-border hydrolytic enzymes of the human digestive tract. It is an integral part of the daily diet, and it exerts direct and indirect physiologic effects throughout the gastrointestinal tract. There are claims that a relative lack of fiber in diet may play a role in a wide range of human disorders such as colonic cancer or hypercholesterolemia and atherosclerosis" (Sobotka *et al.*, 1997).

Dietary fiber belongs to the broad category of carbohydrates. They can be classified into soluble (e.g., gums, pectins), insoluble (e.g., cellulose), or mixed (e.g., bran), fermentable and nonfermentable. Insoluble, nonfermentable fibers are known for their bulking effect that decreases transit time and increases fecal mass. The extent of fermentation of soluble fibers depends on their physical and chemical structure. Fermentation decreases intraluminal pH and stimulates proliferation of colonic epithelial cells. Dietary fiber is fermented by colonic anaerobic bacteria leading to the production of lactic acid, short-chain fatty acids (SCFA—acetate, propionate, and butyrate) and gases (H_2, CO_2, and CH_4) (Roberfroid, 1993). Modulating effects of fiber considered beneficial for overall health are: (a) increased water-holding capacity of the stool, (b) increase in stool volume, (c) impact on transit time, (d) increase in the colonic bifidobacteria and lactobacilli counts, with decrease in the pathogenic types of bacteria, (e) fermentation giving rise to absorption of certain organic compounds, and (f) histological and functional changes in the intestinal epithelium (Gibson *et al.*, 1995; Roberfroid, 1993).

Naturally occurring inulin is found in a variety of edible fruits and vegetables, such as wheat, onions, leeks, garlic, asparagus, artichokes, and bananas and therefore has been part of human sustenance for centuries. An official method (997.08) that quantitates inulin-type fructans in plants and food products has been accepted by the Association of Official Analytical Chemists (AOAC, 1998). Inulin and oligofructose, generally referred to as fructans, are soluble and fermentable dietary fibers. They are not digested in the upper part of the gastrointestinal (GI) tract by α-amylase and hydrolytic enzymes such as sucrase, maltase, and isomaltase (Oku *et al.*, 1984). The average daily consumption for inulin and oligofructose is estimated to be between 1 and 4 g in this country, with a higher intake of 3 to 11 g seen in Europe (Van Loo *et al.*, 1995).

Fructans are believed to be nutritionally important due to their physiologic effects stemming from lower GI tract fermentation. This process is typically complete

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as demonstrated by their lack of detection in the feces (Wang and Gibson, 1993). Their bland to slightly sweet flavor and "fat-like" mouth feel provide new opportunities for achieving increased intake of dietary fiber. Currently, inulin and oligofructose obtained by hydrolysis from inulin are accepted and classified as safe food ingredients by the regulatory authorities in almost all developed countries worldwide.

To determine whether inulin/oligofructose are safe at increased levels of intake, critical review and evaluation of the animal toxicological data are undertaken in this paper, along with an extensive evaluation of clinical studies. The clinical studies reviewed were conducted for various reasons; not all of them had assessing tolerance as their principal objective. We have reviewed and evaluated them with attention both to the original objectives of the studies and to their value in establishing human tolerance. The extent to which the studies are useful in drawing conclusions concerning levels of inulin/oligofructose in the human diet is also discussed.

CHEMICAL ASPECTS

Fructan is a general term used for naturally occurring plant oligo- and polysaccharides and refers to any carbohydrate compound in which one or more fructosyl–fructose links comprise the majority of glycosidic bonds. Fructans are linear or branched fructose polymers which are joined by two types of linkages: $\beta(2 \rightarrow 1)$ seen in inulin or $\beta(2 \rightarrow 6)$ seen with levans-type fructans. Within the inulin-type fructans are two general groups of materials, inulin and its subsets, oligofructose and fructooligosaccharides (FOS). The nomenclature for each of these materials is given below.

Inulin is a polydisperse carbohydrate consisting mainly of $\beta(2 \rightarrow 1)$ fructosyl–fructose links. Inulin contains both GF_n and F_m compounds, where n or m represents the number of fructose units (F) linked to each other, with one terminal glucose (G), that can vary from 2 to 70. The presence of F_m in inulin extracts has been demonstrated. Total hydrolysis of inulin yields fructose and glucose. Since all commercial inulin products are in fact mixtures of molecules with differing chain lengths, it is possible to describe these products in terms of an average degree of polymerization (DP). Commercial inulin products, obtained by hot water extraction of the chicory root, contain 92% inulin with an average degree of polymerization of about 10. A physical separation process can remove the oligosaccharides, in which case the average DP can increase to about 25.

Oligofructose and FOS are synonymous terms used to refer to inulin-type fructans with a maximum DP of less than 10. Their names derive from oligosaccharides (carbohydrates consisting of less than 10 monosaccharide units) that are predominantly composed of fructose. Oligofructose is most commonly used in the literature to describe short-chain inulins obtained by partial enzymatic hydrolysis of chicory inulin. These products contain GF_n- and F_m-type oligomers, with an average DP of about 4. FOS tends to describe mixtures of short-chain inulin-type fructans synthesized from sucrose. This type of FOS consists of sucrose molecules to which one, two, or three additional fructose units have been enzymatically added by $\beta(2 \rightarrow 1)$ linkage to the fructose unit of sucrose. These synthesized materials are of the GF_n type and have an average DP of 3.5.

For the purpose of this review, any inulin-type fructan which contains molecules with an average DP 10 and above will be referred to as inulin. Any inulin-type fructan containing only molecules with a DP less than 10 and derived from chicory will be referred to as oligofructose. Those materials synthesized from sucrose will be referred to as FOS. Whenever available, the average DP of the material used in a study will also be cited.

Inulin, oligofructose, and FOS are chemically similar entities demonstrating like nutritional properties. Their chemical and nutritional similarities are due to the basic structure they share, $\beta(2 \rightarrow 1)$ linkage of fructosyl units sometimes ending with a glucosyl unit, and to their common metabolic pathway (fermentation by the microflora of the colon).

The only difference between inulin/oligofructose and synthetic FOS is in the degree of polymerization (the number of individual monosaccharide units which make up the molecule). The average DP and DP range for major commercial fructans are listed below:

Inulin (RAFTILINE), average DP 10 or higher; range 2–60;

Oligofructose (RAFTILOSE), average DP 4–5; range 2–8; and

FOS (Neosugar, Nutraflora), average DP 3.5; range 2–4.

Sources and Production

After starch, fructans are the most abundant nonstructural polysaccharides found in nature. They are present in a wide variety of plants and in some bacteria and fungi. The food industry is able to utilize three plant species for large-scale production of inulin: agave (*Agave azul tequilana*), Jerusalem artichoke (*Helianthus tuberosus*), and chicory (*Cichorium intybus*).

Chicory is a biennial plant. The inulin content in the plant is high and fairly constant from year to year for a given region. The chicory used for inulin production is the same variety as the one used for the production of the coffee substitute. Chicory inulin is generally extracted from fresh chicory roots, paying specific attention to inhibiting the plant's own inulase activity and acid hydrolysis (Roberfroid and Delzenne, 1998). Through two different methods, the food industry produces two types of products from chicory. Inulin, containing a mixture of short- and long-chain fructans, is

produced by hot water extraction from chicory plant (De Leenheer, 1996). Chicory roots are washed, diced, and extracted. The semi-refined syrup is then purified using physical separation techniques to yield a product of over 99% purity. Oligofructose, containing only molecules with DP less than 10, is produced by a similar method with the addition of partial enzymatic hydrolysis of the inulin processing stream after the initial extraction.

USES

Dietary Fiber

During the past decade, Americans have changed their outlook on health. Their quest for improved quality of life and increased longevity shifted from a focus on supplementation to meet daily requirements to one of disease prevention and enhanced performance. However, the reality is that most of the U.S. population already has risk factors for various illnesses. As estimated by the Census Bureau, the number of people ages 45 and older will grow from 91.5 million (34% of the present U.S. population) to 144.6 million (42%) by the year 2030. This change will also reflect an increase in the morbidity seen in the geriatric age group. Chronic diseases or their risk factors already afflict many in the younger generations. This is demonstrated by the 10 million clinically obese children, 2.2 million with hypertension, and 27.4 million under the age 19 with high cholesterol (AHA, 1997). Furthermore, approximately 17% of the American households have a member requiring a modified diet to manage a health condition.

Even with the current changing nutritional trend, most individuals consume significantly less dietary fiber than the recommended daily value (RDV) in the United States of 25 g. Inulin and oligofructose, while calorically low, have the added advantage of being as much as 0.3 times as sweet as sugar, showing synergistic sweetening with intense sweeteners (Wiedmann and Jager, 1997). Therefore, they are attractive ingredients for use as part of nonnutritive sweetener systems not only for the average consumer, but also for weight-conscious individuals and diabetics (Stone-Dorsow and Levitt, 1987).

Caloric Value

There are four studies available which determined the caloric value of inulin and FOS in human subjects using different methodologies. The study by Molis *et al.* (1996) uses the factorial method to determine a caloric value of 2.3 kcal/g. In an earlier study, Hosoya *et al.* (1988) uses a radiorespirometric method to determine a caloric value of 1.5 kcal/g. In addition to these two studies, Castiglia and Vermorel (1993) and Castiglia-Delavaud *et al.* (1998) conducted energy balance experiments using whole-body indirect calorimetry methods in which these authors reported caloric values for inulin ranging from 2.1 to 2.8 kcal/g. However, most of these human studies have overestimated the caloric value of FOS due to constraints in experimental methodology and, in cases where the factorial equation was used, limitations in theoretical estimates. Although the report by Molis *et al.* (1996) is the best human study available for determining a caloric value for FOS, its reported caloric value of 2.3 kcal/g has some limitations. First, the methodology used to collect ileal samples (i.e., intubation) tends to overestimate absorption of FOS in the small intestine. Second, Molis *et al.* (1996) used a general factor of 0.5 to determine the proportion of carbohydrate energy from fermentation. Roberfroid *et al.* (1993) have demonstrated that the caloric value of FOS can be evaluated by using more specific values to determine the proportion of energy from carbohydrate fermentation available to the host. When these factors are applied to the factorial analysis and results of Molis *et al.* (1996), they provide a more definitive caloric value for FOS, i.e., 1.5 kcal/g.

In summary, the determination of Molis *et al.* (1996) using the factorial method, combined with the specific factors discussed by Roberfroid *et al.* (1993) and the corrected small intestinal absorption factor, leads to a caloric value of 1.5 kcal/g for FOS. The same value is reported in the study conducted by Hosoya *et al.* (1988), using the radiorespirometric method to determine the caloric value of FOS in human subjects. Since inulin is composed of FOS, this caloric value is applicable to both inulin and FOS (i.e., oligofructose). Finally, as described above, higher values by the calorimetry method are variable and less reliable.

Therefore, overall, studies in humans and review of the literature support the conclusion that the caloric value of inulin and oligofructose is 1.5 kcal/g.

TOXICOLOGICAL STUDIES

Given the chemical and physiological similarities of inulin, oligofructose, and FOS, the toxicological studies performed with FOS and their results are predictive of the effects of inulin and oligofructose in demonstrating safety. Takeda and Niizato (1982) conducted the following toxicological studies, with the results being presented at the Proceedings of the First Neosugar Research Conference, in 1982.

Acute Toxicity

The objective of this study was to evaluate any possible acute toxicity in mice and rats exposed to dietary FOS (average DP 3.5). Four-week-old male and female JcL-IcR mice (SPF) and 6-week-old male and 10-week-old female Sprague–Dawley rats were used in four groups of 6 for both mice and rats (a total of 24 M + 24 F mice and a total of 24 M + 24 F rats). The groups were administered 0, 3, 6, and 9 g/kg FOS by gavage.

The concentrations were adjusted so that the total volume of solution given per animal was 0.5 ml for mice and 2 ml for rats. Following a single oral dose the animals were observed for any signs of toxicity until the seventh day. No abnormalities were seen in the general state of health of male and female mice and rats. There were no deaths and increase in body weight after 7 days was the same as that for controls. It was concluded that FOS did not affect mortality or the general state of health or gain in body weight of mice and rats when administered as single doses of up to 9 g/kg. The LD_{50} for FOS was determined to be greater than 9 g/kg.

Subacute Toxicity (6-Week Gavage Study)

Male Wistar rats (SPF), 6 to 7 weeks old, were used in groups of 18. FOS (average DP 3.5) were used as test substances, while sucrose and glucose were the control substances. Test and control substances were given daily by gavage at doses of 1.5, 3, and 4.5 g/kg for 6 weeks. On the second, fourth, and sixth weeks, blood samples were obtained from 6 animals in each group. At sacrifice, the liver, pancreas, adrenal glands, kidneys, brain, cerebellum, heart, lungs, spleen, pituitary gland, and testes were removed and fixed in formalin and H&E-stained slides were prepared. Results revealed that there were no abnormalities or deaths during the study. A slight increase in body weight was observed in the 3 and 4.5 g/kg groups compared to controls. The other group showed the same trend as the controls.

No consistent, treatment-related changes in serum chemistries were seen across groups, although significant fluctuations were observed occasionally. These did not correlate with either dose or time of administration and are, therefore, considered chance occurrence. On dissection, swelling of the appendix was noted in the rats receiving FOS, while this abnormality was not seen in the other groups.

It was concluded that there is no treatment-related toxicity in any of the FOS-treated groups up to a dose of 4.5 g/kg administered orally for 6 weeks.

Subacute Toxicity (6-Week Feeding Study)

Male Wistar rats (SDP), 6 to 7 weeks old, were used in groups of 18. FOS (average DP 3.5) were used as samples, while sucrose, glucose, and sorbitol served as controls. A dietary admixture was prepared with Refined Feed of Oriental Kobo Kogyo, by leaving out the 5% sucrose and the 5% starch and adding 5 or 10% of the test compounds. The feed was provided *ad libitum* for 6 weeks. On the second, fourth, and sixth weeks, blood samples were obtained from 6 of the subjects from each group. At the same time, at necropsy, the liver, pancreas, and adrenal glands were removed for further histopathologic examination. On the sixth week, kidneys, brain, cerebellum, heart, lungs, spleen, pituitary gland, and testes were removed at necropsy. The specimens were fixed in formalin and H&E slides were prepared.

The results of the study revealed no treatment-related abnormalities or deaths. Diarrhea was observed on the 3rd day for the sorbitol group and on the 10th day for FOS. Sorbitol and FOS groups showed a lower body weight in the 1st to 5th week, but the growth trends near completion of the study were the same as those of the control groups. The only blood chemistry finding that appeared to be treatment-related was the reduction in cholesterol for the FOS groups. On the 2nd- and 6th-week necropsies, swollen appendices were identified. A few hepatic specimens, from all groups including controls exhibited slight necrosis and infiltration of round cells. Renal changes and cases of degeneration of the proximal tubular epithelial cells were seen in all sucrose-, glucose-, sorbitol-, and FOS-treated groups. The changes were greatest with sorbitol and sucrose. Cases in the FOS and glucose groups also demonstrated dilatation of the proximal renal tubules. Calcium deposits were found in some cases in the FOS, sorbitol, and control groups.

Feeding diets with added FOS caused a decrease in body weight, a reduction in cholesterol, and swelling of the appendix while, in few cases, pathological changes of the kidneys and liver were observed. These changes were similar to those noted in the control groups. The changes seen in the proximal renal tubules were less severe in the FOS group than in the sucrose group, while the calcium deposits observed in the cortex were also identified in the control groups. It was concluded that FOS showed no toxicity compared with existing sugars commonly used in the food supply. The study also demonstrated that the blood glucose is not raised significantly by a single oral dose administration of FOS. Therefore, it is believed that the reduction in body weight is due to the low caloric content of FOS.

Chronic and Carcinogenicity Studies

A comprehensive long-term/carcinogenicity study was performed with 50 male and 50 female Fischer 344 rats for 104 weeks (Clevenger *et al.*, 1988). After a week of acclimatization, the test animals began receiving FOS (average DP 3.5) with their diet at concentrations of 0, 8000, 20,000, and 50,000 ppm. All animals were observed twice daily and body weights were determined weekly in the first 26 weeks and biweekly thereafter. Food consumption was determined for all animals weekly. Food efficiency and FOS intake were determined from body weights and food consumption data. At the conclusion of the study, blood samples were obtained from all rats prior to sacrifice. Brain, adrenal gland, heart, lung, spleen, liver, kidney, testis, and ovary weights were recorded. Tissues were fixed in formalin, sectioned, and stained with H&E in a standard fashion.

Results indicated that survival of both sexes, in all test groups, was unrelated to treatment. Although a decreased rate of survival in the male 20,000-ppm dose group was observed, this was not considered treatment-related since it was an isolated occurrence and no dose–response relationship was evident. Body weight gain, food intake, and organ weights for both sexes were unaffected by FOS supplementation. Overall, food efficiencies by FOS-treated male and female groups were comparable to their control groups. FOS intake by the 8000-, 20,000-, and 50,000-ppm groups was equivalent to 341, 854, and 2170 mg/kg/day, respectively, for male rats and 419, 1045, and 2664 mg/kg/day, respectively, for female rats.

Hematology parameters were not influenced by FOS supplementation. Blood chemistry results demonstrated a slight, but significant elevation of Na and Cl in male rats. Male rats in the 20,000-ppm FOS group had slightly elevated levels of blood glucose and creatinine. The males in the 50,000-ppm group, in turn, showed a decrease in creatinine levels. All other parameters for the male treated groups were similar to controls. In females, all blood chemistry parameters were similar to those of controls except for slight elevation of uric acid in the 8000- and 20,000-ppm groups.

No treatment-related macro- or microscopic changes were found in either males or females. The only nonneoplastic lesions observed were common to aging rats of this strain as demonstrated by their historical control incidence.

It is well known that a variety of neoplastic lesions tend to occur spontaneously in F-344 rats with relatively high incidence (e.g., pheochromocytomas, thyroid C-cell adenomas, leukemias, and pituitary adenomas) (Haseman *et al.*, 1990). In the FOS-treated animals the incidence of rare tumors (defined as those tumors with incidences below 1%) in male or female rats was not increased. The incidence of spontaneous tumors in the FOS-treated animals was comparable to that of controls, with the exception of pituitary adenomas. In the male rats, the incidence of pituitary adenomas for the 0-, 8000-, 20,000-, and 50,000-ppm dose groups was 20, 26, 38, and 44%, respectively. The historic incidence of pituitary adenomas in the control F-344 male rats from the test laboratory ranges from 1 to 49% (Haseman *et al.*, 1990). While the incidence of this tumor in the present study was well within historical range for all male rats, the incidence in the two highest dose groups (20,000 and 50,000 ppm) was significantly greater than the incidence in controls. Further statistical analysis was carried out employing two generally accepted trend tests. The Cochran–Armitage chi-square indicated a dose–response trend ($P = 0.007$), whereas the logistic regression analysis showed no such trend ($P = 0.51$), giving an overall equivocal result. In the female rats, a negative trend in the incidence of pituitary adenomas was recorded. Based on this analysis it is concluded that higher incidence of pituitary adenomas in males is not treatment-related.

Furthermore the incidence of neoplasms is not affected by FOS treatment and that FOS lacks carcinogenic potential.

Developmental and Reproduction Toxicity

Henquin (1988) demonstrated the lack of developmental toxicity of FOS fed to rats at dietary levels of 20%. Twelve female Wistar rats with a copulation plug were fed a diet containing 20% FOS from day 1 to 21 of gestation. A separate group of 17 female Wistar rats with a copulation plug were fed a control diet for the same period of time. In the first 6 h after birth, the litters were numbered, sexed, and weighed. Thirty-six hours after delivery, the newborns were equally distributed (9/mother) between the lactating mothers, which were continued on their gestational diet. No effect on the number of pregnancies was seen in the FOS groups; however, a reduction in body weight gain of the pregnant rats was identified. This could be due to a lower caloric value for FOS, decreased intake of food for this group, and/or diarrhea observed in the first week and softer stools in the second and third weeks. Despite the reduction in body weight gain of the pregnant rats, the fetuses and newborn weights were not affected. However, during the nursing period, a growth delay was observed for the pups (specifically males) in the test group. This may be indicative of the restricted nutritional status of the lactating mothers. The study concluded that a diet containing 20% FOS has no significant effects on the course of pregnancy in rats and on the development of their fetuses and newborns.

In a separate study, Sleet and Brightwell (1990) also evaluated possible maternal and developmental toxicity in the rat (strain Crl CD (SD) BR) following administration of FOS during gestation. Four groups of 24 to 27 pregnant females were pretreated with FOS at a dietary level of 4.75%, from day 0 to 6 postcoitum, in an attempt to avoid diarrhea observed with earlier studies. A fifth group received a FOS-free diet throughout the entire study. On postcoital day 6, the FOS pretreatment diet was replaced, with each group receiving the following diets: FOS-free diet, 5, 10, and 20% FOS. This regimen was continued until day 15 when all pregnant females were placed on a FOS-free diet.

On day 20, the rats were sacrificed and litters examined. Approximately half of the fetuses were dissected and examined fresh prior to skeletal staining, while the rest were fixed in Bouin's solution. Results of the experiment revealed no treatment-related effects during pretreatment (days 0 to 6 postcoitum) and treatment (days 6 to 15 postcoitum) with FOS at dietary levels up to 20%. No diarrhea was observed in any of the test animals and no deaths were recorded. FOS administered during the pretreatment period did not affect body weight and body weight change in any of

the groups. However, 2 days after the start of the treatment (postcoital day 8), body weight was reduced in all FOS groups relative to controls. Additionally, body weight change in the treatment groups decreased in a dose-related manner. The 5% group exhibited a lower weight gain relative to that of controls, whereas the 10 and 20% groups lost weight. From day 11 to the end of the study, the body weight and body weight change were similar among groups, with the exception of the 20% FOS group which remained below controls.

At necropsy, the findings in dams were unremarkable. The number of pups/litter, the sex ratio, and viability of both the embryo and the fetus were not affected by the dietary supplementation with FOS. Litter and fetal weights were not reduced, while the fetal weight of the 20% group was statistically greater than that of the controls. Structural development of the fetuses was unremarkable.

It was concluded from the study that dietary supplementation with FOS, at concentrations up to 20%, did not cause adverse effects (e.g., diarrhea) or negatively affect the pregnancy outcome or *in utero* development of the rat. The only treatment-related effect was the alteration in the body weight of the dams, with a moderate reduction seen in the 20% FOS group.

Genotoxicity

FOS (average DP 3.5) was tested for genotoxicity in three assays: (1) microbial reverse mutation assays in *Salmonella typhimurium* (Ames assay) strains TA1535, TA1537, TA1538, TA98, and TA100 and *Escherichia coli* WP2 *uvr* A, (2) the L5178Y mouse lymphoma $TK^{\pm}$ mammalian cell mutation assay, and (3) an assay for the induction of unscheduled DNA synthesis in human epithelioid cells (HeLa S3). A wide range of test doses was used for each assay, with and without metabolic activation. The results indicated no genotoxic potential from the use of FOS (Clevenger *et al.,* 1988).

In conclusion, the nonclinical toxicological data presented revealed no evidence of increased morbidity, mortality, or target organ toxicity. There was no change in food consumption, physical appearance, or behavior of test animals. There were no treatment-related changes in reproduction or developmental parameters, no evidence of mutagenicity or genotoxicity, and no treatment-related increases in proliferative lesions in the treated animals.

Diarrhea Tests

Due to the nature of the test compound, studies to assess possible laxation with use of FOS (average DP 3.5) were carried out. Test animals were 7-week-old male Sprague–Dawley rats, divided into groups of six. Positive controls were sorbitol and maltitol, while glucose acted as negative control. The test substance was dissolved in distilled water and the concentration was adjusted so that each animal received 2 ml. A single oral dose of 3 or 6 g/kg was given. Each rat was then placed in its own cage and the number and consistency of the stools produced were monitored at the 6th and 24th hour following administration. Food and water deprivation was implemented until the end of the experiment.

The following results were reported. From the 0 to the 6th hour no diarrheal stools were seen with any groups. From the 6th to the 24th hour, half of the animals had diarrhea in the 3 and 6 g/kg FOS groups. One to 2 diarrheal stools were observed in the 3 g/kg group, while 2 to 4 such stools were recorded in the 6 g/kg groups. With glucose, 1 stool was seen in a single animal in the 3 g/kg group and none in the 6 g/kg group. With sorbitol, 1 to 5 stools were noted in four animals in the 3 g/kg group, while all animals had diarrhea stools in the 6 g/kg group. A watery consistency of the stools was identified. With maltitol, four animals had 1 to 5 diarrheal stools in the 3 g/kg group and 1 to 14 diarrheal stools were seen in five animals in the 6 g/kg group.

Based on the study, it was concluded that the induction of diarrhea was as follows: sorbitol (watery) $\gg$ maltitol $>$ FOS $\gg$ glucose $= 0$. As FOS is expected to have a weaker osmotic effect than sugar alcohols, it is not surprising that the diarrheal effect of FOS is less than that of the tested sugar alcohols.

TOLERANCE

Tolerance by Intravenous Infusion

Inulin, administered intravenously as a sterile solution, has been used for many years as a diagnostic agent to measure glomerular filtration rate (GFR). Due to its small molecular weight, similar to plasma and crystalloids, it passes freely through the glomerular membrane. The concentration of inulin is the same for plasma and glomerular filtrate in the healthy individual (Guyton, 1981). Inulin represents an ideal substance for renal clearance testing since it is filtered by the glomerulus; it is not absorbed or excreted by the renal tubules and is not metabolized. As described in the medical literature, inulin, even when given intravenously at priming doses as high as 50 mg/kg, causes no known adverse reactions or toxic effects (PDR, 53rd ed., 1999). Applying these clinical findings further, an investigation to demonstrate possible placental transfer of inulin from mother to the first-trimester fetus was undertaken (Jauniaux *et al.,* 1997). Inulin is thought to cross the placental barrier by an extracellular porous route dependent on the molecular size of the substance. A study done with healthy pregnant females who received an IV bolus of inulin demonstrated that inulin crosses the human placenta in quantities that yield measurable concentrations in both the coelomic and the amniotic fluids.

The long-standing intravenous/systemic use of inulin in routine medical testing demonstrates its safety and nontoxic effects in humans, even under the most precarious medical conditions (e.g., renal failure).

Tolerance by Ingestion

While alike in structure, inulin, oligofructose, and FOS have somewhat different effects on tolerance due to their dissimilar chain lengths. These effects generally present as GI symptoms. Flatulence, bloating, abdominal distention, and rumbling are known and accepted occurrences with dietary intake of fruits and vegetables and have also been observed with ingestion of inulin and oligofructose. Nonetheless, these common but otherwise subjective complaints are difficult to measure and quantitate. The various effects of fructans on the GI tract can be explained by the following principles:

smaller molecules have a higher osmotic colonic pressure; and
slower fermenting compounds are more easily tolerated than faster ones.

For instance, inulin (average DP 10) has better GI tolerance and less potential for osmotic diarrhea than do FOS (average DP 3). Furthermore, through animal studies it has been documented that FOS induce less diarrhea than maltitol and significantly less than sorbitol (sorbitol $\gg$ maltitol $\geq$ FOS $\gg$ glucose = 0) (Takeda and Niizato, 1982). Inulin, as a slower fermenting compound, has better GI tolerance than oligofructose, and all three carbohydrates present with fewer signs of intolerance if given in divided doses (Alles *et al.*, 1996; Garleb *et al.*, 1996; Kleessen *et al.*, 1997; Luo *et al.*, 1996; Stone-Dorshow and Levitt, 1987).

Physiologically, the malabsorption of carbohydrates in the upper GI tract may lead to some forms of intolerance (e.g., lactose intolerance). The colonic flora ferment undigested carbohydrates resulting in the production of gas and, therefore, flatulence. When the capacity of the colonic bacteria to ferment carbohydrates is exceeded, diarrhea may develop. Hence, flatulance and diarrhea are two potential problems that can develop when using fructans in the diet and the amount ingested is critical in avoiding any problems with intolerance (Stone-Dorshow and Levitt, 1987).

A recent clinical study is probably the first to compare the physiologic responses after ingestion of two different chain lengths fructans (Rumessen and Gudmand-Høyer, 1998). The responses were further compared to those after ingestion of lactulose, fructose, and sorbitol. In a single blind randomized study with 10 healthy adults, intestinal absorption, transit time, and fermentation after ingestion of 10- to 30-g inulin-type fructans (short- and long-chain) were studied. The short-chain fructans (FASC; oligofructose) used had an average DP $<$ 10 (median DP = 3), while the long-chain fructans (FALC; inulin) consisted of fructose chains of different lengths (51% with an average DP $>$ 12 and 42% with an average DP $>$ 21). GI symptoms were recorded and scored. The symptoms included flatulence, distension, borborygmi, abdominal pain, nausea, and diarrhea and were rated in severity from 0 (none) to 3 (severe). They were recorded at fixed (0.5 h) intervals for 7 h after ingestion. A total symptom score for each test was then calculated. The results showed that the highest cumulative and mean maximum symptom scores for all participants were seen after 30 g FASC (oligofructose) and 20 g lactulose, while the lowest were after 20 g fructose. Seventy-five percent of the scores were due to mild gas complaints (distension, borborygmi, and flatulence). Diarrhea was not reported after 10 or 20 g FASC (oligofructose) or after 20 g fructose. Abdominal pain was seen in 15% of symptom scores. For the 10–30 g FASC (oligofructose) alone, 77% of scores were for gas problems and 17% were for abdominal pain. The transit times of long-chain fructans were longer than those of short-chain fructans. The authors concluded that with the ingestion of fructans, abdominal symptoms increased with increasing dose and decreasing chain length.

It has been demonstrated, both *in vitro* and *in vivo*, that inulin-type fructans, due to their β-configuration, are nondigestible in the upper GI tract. Clinical studies support the *in vitro* findings that these fructans withstand hydrolysis by human digestive enzymes (α-glucosidase, maltase–isomaltase, and sucrase) that are specific for α-glycosidic linkages. In addition, they are also resistant to the action of salivary glands, pancreatic enzymes, and gastric acids, reaching the large bowel intact, where extensive fermentation and breakdown occurs. By resisting digestion in the upper part of the GI tract, inulin-type fructans are not absorbed to any significant extent (Cummings *et al.*, 1997; Ellegärd *et al.*, 1997; Molis *et al.*, 1996). Absorption of inulin or a smaller DP of inulin does not raise a safety issue given the experience with its use systemically for measuring glomerular filtration (see Tolerance by Intravenous Infusion). The extent of fermentation of soluble fibers depends on their chemical structure, further affecting intraluminal pH and colonic proliferation of epithelial cells. Dietary fiber is fermented by colonic anaerobic bacteria leading to the production of lactic acid, short-chain fatty acids (acetate, propionate, and butyrate), and gases (H_2, CO_2, and CH_4) (Roberfroid, 1993). The gases may be expelled in the breath and/or flatus, while the SCFAs are partially absorbed by the intestinal epithelium and further metabolized by the body. Acetate provides energy for the tissues. The exact role of propionate in humans is not fully elucidated. Butyrate is metabolized by colonocytes and has been demonstrated to regulate cell growth, induce differentiation, and affect apoptosis. This is considered to be protective against possible malignant transformation

of colonocytes (Cummings *et al.*, 1997). SCFAs may also have a preventive role in certain kinds of colitis (Roberfroid, 1993).

The H_2 produced by colonic fermentation is absorbed and then excreted in expired air serving as a semiquantitative indicator of the rate of fermentation. Various types of fiber may influence small intestinal transit time differently. For example, pectin, a viscous-type polysaccharide, causes a mouth-to-anus transit delay of glucose, while wheat bran tends to increase it. Using breath-H_2 excretion as an indirect assessment of transit time, it is found that insoluble fibers may increase, while soluble fibers may decrease, transit time (Stone-Dorshow and Levitt, 1987).

Numerous studies have been performed with adults demonstrating tolerance to FOS in doses of 20 to 30 g/day (Hata and Nakajima, 1984; Stone-Dorshow and Levitt, 1987; Briet *et al.*, 1995; Table 1), but such data were not available in the pediatric age groups. A study was carried out with school-age children to further assess possible GI intolerance from use of oligofructose (DP 4–5) in this age group (Cadranel and Coussement, 1995, unpublished data). Two schools participated in the study with a total of 43 healthy boys and girls, ages 10 to 13. A noncontrolled diet was supplemented with oligofructose mixed in chocolate drinks, apple juice, and gummy bears. Side effects (headaches, belching, stomach cramps, and sense of heaviness, intestinal noises, gas expulsion, intestinal cramps, and diarrhea) were assessed through questionnaires during 12 h after intake. Breath hydrogen excretion was measured every 60 min for 5 h. Oligofructose increased breath hydrogen excretion in a dose-dependent manner in almost all children. Liquid carriers of oligofructose produced a higher peak hydrogen excretion (79 ppm) and were somewhat less tolerated than solid carriers (37 ppm).

At one participating school (21 children), the tolerance of one or two chocolate drinks containing 3.6 g oligofructose was tested. One group of children received 200 ml chocolate drink containing 3.6 g oligofructose at mid-morning break. A second group received the same drink mid-morning and at lunch (for total of 7.2 g oligofructose), while a third group (control) received 200 ml standard chocolate drink. At the other school, 22 children were tested with higher doses of oligofructose and a solid food. One group received 200 ml apple juice with 9 g oligofructose at mid-morning break. The second group received 200 ml standard apple juice (control), while the third group of children ingested three gummy bears containing 9 g oligofructose over 30 min mid-morning.

Overall, the results demonstrated good tolerance to oligofructose. There were no recorded complaints of flatulence, abnormal stools, or diarrhea. Most complaints were broadly labeled as "stomach cramps" and were frequently noted in the controls as well as in the test groups. The test groups reporting "stomach cramps" did not have associated flatulence or intestinal noises.

The subjects were also followed with hydrogen breath tests that measured peak hydrogen concentration in the expired air. The results showed that the breath hydrogen concentration is proportional to the amount of oligofructose intake and liquid products caused higher breath hydrogen concentrations than the solid ones. In many cases, the breath hydrogen concentration was found to continue rising even after the last observation was made, while in the control groups it remained constant throughout. In approximately 50% of the observations, a breath hydrogen peak was observed within 3 to 4 h from administration, regardless of amount or method of delivery (liquid vs solid).

Advantages and disadvantages were seen with the present study design. The ability to test children of school age generally represents a significant advantage and a difficult task. The results indicate that doses up to 9 g of oligofructose are well tolerated by children between ages 10 and 13. The doses of oligofructose used in the study (3 to 9 g) reflect actual dosing with commercial products. One disadvantage seen in the study design represents the use of a noncontrolled diet. This makes allocation of individual symptoms to the tested substance difficult, if not impossible. It further influences the results obtained from the hydrogen breath tests, allowing for only rough conclusions to be drawn.

The side effects reported (e.g., stomach cramps) were broad, vague, and overall low in frequency without associated symptoms, such as flatulence. In view of the above, the authors concluded that the objectivity of the reported complaints is questionable, possibly psychosomatic in nature and not related to oligofructose intake. It was further observed that oligofructose was better tolerated if incorporated into solid food as opposed to liquid.

Despite the noncontrolled diet, which may contain other ingredients that may lower tolerance to oligofructose, the overall findings support the conclusion that doses of up to 9 g oligofructose are well tolerated by children ages 10 to 13.

Effect on Mineral Absorption

Clinically, there has been concern of possible impairment of the small intestine's absorption of minerals caused by nondigestible fiber. The effects of dietary fiber and some associated substances, such as phytate, were shown *in vitro* to have mineral binding capabilities, specifically on zinc retention and calcium absorption (Person *et al.*, 1991). It has also been demonstrated that humans are unable to adapt to chronic high intake levels of phytate, therefore placing themselves at risk for mineral (e.g., iron) deficiencies (Brune *et al.*, 1989). The minerals that remain bound are

TABLE 1
Inulin-type Fructans[a] and GI Symptoms

Author and year	Number of subjects	Amount of (I), (O), or (FOS)[b]	GI symptoms and tolerance
Alles *et al.*, 1996	24 (healthy men)	0, 5, and 15 g/day (O)	Flatulance at 15 g/day dose
Briet *et al.*, 1995	14 (8M + 6F, healthy young subjects)	30, 40, and 50 g/day (FOS) throughout the day	Flatulus at >30 g/day; bloating at >40 g/day; cramps and diarrhea at 50 g/day
Bouhnik *et al.*, 1996	12 (7M + 5F)	18 g/day (I) + (BFM) in 3 divided doses	Increased bifidogenicity with (BFM), but concurrent administration of (I) does not enhance the effect. No mention of GI intolerance
Cadranel and Coussement, 1995, unpublished	43 (healthy children ages 10 to 13)	3.6 g (O) × 1 dose—liquid; 3.6 g (O) × 2 doses—liquid; 9 g (O) × 1 dose—liquid and solid	Doses up to 9 g (O) well tolerated with no evidence of diarrhea, abnormal stools, or flatulence. Fewer side effects with solid than liquid
Coudray *et al.*, 1997	9 (healthy men); controlled, crossover design study	40 g/day (I) (supplemented in daily diet)	Significantly increased the absorption of Ca and its balance, possibly by solubilizing this element in the colon, without adverse effects on mineral retention. No mention of GI intolerance
Davidson *et al.*, 1998	21 (M + F); randomized, double-blind, crossover study	18 g/day (I) in 3 doses	May blunt hypercholesterolemic effects; the total incidence of reported GI symptoms (mild flatulance, bloating, cramping, and loose stools) was significantly higher in the (I) period
Ellegärd *et al.*, 1997	10 patients with ulcerative colitis and ileostomy	17 g/day (I); 17 g/day (O); 7 g/day sucrose	(I) and (O) are not digested in the small intestine. They do not effect mineral excretion or absorption (Ca, Mg, Zn, and Fe). They do not increase fat or nitrogen excretion from small intestine
Garleb *et al.*, 1996	9 × 3 groups (healthy males); randomized, double-blind study	0, 15, and 31 g/day (FOS) in 3 doses	Increased bifidogenicity; slight flatulus and distention with 15 g/day dose, more flatulus and diarrhea (more than 3 watery BMs/day) with 31 g/day
Gibson *et al.*, 1995	8 (7M + 1F, healthy subjects)	15 g/day (O) divided doses; 15 g/day (I) divided doses (4 of the 8 subjects)	Increased bifidogenicity with both (I) and (O); Decreased clostridia, bacteroids, and fusobacteria (O); Decreased Gram-positive cocci (I). One subject c/o intermittent flatulance throughout and a second c/o flatulance and abdominal pain (O)
Kleessen *et al.*, 1997	25 (elderly, constipated females); 15 received lactulose; 10 received inulin	20 g increased to 40 g/day (I) in 2 doses	Decreased functional constipation with minimal flatulance
Luo *et al.*, 1996	12 healthy males; randomized double-blind crossover study	20 g/day (FOS) supplemented to diet throughout the day	FOS decreased basal hepatic glucose production, with no effect on insulin-stimulated glucose metabolism in healthy subjects. No mention of GI symptoms
Molis *et al.*, 1996	6 healthy (3M + 3F)	20 g/day (FOS) given in 3 equal doses	Measured distal ileal output of FOS and FOS excreted in stool and urine. 89 ± 8.3% FOS was not absorbed in the small intestine and none was excreted in stool. The mean estimated energy value of FOS was 9.5 kJ/g. No GI symptoms seen at this dose
Pedersen *et al.*, 1997	64 healthy females, nornolipidemic; randomized double-blind crossover study	14 g/day (I) spread throughout the day	No significant difference in plasma cholesterol, HDL, LDL, and triglycerols. Statistically higher GI symptoms. Flatulance was the most profound symptom, followed by rumbling, cramps, bloating. No intestinal adaptation was seen at this level of intake for inulin
Rumessen and Gudmand-Høyer, 1998	10 (5M + 5F) healthy subjects	10–30 g inulin-type fructans (FASC and FALC)	77% had c/o gas and 17% had c/o abdominal pain. No diarrhea at 10 and 20 g. No differences seen between 30 g FASC, 20 g lactulose, and 20 g fructose. Increased orocecal transit time (OCTT) with FALC
Stone-Dorshow *et al.*, 1987	15 healthy males	15 g/day (FOS) in 3 divided doses	Increased flatulance and mild abdominal discomfort and bloating
Sobotka *et al.*, 1997	9 patients requiring enteral nutrition; single-blind study	30–35 g/day (I) continuous infusion through jejunostomy	Significant increase in flatulance. No change in SCFA concentration in the stool. No influence in intestinal permeability

TABLE 1—*Continued*

Author and year	Number of subjects	Amount of (I), (O), or (FOS)[b]	GI symptoms and tolerance
Van den Heuvel *et al.*, 1998	12 healthy, nonanemic males; randomized, crossover study	15 g/day (I, FOS, GOS) given in 3 divided doses	No negative effect on iron and Ca absorption with 15 g/day of the above. No GI symptoms recorded
Van den Heuvel *et al.*, 1999	12 adolescent males (14–16 years old); randomized, double-blind, crossover study	15 g (O) given in 3 divided doses for 9 days	Increased in fractional calcium absorption 26% compared to sucrose. GI complaints similar in treatment and control groups

[a] See text for further definition of test article.
[b] Inulin (I); oligofructose (O); fructooligosaccharides (FOS).

therefore not absorbed in the small intestine. Colonic fermentation of nondigestible fibers leads to the formation of high concentrations of short-chain carboxylic acids that can facilitate the absorption of Ca^{2+} and Mg^{2+}. Some authors consider that the presence of these two minerals in the colonic environment has a beneficial effect on the large bowel. This may be due to the possible impact on the rate of cell turnover, while the high concentrations of Ca^{2+} may lead to the formation of insoluble bile and fatty acids salts, therefore decreasing their damaging effect on the colonic epithelium (Roberfroid and Delzenne, 1998; Wargowich *et al.*, 1984).

Mineral absorption and balance is reported to be improved by inulin-type fructans, possibly because of an increased osmotic effect. This intensified water transfer into the large bowel allows for increased mineral solubility. Furthermore, the process of passive diffusion is also enhanced. The extensive fermentation of these carbohydrates causes acidification of the colonic contents and raises the concentration of ionized minerals (Coudray *et al.*, 1997; Roberfroid and Delzenne, 1998; Younes *et al.*, 1996).

In a randomized, crossover study carried out with 12 healthy, young males, Van den Heuvel *et al.* (1998) conclude that 15 g/day of inulin (DP range 2–60), FOS (DP range 2–8) (oligofructose from chicory), or galactooligosaccharide (DP range 2–6) does not have a negative impact on iron and calcium absorption. No signs of intolerance (first sign being flatulance) were identified with 15 g/day of the tested substances administered in divided doses, while they were anticipated at >30 g, as also documented by Briet (1995). In a follow-up study aimed at validating the measurement technique, the same author administered 15 g/day oligofructose (DP range 2–8) to 12 male adolescents (ages 14–16) and saw an increase in fractional calcium absorption of 26% compared to the sucrose (control) group. Reports of GI complaints were similar between the treatment and control groups (Van den Heuvel *et al.*, 1999).

A double-blind crossover comprehensive study, conducted with 10 ileostomy patients, demonstrated that mineral absorption is not impaired. The patients received 17 g inulin (DP range 2–65), 17 g oligofructose (DP range 2–8), and 7 g sucrose added to a control diet during three experimental periods of 3 days each. The study once again confirmed that inulin and oligofructose are not digested in the small intestine, as 88% for inulin and 89% for oligofructose were recovered from the ileostomy effluent. These fructans were well tolerated and did not seem to influence the absorption of nutrients from the small intestine as the excretion of calcium, magnesium, and iron was practically the same in all three diets (Ellegärd *et al.*, 1997).

Animal studies support the clinical findings (Ohta *et al.*, 1994; Younes *et al.*, 1996). A recent rat study employed the method of calcium balance in combination with the ^{45}Ca kinetics as a technique for analyzing calcium metabolism. The results indicate that the increased true calcium absorption and balance produced by FOS feeding might improve bone calcification (Morohashi *et al.*, 1998).

Effect on Glycemic Control

The incidence of diabetes has doubled since the 1950s. Approximately 800,000 new cases are being diagnosed yearly, placing the current diabetic population at 16 million in the United States. Dietary fiber affects the digestion and absorption of various substances, as seen with carbohydrates and starches. For this reason, improvement in glucose tolerance and increased sensitivity to insulin have been identified mainly with soluble dietary fibers. Hyperglycemia and hyperlipidemia often coexist in the diabetic patient. Complex medical regimens are in existence for better control of both insulin- and non-insulin-dependent patients. They include medication, such as oral hypoglycemic agents or insulin, exercise, and diet. The addition of dietary fiber to the standardized diabetic diet has been found to be beneficial.

A 14-day clinical study was performed with non-insulin-dependent diabetics. The subjects had high levels of glucose and/or serum lipids and were poorly controlled. The results indicate that a significant decrease in the fasting blood glucose and total serum cholesterol occurs with a daily intake of 8 g FOS (average DP 3.5) for 14 days. The decrease of total cholesterol was mainly due to a decrease in LDL cholesterol.

It was concluded that regular intake of FOS could benefit diabetic patients (Yamashita *et al.*, 1984). No GI signs or symptoms (e.g., flatulance, diarrhea) were observed in any of the subjects throughout the study. The effects of chronic ingestion of FOS on hepatic glucose production, insulin-mediated glucose metabolism, erythrocyte insulin binding, and blood lipids were evaluated in healthy, nondiabetic males. Twelve subjects received 20 g/day of FOS (average DP 2.7) for two 4-week periods, separated by a 2-week washout interval. The FOS or sucrose were incorporated in cookies and supplemented throughout the day. There were no complaints of GI intolerance. The study concluded that while there was a decrease in basal hepatic glucose production, no detectable effect was identified on insulin-stimulated glucose metabolism in healthy individuals (Luo *et al.*, 1996).

Effect on Constipation

In the Western world, constipation, a relatively common problem, is found in all age groups and significantly more so in the elderly. Expected physiologic changes of aging also affect the GI tract. Some of the changes are due to poor mastication from loss of dentition, atrophic olfactory and gustatory changes, hypochlorhydria, and atrophic gastritis. Decreased intestinal motility and increased transit time may also be due to known side effects from medication and/or decreased physical activity. Other changes are due to alterations in the intestinal microflora, with decrease in beneficial type bacteria (*Bifidobacteria*) and increases in the pathogenic types (enterococci, enterobacteria, and clostridia). The latter changes are believed to increase the toxic burden and the risk for cancer (Kleessen *et al.*, 1997). Considering that these changes in the intestinal flora are well recognized clinically, significant attention has been paid to the possible effects of diet on the GI tract in general and on the colon in particular.

One clinical study evaluating constipation was performed with 25 elderly females with a history of one to two bowel movements/week, hard stools, and abdominal discomfort (Kleessen *et al.*, 1997). Patients were randomly assigned to two groups, each receiving either lactose ($n = 15$) or chicory inulin ($n = 10$), in addition to their regular daily diet, for 19 days. The subjects received a single 20-g dose of lactose or inulin (average DP 10) in the first 8 days of the study. This was followed by a 3-day stepwise increase of the doses to 40 g/day, where patients were maintained for the remaining of the study. The results showed that inulin, regardless of the amount ingested, led to increase in stool frequency (from 1–2/week to 8–9/week). Softening of stools, without evidence of diarrhea, and a mild to moderate amount of flatulance were also observed. Inulin stimulated the growth of bifidobacteria, while suppressing that of pathogenic types, such as enterococci (in number) and enterobacter (in frequency). The bifidogenic effects identified by the study concurred with those of others (Gibson *et al.*, 1995; Wang and Gibson, 1993). However, no demonstrable changes in the indexes of microbial metabolism such as pH, lactate, or short-chain fatty acids, or the activities of β-glucosidase and β-glucuronidase were identified.

Effects on Intestinal Flora (Bifidogenicity)

Some dietary fiber, such as inulin-types fructans, is found to have a beneficial effect on increasing bifidobacteria in the colon (Roberfroid, 1993, 1995), where even small daily intakes of $\leq$5 g have shown a bifidogenic effect (Gibson *et al.*, 1995; Rumessen and Gudmand-Høyer, 1998).

Gibson and Roberfroid presented the concept of "colonic food" in 1994. Colonic food includes a wide variety of food constituents including resistant starch, nondigestible proteins and peptides, live microorganisms (probiotics), and nondigestible oligosaccharides (prebiotics). A prebiotic is defined by the authors as "a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995).

The microflora of the large bowel is composed of a diverse community of bacteria with significant roles in the physiology and nutrition of the human host. One effect of fermentation on the colon is stimulation of bacterial growth (increased biomass). Colonic bacteria are eventually excreted in the feces, therefore contributing to the laxative properties of high-fiber diets. Most authors agree that all carbohydrates reaching the large bowel will have some laxative effect (Cummings *et al.*, 1997; Gibson *et al.*, 1995). A broad and somewhat simplistic categorization of intestinal bacteria can be presented as beneficial, such as *Bifidobacteria* and *Lactobacilli,* or pathogenic as in *Clostridia, Enterobacter,* and Gram-positive cocci. *Bifidobacteria* and *Lactobacilli* are able to create unfavorable growth conditions for the pathogenic types. *Clostridia* and *Enterobacter* are known to have a detrimental effect on the host through an invasive action or through toxin production (Wang and Gibson, 1993).

Bifidobacteria are Gram-positive anaerobic bacteria. They are found in stool shortly after birth and represent a major part of the normal human intestinal flora throughout life. Bifidobacteria can represent as much as 95% of the intestinal microflora in breast-fed infants compared to only 25% seen in adults. This is believed to be the reason why breast-fed infants suffer from fewer GI infections than those being bottle-fed (Gibson *et al.*, 1995). However, the numbers of bifidobacteria are known to decline with the aging process.

To evaluate the effects of oligofructose (average DP 4–5) and inulin (average DP 10) on the microflora and colonic function in the large bowel, a crossover clinical study was performed with eight healthy subjects for a

period of 45 days (Gibson *et al.*, 1995). The study period was divided into 15 days of control diet, 15 days when the 15 g/day sucrose in the control diet was replaced with 15 g/day oligofructose, and then a 15-day second control period with 15 g/day sucrose. A 5-day period of free diet for stool collection to complete fecal marker recovery followed. Four of the subjects went on to participate in another 25-day inulin study, where the 15 g/day sucrose control period was 10 days, followed by 15 g/day inulin for 15 days, and finally concluded by 5 days of stool collection to recover all markers. The tested compounds were given in divided doses throughout the day. Inulin and oligofructose were well tolerated during the study, with only one subject complaining of intermittent flatulence throughout, while another complained of flatulence and abdominal pain during the oligofructose phase of the study. The results indicate that an alteration in the diet, to where 15 g/day of oligofructose or inulin is substituted for sucrose, could significantly change the balance in the colonic microflora by increasing levels of bifidobacteria. This finding was in accordance with those of other authors (Bouhnik *et al.*, 1996; Gibson *et al.*, 1995; Rumessen and Gudmand-Høyer, 1998), but it suggests substitution at a higher amount. The study further demonstrated that the number of pathogenic bacteria decreased with intake of oligofructose. Previous *in vitro* studies also demonstrate that inulin (DP range 2–60) and oligofructose ("defined as the fraction of inulin with a DP below 20") are substrates for bifidobacterial growth (Wang and Gibson, 1993).

Effects on Risk of Colon Cancer

The risk for colon cancer, for both men and women, is high, making colorectal cancer one of the leading causes of mortality in the Western world (Parker *et al.*, 1997). Currently the etiology for this type of cancer is considered to be multifactorial, with diet playing an important role. Dietary fiber, depending on the amount and type, has been credited with decreasing the risk for several cancers, including that of the colon. Colonic microflora are considered to play an important role in the process of colorectal carcinogenesis. The evidence comes from various published works indicating that germ-free experimental animals have a lower incidence of chemically induced colon cancer (Goldin *et al.*, 1981; Reddy *et al.*, 1974). Furthermore, some colonic microflora have the ability to produce carcinogens and promoters (Hill, 1988). However, not all bacteria have a detrimental effect on the host. As already discussed, *Bifidobacteria* and *Lactobacilli*, which are acid-producing bacteria, appear to have a protective effect against colon cancer.

Possible early indicators of colon cancer are aberrant crypt foci (ACF). They are considered to be preneoplastic lesions that could lead to the formation of colonic adenomas and carcinomas in humans and some animals (Reddy *et al.*, 1997). Their presence and number are considered to be predictors of tumor outcome. In a rodent study, Rao *et al.* (1998) employed a colonic ACF assay to evaluate possible preventive properties of certain agents (e.g., coffee fiber, inulin, and pectin) on colon cancer. The study demonstrates that inulin-containing diet reduces the azoxymethane (AOM)-induction effect on the number of ACF/cm^2 in the colon. Inulin also increases by three- to fivefold the production of cecal short-chain fatty acids, which have been shown to increase apoptosis. Similar findings were reported by a different study, also showing a 50% suppression in the 2-amino-3-methylimidazol[4,5-*f*] quinoline (IQ)-induced mammary carcinogenesis in F344 female rats (Reddy, 1998).

In a separate study, 60 male Sprague–Dawley rats were used to assess the effects of *Bifidobacterium longum* and inulin on carcinogen-induced aberrant crypt foci caused by the colon carcinogen AOM (Rowland *et al.*, 1998). The study also measured the concentration of ammonia produced by bacterial degradation of protein and urea, β-glucoranidase, and β-glucosidase, known as possible tumor promoters. The consumption of either inulin or *Bifidobacterium longum* was associated with a decrease (26 and 41%, respectively) in AOM-induced small ACF (1–3 crypts per focus). The simultaneous administration of inulin and *Bifidobacterium* led to 80% inhibition of small ACF and decreased the incidence (by 59%) of large ACF (>4 crypts per focus), which are considered to be predictive of tumor incidence. It was also determined that since both inulin and *Bifidobacterium longum* were started 1 week after treatment with the carcinogen, they may function by inhibiting the early promotion stage of the carcinogenic process. Decreases in ammonia concentration and β-glucoranidase activity were seen as consistent with the beneficial effect on colon cancer prevention.

Using the same premises, an earlier study investigated the effects of FOS (average DP 3.5) and that of *Bifidobacteria* on the process of chemically induced colonic carcinogenicity. Three groups of CF_1 female mice were devised for the experiment. One group (n = 20) received 1,2-dimethylhydrazine (DMH) and was fed *Bifidobacteria* and a bifidogenic diet; the second group (n = 21) received DMH and a normal diet; while the controls (n = 5) did not receive DMH and were fed a normal diet. Results revealed that the incidence of aberrant crypts and foci was significantly lower 38 weeks after the last injection of the carcinogen in mice fed *Bifidobacteria* than in animals treated with carcinogen alone. The decrease in precursor lesions for colonic cancer is presumed to be due to the increased number of *Bifidobacteria* and their effect on lowering intraluminal pH, while displacing pathogenic type bacteria (Koo and Rao, 1991).

Effects on Lipid Metabolism

Coronary heart disease represents the number one source of morbidity and mortality in the United States. According to the American Heart Association, more than 60 million people have been diagnosed with some type of cardiovascular disease, while 1 of 2 remaining individuals are at risk. As a preventive effort, health care agencies have set guidelines and offered recommendations for decreased consumption of saturated fat and cholesterol and increased intake of foods high in fiber, especially soluble fiber (The Surgeon General's report, 1988; National Cholesterol Education Program, 1990).

To assess the effects of diets high in soluble fiber on serum lipid levels, a clinical study was undertaken (Jenkins *et al.*, 1993). Addressed by the study was the question of whether a high intake of fiber can reduce plasma lipids when diets low in saturated fat and cholesterol are used. The subjects were 43 otherwise healthy individuals (15 males and 28 postmenopausal females), with a history of mild to severe hyperlipidemia. They were divided into two groups, 22 subjects receiving the soluble fiber (lentils, peas, barley, and breakfast cereals enriched with psyllium) diet first, and 21 received the insoluble fiber (wheat-bran breakfast cereals, high-fiber crackers, and high-fiber bread containing ground wheat and gluten) diet first. The study lasted for 16 weeks, separated by a 2-month period of a low-cholesterol diet. The results demonstrated that a diet containing a high amount of soluble fiber, compared to that of insoluble fiber, reduced plasma LDL cholesterol and apolipoprotein B levels. It indicated that further reduction of blood lipid levels could occur with the consumption of foods high in soluble fiber, even after saturated fat and cholesterol have been reduced in the diet.

The exact mechanisms by which soluble dietary fibers may impact serum lipid concentrations are not fully elucidated. Some of the working hypothesis include the following:

The binding of bile acids by some dietary fibers, with impairment of micelle formation in the upper GI tract. This process may affect the amount of fat and cholesterol absorption and possibly cause increased excretion of bile acids.

Soluble dietary fiber may contribute to satiety by displacing saturated fatty acids in the diet, therefore impacting the amount and/or time of food intake (Jenkins *et al.*, 1989; Swain *et al.*, 1990).

The short-chain fatty acids, produced by the anaerobic colonic metabolism of the soluble dietary fibers reaching the portal blood system, may partially impair the hepatic cholesterol synthesis (Delzenne and Roberfroid, 1994; Levrat *et al.*, 1994).

The effects of daily ingestion of 14 g of inulin (average DP 11–12) on blood lipid concentrations and GI disturbances were studied by Pedersen *et al.* (1997). Sixty-four healthy, normolipidemic females participated in this randomized double-blind crossover study. Inulin showed a lack of effect on blood lipid concentrations. Gastrointestinal disturbances were ranked numerically from absent (0) to severe (3). Those complaints were significantly higher in the inulin test group compared to controls. Flatulance was ranked the highest, while 12% of the participants also ranked it as severe. No laxation was recorded with this intake amount.

The study conducted by Ellegärd (1997) with 10 patients with ileostomy further demonstrates that there is no effect on cholesterol absorption or excretion when inulin and oligofructose are supplemented in the diet.

DISCUSSION

No evidence of treatment-related toxicity, carcinogenicity, or genotoxicity was observed from standard toxicity tests conducted at doses far above anticipated human exposure. Human and animal studies demonstrated that inulin-type fructans do not adversely affect mineral absorption, glycemic control, lipid metabolism, or intestinal flora.

Critical clinical information on intake and tolerance of fructans in humans is summarized in Table 1. These and other data indicate that the ingestion of fructans will be limited by its affect on the bowel function. The various GI symptoms that have been identified include abdominal pain and bloating, flatulence, and/or osmotic diarrhea. Amounts of 5 g/day are well tolerated. Abdominal complaints seem to occur after a single dose of fructans over 20 g (Rumessen, 1998). Other studies have demonstrated that the ingestion of fructans shows the first signs of intolerance (e.g., flatulence) at over 30 g (Briet *et al.*, 1995). School-age children tolerate supplementation of the diet with oligofructose at levels as high as 9 g/day. This amount is better tolerated if given with solid food, as opposed to liquid (Cadranel and Coussement, 1995, unpublished report). Furthermore, the intake of fructans is better tolerated when given in divided doses throughout the day.

CONCLUSIONS

Inulin and oligofructose are soluble, fermentable dietary fibers that have demonstrated no evidence of toxicity based on toxicological and clinical studies. In regard to their food use, the real issue is not that of safety, but rather of GI tolerance. Signs of intolerance can be seen with intakes above 20–30 g (depending on the study). This is equivalent to a daily value of fiber which consumers currently have had trouble attaining. Given present dietary fiber labeling requirements, consumers will be able to make appropriate and individual choices on daily intake.

It is concluded that inulin-type fructans are safe for human consumption under intended conditions of use as a dietary fiber.

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