GR

ORIGINAL SUBMISSION

GRAS NOTIFICATION

I. Claim of GRAS Status (Revised 5/21/2010)

A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

Fugeia NV (the notifier) has determined that its Wheat Bran Extract, a water soluble fiber derived from wheat bran, is Generally Recognized As Safe, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food. Therefore, the use of Wheat Bran Extract is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Date 5/21/10

Edward A. Steele

Agent for:

Fugeia NV
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C. Common or usual name of the notified substance:

Wheat Bran Extract

D. Conditions of use:

Wheat Bran Extract, mainly comprised of xylo- and arabinoxylo-oligosaccharides derived from arabinoxylan, the main fiber of wheat bran, is intended for use as a food ingredient in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods at use levels ranging from 2.4 to 3.2 g Wheat Bran Extract *per* serving (reference amounts customarily consumed, 21CFR 101.12). The intended use of Wheat Bran Extract in the above mentioned food categories is estimated to result for "users only" at mean and 90th percentile intakes of 5.0 g/person [97 mg/kg body weight (bw)/day] and 10.1 g/person (217 mg/kg bw/day), respectively.

E. Basis for GRAS Determination:

In accordance with 21CFR 170.30, the intended use of Wheat Bran Extract has been determined to be generally recognized as safe (GRAS) based on scientific procedures. A comprehensive search of the scientific literature was also conducted for this review. There is sufficient qualitative and quantitative scientific evidence, including human and animal data, to determine safety-in-use for Wheat Bran Extract. The safety determination of Wheat Bran Extract is based on the totality of available evidence, particularly human observations, including clinical trials and a variety of animal, as well as *in vitro* studies further corroborating its safety. In clinical trials, no adverse effects of Wheat Bran Extract were noted at doses of up to 13.9 g/day. Results from a subchronic rat study show that Wheat Bran Extract had no adverse effects at dose levels up to 7.5% (4354 mg Wheat Bran Extract/kg bw/day), the highest dose tested. The estimated daily intake of Wheat Bran Extract from the intended uses at the 90th percentile is 10.1 g/person (217 mg/kg bw/day). This amount is similar to that used in clinical trials in humans and is approximately 20-fold lower than the demonstrated safe levels of intake in animal studies of 4354 mg of Wheat Bran Extract/kg bw/day. The estimated daily intake, if ingested daily over a lifetime, is safe.

F. Availability of Information:

The data and information that forms the basis for this GRAS determination will be provided to Food and Drug Administration upon request and are located at the offices of: Edward A. Steele, EAS Consulting Group, LLC, 1940 Duke Street, Suite 200, Alexandria, VA 22314; Phone: (703) 684-4408; Fax: (703) 684-4428; E-mail: esteele@easconsultinggroup.com

The primary toxicologist, Dr. Madhusudan G. Soni, responsible for the preparation of this GRAS monograph and who is also a member of the expert panel can also be contacted for the data and information that forms the basis for this GRAS determination at the following address: Madhusudan G. Soni, Ph.D., FACN, 749 46th Square, Vero Beach FL, 32968; Phone: (772) 299-0746; Fax: (772) 299-5381; E-mail: sonim@easconsultinggroup.com

II. Detailed Information About the Identity of the Notified Substance:

A. Chemical name

Wheat Bran Extract. The product is composed mainly of xylo-oligosaccharides, arabinoxylo-oligosaccharides and β-glucans. In the following sections, AXOS (arabinoxylan oligosaccharides) will be used as an abbreviation for preparations that contain mixtures of xylo-oligosaccharides and arabinoxylo-oligosaccharides.

B. Common Name:

The subject of this notification will be marketed as Wheat Bran Extract.

C. Chemical Abstract Registry Number:

84012-44-2; EINECS No.: 281-689-7

D. Chemical Formula:

Not applicable

E. Structure:

The main components of Wheat Bran Extract, xylo- and arabinoxylo-oligosaccharides both have a backbone of β-(1-4)-linked D-xylopyranosyl residues (xylose), which in case of arabinoxylo-oligosaccharides is substituted with α-L-arabinofuranosyl residues (arabinose) linked at some of the O-2, O-3 or at both the O-2 and O-3 positions of the xylose residues (Figure 1).

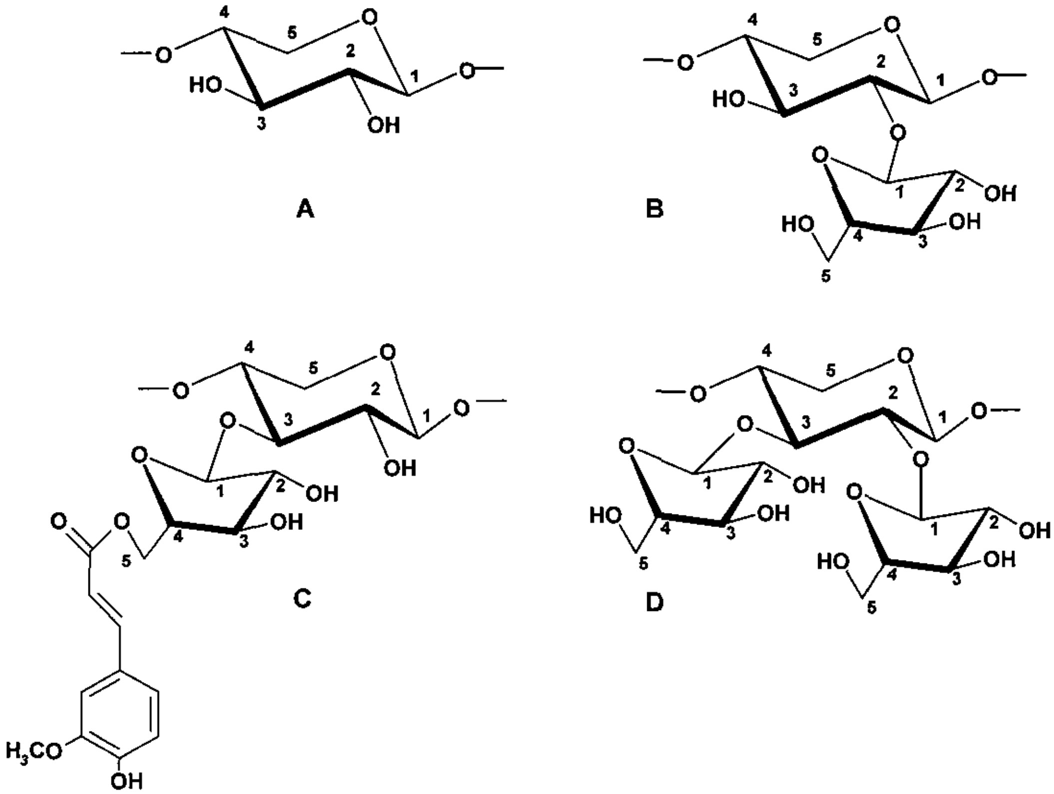

Figure 1: Key structural elements of arabinoxylo-oligosaccharides: A: unsubstituted D-xylopyranosyl residue (xylose), B. xylose residue substituted at the O-2 position with L-arabinofuranose (arabinose), C: xylose residue substituted at the O-3 position with arabinose, and D: xylose residue substituted at the O-2 and O-3 position with arabinose Structure C shows the link of ferulic acid to the O-5 position of an arabinose residue.

F. Molecular Weight

Molecular weight of xylo- and arabinoxylo-oligosaccharides varies depending on the degree of polymerization. The degree of polymerization of the main AXOS fraction varies between 2 and 15 (average degree of polymerization: 3-8), therefore the AXOS molecular weight is likely to vary between 282 and 1998.

G. Physical Characteristics

Wheat Bran Extract is an off-white crystalline powder.

H. Typical Composition and Specifications

Typical specifications of Wheat Bran Extract are presented in Table 1. Analyses of 5 independently produced, representative, batches (Table 2) of Wheat Bran Extract demonstrate that the manufacturing process and final product are both highly reproducible and that the process is capable of producing material that consistently meets the specification. Representative HPAEC-PAD[1] patterns of Wheat Bran Extract are given in Appendix I. Wheat Bran extract is composed of AXOS (>70%) and other bran-extracted poly- and oligosaccharides (~20%). The other poly- and oligosaccharides primarily include β-glucans (10%-14%). Wheat Bran Extract also contains ~1.5% ferulic acid and ~1% glucuronic acid that are primarily bound to AXOS. As shown in Table 2, the sum of all analyzed components demonstrates that Wheat Bran Extract is fully characterized (almost 100%) for its constituents. Extensive analysis of potential external contaminants of Wheat Bran Extract such as mycotoxins, heavy metals, pesticide residues, and microbes that are generally associated with wheat products, from five batches [redacted] (b)(4) revealed either the absence of these contaminants or their presence at very low levels that are unlikely to cause any adverse effects.

Table 1. Physical, chemical and microbiological specifications of Wheat Bran Extract

Parameter	Specifications	Assay method
Physical parameters		
Appearance	Off-white crystallıne powder	Vısual
Taste	Slight sweet	
Chemical parameters		
Arabinoxylan oligosaccharıdes (AXOS)*	Min. 70% of dm	Courtin et al , 2009a
Average DP** of AXOS	3-8	Courtın et al., 2009a
Ferulic acid (bound to AXOS)	1-3% of dm	Hartmann et al., 2005
Total poly/oligosaccharides***	Min 90 %	Courtin et al., 2009a
Proteın	Max. 2% of dm	AOAC Method 990.03
Ash	Max. 2% of dm	AACC Method 08-01
Dry matter (dm)	Mın 94 %	AACC Method 44-19
B-glucan	Mın. 7% of dm	AACC Method 995.16
Heavy metals		
Lead	<0 1 ppm	
Arsenic	<0.1 ppm	
Cadmium	<0.1 ppm	
Mercury	<0.1 ppm	
Microbiological parameters		
Mesophilic bacterıa– total count	Max 10,000/g	ISO 4833 or equi
Yeasts	Max. 100/g	ISO 7954 or equi
Fungi	Max 100/g	ISO 7954 or equi
Salmonella	Absent in 25g	ISO 6579/cor1 or equi
Bacillus cereus	Max 1000/g	ISO 7932 or equı
Clostrıdium perfringens	Max. 1000/g	ISO 7937 or equi

*Contain mixtures of xylo-oligosaccharides and arabinoxylo-olıgosaccharides, **DP = Degree of polymerızation; ***Wheat Bran Extract may comprıse up to 20% other poly/olıgosaccharides (12 and 16% glucose as part of poly/oligosaccharides)

[1] High-performance anıon-exchange chromatography with pulsed amperometric detection (see Appendix I)

Table 2. Compositional analysis of five different batches of Wheat Bran Extract.

Parameters	Specification	WBE Batch A	WBE Batch B	WBE Batch C	WBE Batch D	WBE Batch E
Physical parameters						
Appearance	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder
Chemical parameters						
Arabinoxylan oligosaccharides (AXOS, %dm)	Min 70	79 0	81 6	78 3	78 2	76 8
Of which xylo-oligosaccharides (XOS_{DP2-9}, %dm)		39 5	43 6	40 9	39 3	37 7
Of which xylobiose (XOS_{DP2}, %dm)		22 2	19 5	22 7	19 6	18 8
Average DP[a] of AXOS	3-8	5	5	4	5	5
A/X[b] ratio of AXOS		0 19	0 20	0 20	0 20	0 21
Glucuronic acid bound to AXOS (%dm)		1 0	0 9	1 0	1 0	1 1
Ferulic acid bound to AXOS (%dm)[c]	1 5 ± 0 5	1 5	1 6	1 5	1 5	1 5
Glucose as part of poly/oligosaccharides (% dm)		12 2	15 9	16 0	15 7	15 3
Of which β-(1,3)(1,4)-glucan (%dm)		10 7	12 8	12 6	13 5	13 3
Of which cellobiose (%dm)		0 7	0 9	2 6	1 4	1 1
Of which laminaribiose (%dm)		<0 1	<0 1	<0 1	<0 1	<0 1
Galactose as part of poly/oligosacharides (% dm)		0 5	0 5	0 5	0 5	0 5
Mannose as part of poly/oligosacharides (% dm)		0 2	0 2	0 3	0 2	0 2
Total poly/oligosaccharides[d] (%dm)	Min 90	94 5	100 7	97 6	97 1	95 4
Total free monosaccharides (% dm)		0 5	0 4	1 1	1 2	1 2
Protein [Nx6 25] (%dm)	Max 2 0	0 6	0 7	0 6	1 2	1 1
Total Fat (%dm)		<0 5	<0 5	<0 5	<0 5	<0 5
Ash (%dm)	Max 2 0	0 2	0 2	0 2	0 4	0 6
Of which sodium (%dm)		0 04	0 02	0 06	0 13	0 20
Dry matter (dm, %)	Min 94	96 4	97 8	99 0	98 0	97 6
Sum of analysed components (%dm)		**95 8**	**102 0**	**99.5**	**99.9**	**98.3**

[a] DP = degree of polymerisation

[b] A/X = arabinose to xylose ratio

[c] Calculated from total and free ferulic acid as described in Annex A

[d] Sum of AXOS and the bound ferulic and glucuronic acid, and the glucose-, galactose- and mannose as part of poly/oligosaccharides

I. Manufacturing process

Wheat Bran Extract is produced by enzymatic (partial) depolymerization of the water-unextractable arabinoxylan component of wheat bran. The production procedure uses wheat bran as the starting raw material and combines gentle enzymatic extraction techniques with physical separation methods, including filtration, evaporation and spray drying. The Wheat Bran Extract production process is schematically represented in Figure 2. In the first step, wheat bran is suspended in water containing α-amylase to cleave the bran-contained starch. In the second step, the destarched bran comprising the water-unextractable hemicelluloses is separated from the solubles, washed and resuspended in water in the presence of a hemicellulase enzyme preparation, which releases and solubilizes the AXOS. Subsequently, in the third step, the AXOS-containing liquid fraction is filtered and further purified by ion exchange resin treatment. In a final fourth step, the purified liquid fraction is concentrated and spray-dried.

All equipment and materials used in the production process have a history of use in food processing. In particular the enzymes used in the production process, food-grade α-amylase and hemicellulase (endoxylanases, EC 3.2.1.8), are routinely used in the processing of cereal-based foods. Both enzymes from non-genetically modified organisms are commonly used in beer brewing in an analogous manner as in the Wheat Bran Extract production (during brewing, malt is suspended in the presence of these enzymes and following an incubation of the brewing mash, the wort comprising the solubles is isolated and processed into beer). Similarly, the production of Wheat Bran Extract involves the incubation of wheat bran in the presence of these enzymes, followed by isolation of a liquid fraction which is subsequently processed to obtain Wheat Bran Extract. Wheat Bran Extract is manufactured in accordance with current good manufacturing practices (cGMP).

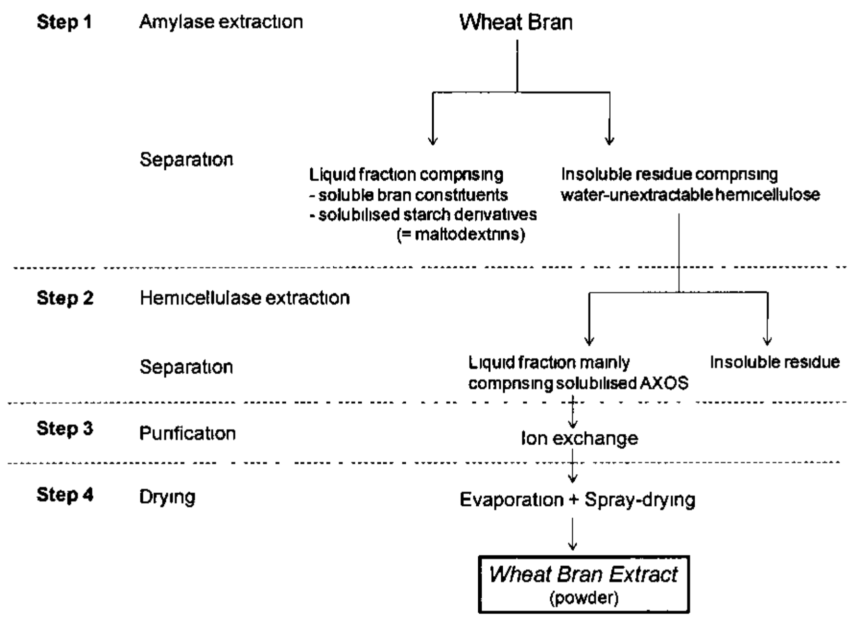

Figure 2. Manufacturing process of Wheat Bran Extract

J. Intended Technical Effects

The intended technical effect of Wheat Bran Extract is as a texturizer. Wheat Bran Extract's appealing textural characteristics and neutral flavor, together with a high level of Total Dietary Fiber (values >70%), offer several food applications. These applications include, but are not limited to, raising total dietary fiber, reducing caloric content, controlling water activity and modifying the rheological properties of food systems. The most compatible foods for use of Wheat Bran Extract include baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods. Although the main constituents of Wheat Bran Extract are present at varying levels in the diet, its addition to food aims at gaining certain health benefits associated with the use of prebiotic ingredients.

III. Summary of the Basis for the Notifier's Determination that Wheat Bran Extract is GRAS

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Fugeia NV to determine the Generally Recognized As Safe (GRAS) status of Wheat Bran Extract intended for use as a multipurpose food ingredient, particularly as a source of dietary fiber. A comprehensive search of the scientific literature was also conducted to identify relevant safety studies for this assessment.

Based on a critical evaluation of the pertinent data and information summarized herein, the Expert Panel members have individually and collectively determined by scientific procedures that the addition of Wheat Bran Extract to baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods, meeting the specifications cited above and manufactured according to current Good Manufacturing Practice, is Generally Recognized As Safe (GRAS) under the conditions of intended use in selected foods, as specified herein.

In coming to its decision that Wheat Bran Extract is GRAS, the Expert Panel reviewed published toxicology and clinical studies and other relevant corroborating information relating to the safety of the product and concluded that neither Wheat Bran Extract nor any of its degradation products pose any toxicological hazards or safety concerns at the intended use levels. . It is also their opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

IV. Basis for a Conclusion that Wheat Bran Extract is GRAS for its Intended Use.

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF WHEAT BRAN EXTRACT AS A FOOD INGREDIENT

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DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF WHEAT BRAN EXTRACT AS A FOOD INGREDIENT

1. INTRODUCTION

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[2], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by EAS Consulting Group, LLC, at the request of Fugeia NV, Belgium, to determine the Generally Recognized As Safe (GRAS) status of Wheat Bran Extract as a food ingredient in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods at use levels ranging from 1 to 3 g Wheat Bran Extract *per* serving. The main components of Wheat Bran Extract are xylo-oligosaccharides and arabinoxylo-oligosaccharides. In the following sections, xylo-oligosaccharides will be abbreviated as XOS when it concerns preparations that comprise mainly xylo-oligosaccharides, while AXOS (arabinoxylan oligosaccharides) will be used as an abbreviation for preparations that contain mixtures of xylo-oligosaccharides and arabinoxylo-oligosaccharides. A comprehensive search of the scientific literature for safety and toxicity information on Wheat Bran Extract and its main component xylo-oligosaccharide and arabinoxylo-oligosaccharides was conducted through November 2009 and made available to the Expert Panel. Fugeia NV assures that all unpublished information in its possession and relevant to the subject of this determination has been provided for this assessment and has been summarized in this GRAS monograph. The Expert Panel independently and critically evaluated materials submitted by Fugeia NV and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

1.1. Background

It is well recognized that adequate dietary fiber consumption promotes overall health. Protective effects of dietary fiber have been reported against heart disease, obesity, bowel diseases, and cancer (Timm et al., 2008). The Food and Nutrition Board of the Institute of Medicine established recommended intake levels for dietary fiber in 2001 (IOM, 2002). For adults (≤50 years of age), the adequate intake recommendation for total fiber is 38 g/day for men and 25 g/day for women. Additionally, the daily reference value for dietary fiber is 25 g (for a 2000 calorie diet) [21 CFR 101.9(d)]. Dietary fiber intake in the U.S. averages from 16-18 g/day for men and 12-14 g/day for women, which is well below recommended intake levels (IOM, 2002). Similarly, other national and international nutrition guidelines emphasize that a healthy diet should provide a minimum daily amount of dietary fiber, varying from 20 to 40 g/day. However, several surveys have shown that in the United States and Western Europe the average fiber intake is markedly lower than these recommended amounts (Gray, 2006; Lairon et al., 2003).

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[2]Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended See also attachments (curriculum vitae) documenting the expertise of the Panel members.

The sub-optimal fiber intake in industrialized countries is attributed to a shift from a diet based mainly on whole cereals, pulses, fruits and vegetables towards a diet based on animal foodstuffs and refined cereals, which are often incorporated into processed foods and result in a low fiber content and daily intake of fiber. Fugeia NV intends to market a well-characterized extract of wheat bran, under the name Wheat Bran Extract, as a food ingredient that would contribute to a desirable increase in the average fiber intake at a population level. Additionally, the physico-chemical characteristics of the Wheat Bran Extract allow for its incorporation into a wide variety of food products without substantially affecting their taste or texture.

1.2. Chemistry and Biological Activity

Cereals are known to contain about 5-10% of arabinoxylan, which along with cellulose, starch, and β-glucan constitute the most abundant cereal carbohydrates. Generally, wheat bran is composed of arabinoxylan (~19-25%), starch (17-29%), protein (14-18%), lignin (about 3%), β-D-glucan (1-3 %), phytic acid (3-5%), and ferulic acid (0.3%-0.5%) (Bataillon et al., 1998; Maes and Delcour, 2002; Swennen et al., 2006; Hollmann and Lindhauer, 2005; Jenab and Thompson, 1998). In wheat bran, arabinoxylan is the main cell wall component per se. Wheat Bran Extract prepared by partial enzymatic hydrolysis of arabinoxylan (the main fiber type contained in wheat bran) is principally composed of AXOS (>70%). A further 20% of Wheat Bran Extract contains other bran-extracted poly- and oligosaccharides. From a chemical perspective, AXOS are a mixture of low molecular weight xylo- and arabinoxylo-oligosaccharides. Both xylo- and arabinoxylo-oligosaccharides have a backbone of β-(1-4)-linked D-xylopyranosyl residues (xylose), which in the case of arabinoxylo-oligosaccharides is substituted with α-L-arabinofuranosyl residues (arabinose) linked at some of the O-2, O-3 or at both the O-2 and O-3 positions of the xylose residues (Vietor et al., 1992; Izydorczyk and Biliaderis, 1995; Vinkx and Delcour, 1996). Unsubstituted, O-2 mono-, O-3 mono- and O-2,O-3 di-substituted xylose residues are the key structural elements of AXOS (Figure 1). Other, more minor substituents such as glucuronic acid, 4-O-methylglucuronic acid, or acetic acid, are coupled to some of the xylose residues, while hydroxycinnamic acids such as ferulic acid are linked to some of the arabinose residues of AX (Izydorczyk & Biliaderis, 1995; Vinkx and Delcour, 1996). AXOS in Wheat Bran Extract are a polydisperse mixture of oligosaccharides with an average degree of polymerization (DP) varying between 3 and 8. The arabinose to xylose (A/X) ratio, also referred to as the average degree of arabinose substitution, varies between 0.18 to 0.30. XOS with a DP ranging from 2 to 9 represents about 50% of the total fraction of the AXOS, with the remainder being arabinoxylo-oligosaccharides. Representative HPAEC-PAD patterns of Wheat Bran Extract are given in Appendix I. Figure A and B in the appendix show a pattern obtained with untreated Wheat Bran Extract and Wheat Bran Extract treated with acid to de-arabinosylate the arabinoxylo-oligosaccharides, respectively. These patterns and their comparison, chromatographically represented in Figure C (Appendix I), allow one to conclude that the Wheat Bran Extract AXOS have a xylose backbone with a DP ranging from 2 to 9 and that the xylo-oligosaccharides in Wheat Bran Extract mainly have a DP of 2 (xylobiose) and 3 (xylotriose), while most arabinoxylo-oligosaccharides have a xylose backbone with a DP of 4 or 5.

Wheat Bran Extract is composed of ~ 1.5% ferulic acid and about 1% glucuronic acid. These compounds are predominantly bound to AXOS; no free glucuronic acid was detected, while the amount of free ferulic acid was lower than 0.01%. Next to AXOS, Wheat Bran Extract contains β-D-(1,3)(1,4)-glucans (10%-14%). β-D-(1,3)(1,4)-glucans, also referred to as β-glucans, are the second most abundant hemicellulose type found in the cell walls of cereals. β-

Glucans are linear polymers of D-glucose with units of typically 2 or 3 β-(1,4)-linked glucose residues. These units are linked by single β-(1,3) bonds (Woodward et al., 1982; Cui et al., 2000). Further, it has been reported that the β-glucans typically have an average DP of about 8 and that the presence of the DP2 β-glucan oligomer cellobiose was relatively low (0.7-2.6%), while the other DP2 β-glucan oligomer, laminaribiose, was undetectable.

The main fractions of the Wheat Bran Extract oligo/polysaccharides, mainly AXOS and β-glucans have a degree of polymerization of 3 or more and are not digested in the upper intestinal tract. Therefore, Wheat Bran Extract has high dietary fiber content. As shown previously in Table 2, compositional analyses demonstrate that Wheat Bran Extract is comprised of on average, 70% (67-75%) dietary fiber (sum of AXOS, beta-glucan, bound glucuronic acid and bound ferulic acid minus sum of xylobiose, cellobiose and laminaribiose).

The arabinoxylan derived oligosaccharides have been shown to possess prebiotic properties as they selectively stimulate the growth of Bifidobacteria in the colon (Grootaert et al., 2007). Bifidobacteria are generally recognized as beneficial colonic bacteria and are used as probiotic supplements in a wide variety of food products. The prebiotic activity of Wheat Bran Extract is associated with a promotion of colonic butyrate production and a decrease of colonic protein fermentation. Butyrate is the main energy source for colon cells and as such a crucial parameter in colon function and health. Reduced protein fermentation in the colon is desired, because bacterial amino acid degradation results in the production of metabolites some of which have been implicated in different bowel disorders, as well as in cancer (Van Craeyveld et al., 2008).

1.3. Description, Specifications and Manufacturing Process

Wheat Bran Extract is derived from wheat (*Triticum aestivum* L.; synonym *Triticum vulgare* Vill.; Family Poaceae) grain bran. It is an off-white crystalline powder with a high concentration of arabinoxylan-derived oligosaccharides referred to as arabinoxylan oligosaccharides. These non-digestible oligosaccharides are derived by partial enzymatic hydrolysis of arabinoxylan, the main cell wall component of wheat. The resulting extract contains a high concentration of dietary fiber [according to the definition of dietary fiber proposed by the Food and Nutrition Board of the Institute of Medicine (IOM, 2001)]. Wheat Bran Extract has a slightly sweet taste. Food grade specifications of Wheat Bran Extract manufactured by Fugeia NV are summarized in Table 1 (Section II- H). Analytical results of five independently produced, representative batches demonstrate that the Wheat Bran Extract consistently meets these specifications (Table 2).

Extensive analysis for potential external contaminants of Wheat Bran Extract such as mycotoxins [deoxynivalenol, ochratoxin A, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, fumonisin B1, fumonisin B2, zearalenone, HT-2 toxin (fusariotoxin HT-2), and T-2 toxin (fusariotoxin T-2)], heavy metals [cadmium, arsenic, mercury, lead, copper, molybdenium, nickel, zinc, chromium, iron], pesticide residues [47 N-containing pesticides, 77 organo-chlorine pesticides, 116 analyzed organo-phosphorous pesticides], and microbes (including *Salmonella* sp., coliforms, *Staphylococcus aureus*, *Clostridium perfringens*, or *Clostridium botulinum*) that are generally associated with wheat products, from five batches revealed either the absence of these contaminants or their presence at very low levels that are considered as safe.

1.3.1. Manufacturing Process

The Wheat Bran Extract production process involves gentle enzymatic extraction of water-unextractable hemicelluloses (arabinoxylan and β-glucan) from wheat bran resulting in a product comprising over 90% oligo/polysaccharides of which the majority is AXOS with an average DP between 3 and 8. The production process is schematically represented in Figure 2. In the first step, wheat bran is suspended in water containing α-amylase to extract the bran-containing starch. In the second step, the destarched bran comprising the water-unextractable hemicelluloses is separated from the solubles, washed and resuspended in water in the presence of a hemicellulase enzyme preparation, which releases and solubilizes the AXOS. Subsequently, in the third step, the AXOS-containing liquid fraction is filtered off and further purified by ion exchange resin treatment. In a final fourth step, the purified liquid fraction is concentrated and spray-dried.

All equipment and materials used in the production process have a history of use in food processing. In particular, the enzymes used in the production process, food-grade α-amylase (21 CFR 137.105; 137.200; 184.1012; 184.1148), and hemicellulase, are routinely used in the processing of cereal-based foods. Both enzymes are commonly used in beer brewing in an analogous manner as in the Wheat Bran Extract production (during brewing, malt is suspended in presence of these enzymes and following an incubation of the brewing mash, the wort comprising the solubles is isolated and processed into beer). Similarly, the production of Wheat Bran Extract involves the incubation of wheat bran in the presence of the above enzymes, followed by the isolation of a liquid fraction which is subsequently processed to obtain the Wheat Bran Extract.

Processing aids used such as sulfuric acid/phosphoric acid and calcium hydroxide/sodium hydroxide are of food grade quality as specified in Food Chemical Codex 6^{th} Edition (FCC, 2008). Wheat Bran Extract is manufactured in accordance with current good manufacturing practices (cGMP). The steps used in the production process and the raw materials/processing aids used are common in the food industry. Following detailed consideration and extensive analytical assessment of the Wheat Bran Extract, the production process is neither considered likely to generate nor has been demonstrated to generate new potential toxicants, nutritional hazards or sources of microbiological contamination. Fugeia NV has established a HACCP-controlled manufacturing process to monitor critical steps of the process in order to avoid occurrence of any extremes in the processing parameters, including processing temperature.

The use of elevated temperatures in the production process nevertheless led to the consideration of the potential generation of heat-mediated reaction products such as furfural (mainly from pentose monosaccharides), 5-hydroxymethylfurfural (from hexose monosaccharides) and acrylamide. However, at the maximum temperature of the Wheat Bran Extract production process (95°C) the degradation of carbohydrates is limited. Moreover, the production process includes an ion exchange resin treatment step using anion and cation exchange resins, which have been demonstrated to be effective at removing furfural and 5-hydroxymethylfurfural (Carvalheiro et al., 2005). The analyses of furfural, 5-hydroxymethylfurfural and acrylamide in 5 independently produced batches of Wheat Bran Extract indicate that of the three analyzed heat-mediated reaction products only furfural occurs at a concentration above the analytical detection limit, albeit at a very low level (5-22 mg/kg). The furfural levels in Wheat Bran Extract are in the same range as those found in wheat bran itself demonstrating that they are not concentrated or generated in the production process. The furfural levels in Wheat Bran Extract are also similar to those found in wheat bread (up to 26 mg/kg, Adams et al., 1997). From the intended uses of Wheat Bran Extract, the intake of furfural has been estimated as 0.005 mg/kg/day. This intake is 100 fold lower than JECFA acceptable daily intake for furfural related substances (JECFA, 2004). Acrylamide was not detected (detection limit < 30 µg/kg) in any of the batches tested.

1.3.2. Enzymes used in the production

The enzymes, amylase and hemicellulase are used in the production of Wheat Bran Extract. Both enzymes are approved for use in the food and food ingredient industry, including the brewing industry where they are applied in a very similar way as in the production process of Wheat Bran Extract. Following the respective enzymatic reactions the enzymes are inactivated during the Wheat Bran Extract production process. Both residual α-amylase and hemicellulase activity were absent in each of the 5 production batches of Wheat Bran Extract tested using the Megazyme assay kits.

α-Amylase: Several commercially available, food-grade α-amylase containing enzyme preparations from non-pathogenic *Bacillus* species (e.g. *B amyloliquefaciens, B. subtilis, B. licheniformis*) can be used to destarch wheat bran in the Wheat Bran Extract production. These enzymes are produced in compliance with the purity recommendations for food-grade enzymes of the joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) and the Food Chemicals Codex (FCC, 2008). They have been widely used in Europe since the 1970s as processing aids in the starch industry for the production of food-grade glucose syrups and maltodextrins, as well as in the baking, brewing, liquor, sugar, fruit and vegetable industries. In the brewing industry, these enzymes are added to the mash in order to hydrolyze cereal-derived starch into maltodextrins that can be further converted by exo-amylases into fermentable glucose. Such enzymes were formally approved in Denmark for the maltodextrin, glucose, starch, and brewing industries in 1973, and in France for the starch industry in 1977 and for the baking, starch, brewing, sugar, fruit and vegetable industries in 2006. Additionally, α-amylase obtained from *Bacillus stearothermophilus* (21 CFR 184.1012) and from *Bacillus subtilis* or *B amyloliquefaciens* (21 CFR 184.1148) as well as a malt preparation containing α-amylase (21 CFR 184.1443a) are considered as GRAS for food uses. α-Endo-amylase derived from *Bacillus amyloliquefaciens, B. licheniformis*, and *B. subtilis* are also mentioned on the list of the enzymes frequently used in the US food processing industry as compiled by the Enzyme Technical Association (ETA, Washington D.C., USA).

A commercially available, non-GMO α-endo-amylase derived from *B. licheniformis* is used in the manufacturing of Wheat Bran Extract. The enzyme preparation complies with the recommended purity specifications for food-grade enzymes described in FCC (2008). The intended use of this enzyme in the preparation of Wheat Bran Extract is considered safe.

Hemicellulase: Several food-grade hemicellulase enzyme preparations from non-pathogenic fungal *Trichoderma* species (e.g. *T. viride* or *T longibrachiatum, formerly called T. reesei)* can be used to depolymerise water-unextractable arabinoxylans in the destarched bran used in the production of Wheat Bran Extract. The main enzyme activities in these preparations are endo-β-1,4-xylanases (endoxylanases, EC 3.2.1.8) and endo-β-1,3-1,4-glucanases (endo-β-glucanases, EC 3.2.1.6). These enzymes are produced in accordance with the purity recommendations for food-grade enzymes of JECFA (2006) and FCC (2008). They have been used in Europe since the 1980s as a processing aid in the brewing industry and fruit juice industry, to facilitate filtration and in the baking industry to increase loaf volume and softness of breads. Additionally, under 21 CFR 184.1250, cellulase enzyme preparation derived from *T longibrachiatum* is GRAS as a direct human food ingredient. Endoxylanase derived from *Trichoderma longibrachiatum* (formerly called *T. reesei*) is also mentioned on the list of the enzymes frequently used in the US food processing industry as compiled by the Enzyme Technical Association (ETA, USA).

A commercially available, non-GMO xylanase derived from *T. longibrachiatum* is used in the manufacturing of Wheat Bran Extract. The enzyme preparation complies with the recommended purity specifications for food-grade enzymes described in FCC (2008). The intended use of xylanase in the preparation of Wheat Bran Extract is considered safe.

1.4. Stability Studies

The stability of Wheat Bran Extract as such and its primary constituent AXOS in food matrix have been demonstrated in several food matrices. Analysis of a Wheat Bran Extract batch after a storage period of 18 months at room temperature showed excellent stability as it conformed to the specifications for all parameters (Table 1). The stability of the AXOS, the principal component in Wheat Bran Extract, was also assessed in two different liquid food matrices: (1) Wheat Bran Extract was added to chocolate drink (pH 5.9), dispensed aseptically after Ultra High Temperature treatment (140°C, 5 second) in high density polyethylene bottles with an aluminum seal, heat treated after preparation at 120°C for 1 minute (F_0 value of 1.5), and stored at either 4°C or room temperature for up to 16 months; and (2) Wheat Bran Extract was added to orange juice (pH 3.7), dispensed in polypropylene bottles with screw cap, heat treated after preparation at 120°C for 5 minutes, and stored at either 4°C or room temperature for up to 16 months.

The content of AXOS, the arabinose to xylose ratio (A/X ratio) and average degree of polymerization (avDP) of AXOS were measured by gas chromatography (Courtin et al., 2000; 2009a) immediately after preparation and after different periods of storage. In both matrices, the content of AXOS and its A/X ratio and avDP remained unchanged within the experimental error at any of the time points. These observations demonstrate that AXOS are stable for at least 16 months when incorporated in either a neutral or an acidic (pH 3.7) liquid food product stored at 4°C or room temperature. Additionally, the stability of AXOS in terms of DP and A/X ratio was demonstrated in a dry food blend prepared for a dietary toxicology study of Wheat Bran Extract in rats up to the last sampling at 123 days.

1.5. Technical Effects

The intended technical effect of Wheat Bran Extract is as a texturizer. The Wheat Bran Extract's appealing textural characteristics and cereal flavor together with a high level of Total Dietary Fiber (values >70%) offer several food applications. These applications include, but are not limited to, raising total dietary fiber, reducing caloric content, controlling water activity and modifying the rheological properties of food systems. Although the constituents similar to those found in Wheat Bran Extract are likely to be present at certain levels in the diet, its addition to food is aimed at gaining certain health benefits.

Use of Wheat Bran Extract improves the texture and stability of the food product. Additionally, the physico-chemical characteristics of the Wheat Bran Extract are such that it allows for a practical and stable incorporation in a wide variety of food products without substantially affecting their taste or texture. The thermal stability of Wheat Bran Extract and its resistance against acid degradation makes it particularly suitable for use as an ingredient in processed foods. The most compatible foods for use of Wheat Bran Extract include baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods. It is recognized that there are Standard of Identity requirements for some of these foods (i.e., certain bakery products), and therefore, in these cases, it is not the intent to refer to them by their commonly recognized names.

1.6. Intake from Natural Presence in Food

Cereals, and in particular, wheat products are important components in the daily diet of the majority of the consumers in the US. The consumption of cereals naturally results in the intake of cereal bran fractions and of arabinoxylans, the main hemicellulose of cereals. Thus, the source of Wheat Bran Extract and some of its constituents are likely to be consumed by humans. Following ingestion, a substantial portion of the consumed arabinoxylan is fermented by the intestinal microbial flora. As this fermentation involves the formation of low molecular weight arabinoxylan, including AXOS, current arabinoxylan consumption from wheat products constitutes an indirect exposure to AXOS, the primary component of Wheat Bran Extract. Further, the biochemical appearance of arabinoxylan varies depending on the processing applied to cereal-containing foods. Depending on the exposure of a cereal-containing food product to endoxylanase activity during its preparation, part or all of the arabinoxylans contained in this product will be in the form of partially depolymerized low molecular weight (LMW) arabinoxylans (avDP 100 to 300). Bread typically contains LMW arabinoxylans, while the arabinoxylan in beer also contains AXOS. Thus, bread LMW arabinoxylans and beer AXOS are nutritionally comparable to the main constituent of Wheat Bran Extract, AXOS.

1.6.1. Exposure to Wheat Bran

Wheat bran is mainly consumed through whole grain bread and thus the intake of bran from this source can be derived from whole grain bread consumption data. This can be done by taking into account that bran represents about 15% by weight of wholegrain meal, the remainder being endosperm (about 83%) and the germ (about 2%) (Osborne and Mendel, 1919; Belitz et al., 2004). Although bread from refined wheat represents an important fraction of the bread market, the consumption of wholegrain bread is recommended by national authorities worldwide as part of a healthy human diet (Slavin, 2003, Lang and Jebb, 2003; Seal, 2006). A relevant percentage of consumers tend to consciously consume mainly wholegrain bread. Based on USDA, CSFII (Smiciklas-Wright et al., 2002) data, the mean and 90th percentile whole grain and "wheat" bread consumption in the United States is 61 and 105 g/day. Assuming that 15% of the wholegrain bread contains bran, the mean and 90th percentile estimated bran exposures will be 9 and 16 g/day. Other important sources of wheat bran exposure are bran-enriched ready-to-eat cereal products. A popular example of such product category is All-Bran™ Plus from The Kellogg Company, which contains 87% wheat bran[3]. The recommended serving of this product is 40 g, and hence contains 35 g of wheat bran.

[3] Ingredients: Wheat Bran (86%), Sugar, Barley Malt Flavouring, Glucose-Fructose Syrup, Salt, Niacin, Iron, Vitamin B6, Riboflavin (B2), Thiamin (B1), Folic Acid, Vitamin D, Vitamin B12 (http://www kelloggs.co.uk/products/allbran/cereal/all_bran aspx)

1.6.2. Background exposure to Arabinoxylan and AXOS

Cereal grains are the main source of arabinoxylan in the human diet. For instance, the arabinoxylan content in whole wheat grains varies between 4 and 8% (Hashimoto et al., 1987; Hong et al., 1989; Saulnier et al., 1995), in whole barley grains between 4% and 10% (Henry, 1987; Oscarsson et al., 1996; Andersson et al., 1999), in whole rye grains between 8 and 10% (Henry, 1987), and in whole oat grains around 6% (Henry, 1987). In refined (endosperm) flour, the arabinoxylan content is on average 1.9% for a range of wheat cultivars (Gebruers et al., 2008) and 3.6% for a range of rye cultivars (Nystrom et al., 2008). Arabinoxylan occurs in a wide range of cereal-based food and beverage products, such as bread, pastry, pasta, cookies, crackers, pancakes, waffles, ready-to-eat cereals, cereal bars, and beer. Through their arabinoxylan content these products can be considered as indirect sources of AXOS. Indeed, based on experiments in a pig animal model fed whole wheat (Bach-Knudsen et al., 1991) or whole rye (Glitso et al., 1999) it is estimated that in mammals the major part, more than 60%, of the ingested arabinoxylans are fermented by the intestinal flora (Grootaert et al., 2007). As for other carbohydrate fermentation processes the end products of arabinoxylan fermentation are short chain fatty acids (Grasten et al., 2003). Several microbial species occurring in the intestines of animals and humans have been shown to produce endoxylanases[4] as well as other arabinoxylan degrading enzymes such as xylosidases, α-L-arabinofuranosidases, feruloyl esterases, acetyl esterases, and α-glucuronidases (Grootaert et al., 2007). The concerted action of these different arabinoxylan degrading enzymes results in the generation of a polydisperse mixture of arabinoxylan degradation products, including AXOS, within the lumen of the large intestine. Thus it is reasonable to expect that human beings have been exposed to AXOS as a result of the colonic degradation and of arabinoxylan.

Nevertheless, it should be noted that as for dietary fibers the breakdown and fermentation of arabinoxylans vary according to their physicochemical characteristics. Water soluble arabinoxylans are more readily degraded and fermented than water-unextractable arabinoxylans (Glitso et al., 1999). The fermentation of arabinoxylans is further influenced by the degree of ferulic acid cross-linking (Hopkins et al., 2003) and their molecular weight (Hughes et al., 2007). Comparison of the *in vitro* fermentation by human fecal microflora of three arabinoxylan preparations with average molecular masses of 354, 278 and 66 kDa, respectively, indicated that the prebiotic effect increased as the molecular mass decreased, with the 66kDa arabinoxylan material having a prebiotic index, which exceeded that of inulin (Hughes et al., 2007). These findings were supported by Vardakou et al. (2008), who observed that in a comparable *in vitro* system the prebiotic activity of water-unextractable arabinoxylan was significantly enhanced by pre-treatment of this arabinoxylan with a xylanase. Hughes et al. (2007) attributed this higher prebiotic effect of low molecular weight arabinoxylan to the fact that per unit of mass the shorter polysaccharides have more non-reducing ends, which are susceptible to attack by the exo-enzymes produced by the colonic bacteria than higher molecular mass arabinoxylan.

Considering the importance of the physicochemical properties of arabinoxylans with respect to their intestinal breakdown and fermentation, it is important to take these properties into account when assessing the background exposure to arabinoxylan in view of the

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[4] The predominant endoxylanase-producing microbiota in the human colon include species of the Gram-negative genus Bacteroides (relatives of *B ovatus, B intestinalis* and *B dorei*) and of the Gram-positive genus *Roseburia* (relatives of *R. intestinalis*, belonging to the Roseburia/Eubacterium rectale group) (Chassard et al , 2007).

introduction of Wheat Bran Extract. In this context the existing exposure to the highly water soluble, low molecular weight arabinoxylans in bread (average MW between 13,200 and 39,600) and beer (average MW between 2640 and 5280) seem particularly relevant. In the same way as Wheat Bran Extract AXOS, the bread and beer low molecular weight arabinoxylans are obtained by exposing cereal arabinoxylans to exogenous endoxylanases (see section on hemicellulase). Furthermore, in light of Hughes et al. (2007) their molecular weights allow to assume that they also have certain prebiotic effects. Therefore, the effects and digestive fate of bread and beer low molecular weight arabinoxylans is anticipated to be comparable to that of Wheat Bran Extract AXOS.

The amount of dietary arabinoxylan consumed in the US was estimated based on data for consumption of ready-to-eat cereals reported in USDA, CSFII data (Smiciklas-Wright et al., 2002). The average arabinoxylan content of refined cereals is reported as 1.9% (Gebruers et al., 2008) and 6.5% in wholegrain cereals (Hashimoto et al., 1987; Hong et al., 1989; Saulnier et al., 1995). The mean and 90th percentile intakes of ready-to-eat cereals (may include refined and wholegrain cereals) were 56 and 104 g/person/day, respectively. Thus considering 1.9% as the average content of arabinoxylan in cereals, the mean and 90th percentile intakes of arabinoxylan from its presence in ready-to-eat cereals are estimated as 1.1 and 2.0 g/person/day, respectively. The estimated mean and 90th percentile intakes of arabinoxylan from whole grain ready-to-eat cereals will be 3.6 and 6.8 g/person/day, respectively.

An important category within the cereal products is bread. The routine use of endoxylanases in bread preparations results in the formation of considerable amounts of water soluble low molecular weight (LMW) arabinoxylans (avDP 100 to 300). Addition of endoxylanases at optimal doses during bread-making has the desirable effect of increasing the viscosity and elasticity of the dough, increasing loaf volume after baking, and resulting in a softer crumb structure (McCleary, 1986; Rouau et al., 1994; Courtin and Delcour, 2002). The LMW arabinoxylan content in bread has been characterized for different bread types by Van Haesendonck et al. (2008).

In an attempt to determine the background intake of AXOS or AXOS like compounds (the primary constituent of Wheat Bran Extract), from dietary sources, Fugeia NV performed extensive analyses to estimate the daily human exposure to LMW arabinoxylan.

In these experiments, breads with different cereal flour compositions were prepared with and without addition of commercial endoxylanase preparations at a dose of 0.12 g/kg flour. This dose is in the range of doses recommended by manufacturers of endoxylanases for breadmaking[5]. The total arabinoxylan content, LMW arabinoxylan content and average DP of the LMW arabinoxylan, were determined using the same methodology as used for the characterization of Wheat Bran Extract and reported by Van Haesendonck et al. (2008). These analyses revealed the average LMW arabinoxylan content in bread as 1.32 g/100 g dry matter in refined wheat flour bread and 2.64 g/100 g dry matter for 70/30 rye/wheat flour bread. Based on these analyses, the current average daily intake rates of LMW arabinoxylan from bread consumption ranges from 0.5 g/capita/day for the United Kingdom to 1.7 g/capita/day for Poland for consumers of refined

[5] Novozymes, one of the largest commercial producers of bakery enzymes, recommends the following doses for the following endoxylanase-containing bread improvement products· 0.02-0 12 g/kg flour for Pentopan Mono BG; and 0.02-0.18 g/kg flour for Pentopan 500 BG (Novozymes, Cereal Food Application Sheet. Dough Conditioning 2003-33195-04).

wheat flour bread, and from 0.9 g/capita/day for the United Kingdom to 3.5 g/capita/day for Poland for consumers of rye/wheat flour mix bread. Daily average intake of LMW arabinoxylan from bread in European countries is estimated to range from 0.5 to 3.5 g. In the US, the mean and 90th percentile intakes of Total Yeast Bread (includes total white bread plus total whole grain and "wheat" bread) were reported as 79 and 145 g/day, respectively (Smiciklas-Wright et al., 2002). Assuming that LMW arabinoxylan in bread ranges from 1.32 (wheat bread) to 2.64 (rye bread) g/100 g, the daily mean and 90th percentile intakes of LMW arabinoxylan in the US will range from 1.04 to 3.83 g/day.

As beer is made from wholegrain cereals as the primary starting ingredient, it is a prominent source of LMW arabinoxylan in the human diet. During the mashing process for beer production, the arabinoxylans of the cereals become substrates for endoxylanases (naturally present in cereal or added externally to reduce arabinoxylan-mediated viscosity of wort), resulting in conversion of at least a part of the arabinoxylans to LMW arabinoxylan. The LMW arabinoxylan content in beer ranged from 0.49 g/L in an American light beer to 1.90 g/L in a strong ale (Courtin et al., 2009a). In the US, mean and 90th percentile beer intake is reported as 1035 and 2131 g/day, respectively. Assuming that LMW arabinoxylan in beer ranges from 0.49 to 1.90 g/L g, the daily mean and 90th percentile intakes of LMW arabinoxylan from beer will range from 0.51 to 4.05 g/day. Thus, the estimated intake from consumption of bread and beer will result in the likely intake of LMW arabinoxylan ranging from 1.55 to 7.88 g/person/day.

1.6.3. Summary of Background Intake of AXOS or AXOS-like compounds

The pre-existing consumption of arabinoxylan can be estimated from the intake of cereal products. The estimated mean and 90th percentile intakes of arabinoxylan from whole grain ready-to-eat cereals were determined as 3.6 and 6.8 g/person/day, respectively. This pre-existing arabinoxylan intake to a certain extent constitutes an indirect exposure to AXOS, given that the major part of the ingested arabinoxylan is broken down by the intestinal microbiota, involving the formation of AXOS (Grootaert et al., 2007). A rough estimate of the pre-existing consumption of LMW arabinoxylan is obtained by summing the calculated values for LMW arabinoxylan consumption through beer and bread. The daily mean and 90th percentile intakes of LMW arabinoxylan from the consumption of bread in the US are estimated to range from 1.04 to 3.83 g/day. Similarly, the daily mean and 90th percentile intakes of LMW arabinoxylan from the consumption of beer are estimated to range from 0.51 to 4.05 g/day. From the combined consumption of beer and bread, the mean and 90th percentile intakes of LMW arabinoxylan are estimated to range from 1.55 to 7.88 g/consumer/day.

1.6.4. Background Exposure to β-Glucan

No specific numbers are available on the pre-existing exposure to cereal β-glucans. However, per 21 CFR 101.81, substantiation of health claims for soluble fiber from certain foods and risk of coronary heart disease requires the daily intake of 3 g or more per day of β-glucan soluble fiber from either whole oats or barley, or a combination of whole oats and barley. Therefore, it can be estimated that a diet aiming to reduce the risk of coronary heart disease provides at least 3 g β-glucan/day. Given that oats and barley have a β-glucan content of on average 5 and 7%, respectively (Peterson et al., 1995; Oscarsson et al., 1996; Izydorczyk and Dexter, 2008), it can be calculated that a serving of 50 g wholegrain oat or barley provides 2.5 and 3.5 g β-glucan, respectively.

1.7. Intended Use Levels and Food Categories

Fugeia NV intends to use Wheat Bran Extract as a food ingredient at levels up to 3.2 g/serving in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods. Although some foods with standards of identity are included in the list of foods, the use of Wheat Bran Extract is intended for foods without a standard of identity. Estimates of possible daily intake from the intended use levels of Wheat Bran Extract have been determined using the CSFII 2003-04 (USDA, 2005) database of the food product. Based on the maximum proposed use levels of Wheat Bran Extract, the resulting daily intake at the mean and 90th percentile levels of Wheat Bran Extract have been determined.

1.7.1. Estimated Daily Intake from the Intended Uses

Based on the exposure estimates using the CSFII 2003-04 database (Appendix II), approximately 84% of the total U.S. population were identified as consumers of Wheat Bran Extract (6,951 actual users identified) (USDA, 2005). While infants are included in the intake determinations, Wheat Bran Extract is not intended to be used in products such as baby foods or infant formula that are specifically marketed for use by infants. A summary of the estimated daily intake of Wheat Bran Extract from the intended food categories is presented in Table 3. Consumption of types of food categories intended for addition of Wheat Bran Extract by the total U.S. population resulted in estimated mean all-person and all-user intakes of Wheat Bran Extract of 4.2 g/person/day (0.083 g/kg bw/day) and 5.0 g/person/day (0.10 g/kg bw/day), respectively. The 90th percentile all-person and all-user intakes of Wheat Bran Extract from all intended food-uses by the total population were 9.4 g/person/day (0.20 g/kg body weight/day) and 10.1 g/person/day (0.22 g/kg body weight/day), respectively. For details of the consumption analysis, please see appendix II. The FDA commonly uses the estimated daily intake for the 90th percentile consumer of a food additive as a measure of high chronic dietary intake. Hence, for the safety determinations, the resulting 90th percentile intake of Wheat Bran Extract (10.10 g/person/day) from its intended uses in the above described food categories is considered.

Table 3. Intake of Wheat Bran Extract (g/person/day) based on USDA data*

Population Group	Age Group (Years)	% Users	Actual # of Users	All-Person Consumption (g)		All-Users Consumption (g)	
				Mean	90th Percentile	Mean	90th Percentile
Infants**	0 to 2	69.7	648	3 0	6.7	3 8	7.0
Children	3 to 11	96.4	1,241	5.0	9.4	5 2	9 5
Female Teenagers	12 to 19	86.9	862	4 0	8.9	4.6	9.8
Male Teenagers	12 to 19	84.1	840	4 6	10.1	5.4	11 0
Female Adults	20 and Up	86.0	1,831	4.1	9.1	4 7	9 6
Male Adults	20 and Up	79.3	1,529	4 3	10.4	5.4	11.4
Total Population	**All Ages**	**84.1**	**6,951**	**4.2**	**9.4**	**5.0**	**10.1**

*Detailed analysis and additional explanation is provided in Appendix II, **While infants are included in these estimates, Wheat Bran Extract is not intended for inclusion in infant formula or baby foods

1.8. Summary of Consumption

The estimated daily intake of Wheat Bran Extract from its intended uses in the various food categories identified above was determined using the CSFII 2003-04 database (USDA, 2005). From these determinations, the high users only 90th percentile consumption value of Wheat Bran Extract as a food ingredient in different products [baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods] was considered as the estimated daily intake resulting from the intended use of Wheat Bran Extract. Based on these considerations, the intended uses of Wheat Bran Extract will result in daily 90th percentile exposure of 10.1 g/person (0.22 g/kg bw/day). Taking into account an average content of AXOS in Wheat Bran Extract of 78.6% (Table 2), this corresponds to an intake of 7.9 g AXOS/person/day. LMW arabinoxylans, which are similar to the primary component of Wheat Bran Extract, AXOS, are found in various foods, particularly bread and beer, and its background intake from diet at the mean and 90th percentile is estimated to range from 1.55 to 7.88 g/person/day.

2. SAFETY OF WHEAT BRAN EXTRACT

2.1. Common Knowledge of Safe Use

There is common knowledge of a long history of human consumption of wheat as a food. Wheat and other cereals have been reported to be gathered and consumed for over 19,000 years, while its domestication began about 9500 years ago in the Middle East (Tanno and Willcox, 2006). By 2000 B.C., cultivation and consumption of wheat had spread from the Middle East to North Africa, Europe, and large parts of Asia (Harris, 1996). Today, wheat is grown on more land area than any other crop on earth. Wheat is considered as staple food and approximately one-third of the world's population has been estimated to depend on this grain for their nourishment. Most grains, like wheat and oats, have a hard outer layer and when they are processed, the layer becomes a by-product called bran. Wheat bran is consumed, mainly as a part of wholegrain wheat foods, such as wholegrain wheat bread. Furthermore, isolated wheat bran is added as an ingredient in the production of a broad range of cereal-based food products, such as bread, muffins, cookies, and breakfast cereals, to increase their fiber content. A well-known example of the latter is the breakfast cereal product "All-bran® cereal" from Kellogg's, of which the major ingredient is wheat bran.

In recent years, the health benefits of wholegrain foods as part of the human diet are well recognized (Slavin, 2003, Lang and Jebb, 2003; Seal, 2006). Around the world, governmental and other agencies recommend consumption of wholegrain cereals over refined cereals. The USDA and the American Dietetic Association recommend consuming at least three servings of whole grain per day (Seal, 2006). Because bran makes up the difference between wholegrain and refined cereals products, it is clear that bran is generally recommenced as part of a healthy diet.

Cereals are known to contain about 5-10% of arabinoxylan, which along with cellulose, starch, and β-glucan constitute the most abundant cereal carbohydrates. In wheat bran, arabinoxylan is the main cell wall component per se. Wheat Bran Extract, the subject of this

GRAS determination is derived from wheat (*T. vulgare* Vill.) grain. Wheat Bran Extract mainly comprises xylo- and arabinoxylo-oligosaccharides derived from the arabinoxylan, the main fiber type found in wheat bran. It is composed of AXOS (>70%) and other bran-extracted poly- and oligosaccharides (~20%). The available evidence suggests that humans around the world have been exposed to these types of fibers for centuries. During the preparation of cereal-based food products such as bread and beer, LMW arabinoxylan are formed as a result of enzymatic degradation of arabinoxylan-containing cell wall material. Furthermore, these LMW arabinoxylan, including oligosaccharides, are formed as an intermediate product in the colonic fermentation of ingested arabinoxylan, which is naturally present in most cereal based foods, such as bread, pasta, and beer. Despite this widespread daily exposure to consumers, no adverse effects associated with these oligosaccharides have been reported, indicating that AXOS are well tolerated. Thus, the available evidence provides support for common knowledge of human exposure to the bran fiber oligosaccharides present in Wheat Bran Extract.

2.2. Biological Data

The safety determination of Wheat Bran Extract is based on the totality of available evidence, particularly from human observations, as well as experimental studies of Wheat Bran Extract and those conducted on similar dietary fibers in humans and animals. The human studies of Wheat Bran Extract were designed to evaluate its efficacy as a prebiotic dietary fiber. These studies are reviewed as part of the safety evaluation, since in addition to efficacy, relevant safety endpoints were included. The assessment of efficacy studies is limited to a review of the results related to safety and tolerability. Relevant biological and toxicological studies on Wheat Bran Extract and other similar dietary fibers are included in the following section to provide support for the conclusions reached in this determination.

2.2.1. Nutritional Effects

2.2.1.1. Equivalence to Other Dietary Fibers

As mentioned earlier, Wheat Bran Extract is principally composed of AXOS obtained by partial enzymatic hydrolysis of wheat bran arabinoxylan. Although the degree of polymerization may differ, these AXOS are comparable to arabinoxylan hydrolysis products present in food products such as bread and beer as well as to those formed in the gastrointestinal tract following ingestion of foods containing arabinoxylan. Cereal arabinoxylans are not digested in the stomach and small intestine, but are well fermented by the colonic microbiota (Holloway et al., 1980; Sandberg et al., 1981; Englyst and Cummings, 1985; Bach-Knudsen and Hansen, 1991; Glitso et al., 1999). This fermentation results in the conversion of the arabinoxylan into gases like H_2 and CO_2, and to the short chain fatty acids (SCFA) acetate, propionate, butyrate, and to a lesser extent lactate. The SCFA produced through microbial metabolism in the colon lumen are readily absorbed by the colon epithelial cells (McNeil et al., 1978; Ruppin et al., 1980; Wong et al., 2006). Inside the colonocytes, SCFA, and in particular butyrate, are metabolized to CO_2 by cellular oxidation and thereby represent a major energy source for the colon mucosa (Roediger, 1982). Propionate is in part transported via the blood to the liver where it can be used for gluconeogenesis and acetate is transported to various tissues that use it as an energy source (Wong et al., 2006). As such, the gastrointestinal processing of Wheat Bran Extract AXOS is comparable to that of the arabinoxylan material currently present in most cereal-containing foods, including bread, pasta, and beer (please see criterion 2 of Section 2.2.1.2. and Table 4). Similar to AXOS, the other polysaccharide of Wheat Bran Extract, β-glucans, are resistant to digestion and absorption in the upper gastrointestinal tract and are readily fermented in the colon resulting in the production of the same gases and SCFA's (McBurney, 1991; Queenan et al., 2007).

2.2.1.2. Equivalence to Prebiotic Oligosaccharides

Several non-digestible oligosaccharides and polysaccharides have been shown to act as prebiotic compounds, of which inulin, fructooligosaccharide (FOS) and galactooligosaccharide (GOS) are presently most widely used in food. The criteria for classifying a compound as a prebiotic have been established by Gibson et al. (2004) and are three-fold: 1) resistance to gastric acidity, to hydrolysis by mammalian enzymes, and to gastrointestinal absorption; 2) fermentation by intestinal microbiota; and 3) selective stimulation of the growth and/or activity of those intestinal bacteria that contribute to health and well-being. In a recent article, Broekaert et al. (2010) presented evidence that the main Wheat Bran Extract components, XOS and AXOS, fulfill all three criteria for prebiotics laid out by Gibson et al. (2004), and thus have nutritional effects similar to those of the most documented and most widely consumed prebiotics, i.e. inulin, FOS, and GOS (Roberfroid, 2007). The findings of Broekaert et al. (2010) with respect to the above mentioned criteria are summarized below:

Criterion 1 (resistance to gastric acidity, hydrolysis by mammalian enzymes, and to gastrointestinal absorption) is supported by following evidence.

- Both arabinoxylo- and XOS were found to be stable under pH and temperature conditions mimicking the gastric environment (Courtin et al., 2009b);
- The β-1,4-bond linking the xylose moieties that form the xylan backbone of AXOS resists enzymatic digestion in the upper gastrointestinal tract (Okazaki et al., 1991, Sanchez et al., 2009);
- A survey of all protein sequences deduced from the fully sequenced human genome for the presence of proteins that belong to glycoside hydrolase (GH) families known to contain either endoxylanases, xylosidases or arabinofuranosidases, revealed that enzymes allowing the degradation of AXOS are absent in humans (Broekaert et al., 2010);
- Studies in ileostomy patients (Holloway et al., 1980; Sandberg et al., 1981; Englyst and Cummings, 1985) and animal models (Bach-Knudsen and Hansen, 1991; Glitso et al., 1999) indicate that little if any digestion or absorption of cereal derived arabinoxylan occurs in the stomach and small intestine;
- Considering that the absorption of carbohydrates requires their degradation into monomers, the above implies that AXOS migrate unabsorbed through the small intestine (Broekaert et al., 2010).

Criterion 2 (fermentation by intestinal microbiota) is supported by following evidence.

- *In vivo* studies in a pig animal model showed that arabinoxylan, the source of Wheat Bran Extract AXOS, passed the stomach and the small intestine largely undegraded, while over 80% is fermented in the large intestine (caeco-colon) (Bach-Knudsen and Hansen, 1991; Glitso et al., 1999);
- *In vitro* studies demonstrated that AXOS, including wheat bran derived AXOS, are well fermented by the colonic microbiota and that their fermentation is associated with the formation SCFA (Kabel et al., 2002; Sanchez et al., 2009);
- The supplementation of rat diets with either XOS or AXOS, including wheat bran AXOS, was associated with increased levels of SCFA (see references in Table 4);

- The addition of wheat bran derived AXOS to a test meal significantly increased the cumulative breath H_2 excretion in human volunteers over a period of 10 hours. Cumulative breath H_2 excretion is considered a good marker for the occurrence of anaerobic microbial fermentation in the colon (Cloetens et al., 2008a). In a comparative test performed on human volunteers, wheat bran AXOS and a FOS/inulin mixture were found to stimulate breath H_2 excretion to the same extent (Cloetens, 2009);
- Intervention trials involving the administration of XOS (Na and Kim, 2007) and AXOS (Grasten et al., 2003) preparations to healthy human volunteers indicated that the ingestion of these oligosaccharides concurs with increased fecal SCFA concentrations.

Criterion 3 (selective stimulation of growth and/or activity of beneficial intestinal bacteria) are supported by following evidence:

- Several studies have demonstrated that different *Bifidobacterium* species, when grown in pure cultures, efficiently utilize XOS (Okazaki et al., 1990; Jaskari et al., 1998; Van Laere et al., 1999; Crittenden et al., 2002; Palframan et al., 2003; Moura et al., 2007) as well as wheat bran AXOS (Yamada et al., 1993) or barley AXOS (Van Laere et al., 1999). Other colonic bacterial species, including *E. coli* and *Clostridium* species, were not able to utilize AXOS (Yamada et al., 1993; Van Laere et al., 1999; Moura et al., 2007);
- *In vitro* batch culture fermentations of XOS (Rycroft et al., 2001) and AXOS derived from wheat arabinoxylan (Hughes et al., 2007), entailed a strong increase of Bifidobacteria;
- Eight independent intervention trials in rats revealed that both XOS and AXOS, including a Wheat Bran Extract that closely resembles wheat bran-derived AXOS preparation, have pronounced bifidogenic effects (Table 4). In one rat trial, wheat bran derived AXOS was tested in parallel with XOS, inulin and FOS and all these oligosaccharide preparations caused a similar increase in the number of Bifidobacteria in the caecum (Van Craeyveld et al., 2008);
- The stimulating effect of the ingestion of XOS preparations on counts of fecal Bifidobacteria has been confirmed in four independent human intervention studies (Okazaki et al., 1990; Kajihara et al., 2000; Chung et al., 2007; Na and Kim, 2007).
- An increase of the number of fecal Bifidobacteria following the intake of Wheat Bran Extract that closely resembles wheat bran derived AXOS preparations was seen in two independent experiments involving healthy human volunteers (Cloetens et al., 2009a, 2010).
- The administration of wheat bran derived AXOS, which closely resembles with Wheat Bran Extract, to human volunteers was associated with a decrease of urinary excretion of nitrogen, *p*-phenol and *p*-cresol and an increase of fecal nitrogen excretion, all being indicative of a suppression of the colonic protein fermentation (Cloetens et al., 2008a, 2009a, 2010). In a cross-over study on human volunteers, wheat bran derived AXOS and a FOS/inulin mixture were found to lower urinary nitrogen excretion and raise fecal nitrogen excretion to the same extent at equal doses (Cloetens, 2009). These effects are generally considered to be beneficial for health given that several metabolites of colonic protein fermentation have been associated with the pathogenesis of colon and bladder cancer (Bone et al., 1976; Johnson, 1977; Visek, 1978) and in the exacerbation of diseases such as ulcerative colitis (Ramakrishna et al., 1991). In support of these suggested protective effects, experiments in a rat model indicate that the supplementation of the diet with Wheat Bran Extract is protective against chemically induced preneoplastic lesion in the colon (Femia et al., 2009).

The above described findings from several lines of evidence demonstrate that Wheat Bran Extract possesses typical characteristics of a dietary fiber and is equivalent to other prebiotic oligosaccharides.

Table 4. Overview of the different animal and human intervention studies demonstrating prebiotic activity of AXOS and XOS.

Tested compound	Test species	Specification of test population	Observed effect	Specification of test compound	Source of test compound	Level in diet (numbers in parentheses are corrected for AX purity)	Reference
AXOS	Rats	Not pretreated healthy rats	Increased caecal SCFA (acetate, propionate) Increased caecal Bifidobacteria Reduced caecal E coli and Staphylococcus	DP 3-6, A/X ratio 0 3	wheat bran	5% (4 3%) and 2 5 % (2 15%) in regular rat diet, 21 days	Yamada et al 1993
AXOS/XOS	Rats	Not pretreated healthy rats	Increased caecal Bifidobacteria Increased colonic SCFA (acetate, butyrate) Reduced ammonia levels in caecum Reduced branched SCFA levels in caecum (marker protein fermentation)	range of products with avDP 3 to 61, A/X ratio 0 09-0 69	wheat bran, wheat flour processing byproduct	4 6-5 5% (3 6-4 0%) in humanised rat diet, 14 days	Van Craeyveld et al 2008
AXOS	Mice	Not pretreated healthy mice	Increased caecal SCFA (acetate, propionate, butyrate) Increased caecal Bifidobacteria Reduced caecal E coli, Staphylococcus and Streptococcus	DP 3-6, A/X ratio 0 3	wheat bran	5% (4 3%) and 2 5 % (2 15%) in regular mice diet, 21 days	Yamada et al 1993
AXOS	Chickens	Not pretreated healthy chickens	Increased caecal Bifidobacteria	avDP 15, A/X ratio 0 27	wheat bran	0 35% (0 25%) in wheat/corn-based broiler diet, 14 days	Courtin et al 2008a
AXOS	Chickens	Not pretreated healthy chickens	Increased caecal Bifidobacteria	avDP 9, A/X ratio 0 34	wheat bran	0 25% (0 17%) in wheat/corn-based broiler diet, 14 days	Courtin et al 2008b
AXOS	Chickens	Not pretreated healthy chickens	Reduction of Salmonella Enteritidis colonization of caecum after oral infection Reduced translocation to the spleen	avDP 9, A/X ratio 0 25, avDP 3, A/X ratio 0 25	wheat bran	0 25 - 0 66% (0 20-0 40%), 37 days	Eeckhaut et al 2008
AXOS	Humans	14 healthy subjects	Increased faecal SCFA (acetate, propionate, butyrate)	avDP and A/X ratio not specified	wheat flour processing byproduct	15 g (purity not specified) in bread during 19-22 days	Grasten et al 2003
AXOS	Humans	12 healthy subjects	Decreased ammonium biomarker secretion via urine Increased ammonium biomarker secretion via faeces	avDP 15, A/X ratio 0 27	wheat bran	2 2 g, 1 day	Cloetens et al 2008a
AXOS	Humans	13+11 healthy subjects (24±5 years)	Increased faecal Bifidobacteria Decreased ammonium biomarker secretion via urine Increased ammonium biomarker secretion via faeces	avDP 9, A/X ratio 0 26	wheat bran	8 7 g (5 0 g) and 3 9 g (2 2 g) per day in water, 14 days	Cloetens et al 2010
AXOS	Humans	20 healthy subjects (24±5 years)	Increased faecal Bifidobacteria, increased faecal lactobacilli Decreased urinary p-cresol	avDP 6, A/X ratio 0 26	wheat bran	13 9 g (10 g) per day in orange juice, 21 days	Cloetens et al 2009

Table 4. Overview of the different animal and human intervention studies demonstrating prebiotic activity of AXOS and XOS (continued).

Tested compound	Test species	Specification of test population	Observed effect	Specification of test compound	Source of test compound	Level in diet (numbers in parentheses are corrected for AX purity)	Reference
XOS	Rats	Rats treated with diabetes-inducing drug (streptozotocin)	Increased caecal SCFA (acetate)	>70% DP2 and DP3	birch wood	10% (7 5%) in regular rat diet, 35 days	Imaizumi et al 1991
XOS	Rats	Not pretreated healthy rats	Increased caecal SCFA (acetate, propionate, butyrate and lactate) Increased caecal and faecal Bifidobacteria Increased caecal and faecal total anaerobic bacteria	>80% DP2 and DP3	corn cobs	6% (5 4%) in regular rat diet, 14 days	Campbell et al 1997
XOS	Rats	Wild type rats treated colon cancer promoting drug (1,2-dimethylhydrazine)	Increased caecal Bifidobacteria Reduced caecal E coli	>80% DP2 and DP3	corn cobs	6% (5 4%) in regular rat diet, 35 days	Hsu et al 2004
XOS	Mice	Not pretreated healthy mice	Increased Bifidobacteria, Lactobacilli, Bacteroides and total anaerobes in the colon Increased Bifidobacteria in the ileum Reduced Enterobacteriaceae and sulphite-reducing bacteria in the colon	>80% DP2 and DP3	corn cobs	1 0 % (0 95%) in regular mice diet, 6 months	Santos et al 2006
XOS	Humans	9 healthy subjects, between 50 and 60 years of age	Increased Bifidobacteria in faeces No change in total faecal bacteria Increased SCFA levels in faeces	>70% DP2 and DP3	birch wood	5 g (3 9 g) per day, 3 weeks	Okazaki et al 1990
XOS	Humans	14 subjects with hepatic cirrhosis with mild hyperammonaemia	Increased Bifidobacteria in faeces	>65% DP2 and DP3	corn cobs	3 g (2 8 g) per day	Kiso et al 2001, Kajihara et al 2000
XOS	Humans	13 eldery persons, average age 77	Increased Bifidobacteria in faeces	>80% DP2 and DP3	corn cobs	4 0 g (3 8 g) per day during 21 days	Chung et al 2007
XOS	Humans	14 healthy young women, between 23 and 26 years	Increased SCFA (lactate) level in faeces Increased Bifidobacteria in faeces	avDP not specified	corn cobs	1 4 g (1 3 g) or 2 8 g (2 7 g) per day during 28 days	Na and Kim, 2007
XOS	Humans	26 type-2 diabetes patients, 19 men and 7 women	Decreased plasma levels of glucose, HbA1c, fructosamine, total cholesterol, LDL cholesterol, oxidized LDL and apolipoprotein B	>80% DP2 and DP3	corn cobs	4 g (3 8 g) per day during 8 weeks	Shue et al , 2008

2.2.2. Effects on Digestive Physiology and Nutrient Bioavailability

Although certain indigestible carbohydrates have been associated with altered gastrointestinal transit and modified bioavailability of minerals, as discussed below no adverse impact on either digestive physiology or nutrient bioavailability has been observed for Wheat Bran Extract at recommended levels of daily intake.

2.2.2.1. Digestive Physiology

Certain indigestible carbohydrates are known to alter the rate and extent of digestion and absorption of other nutrients. These effects are related to their physicochemical properties such as viscosity, water-holding capacity and osmolarity (Malkki, 2001). For example, the disaccharide lactulose is known to accelerate oro-caecal transit time (OCTT), which is attributed to its osmotic effect. For this reason, it is used for the treatment of constipation. A shorter OCTT can lead to a shorter contact time of food with digestive enzymes and a lower degree of digestion (Holgate and Read, 1983). Water-holding polysaccharides increase the viscosity of the intestinal content, which might interfere with mixing of the intestinal contents and the diffusion of nutrients towards the epithelial cells. In addition, viscous indigestible carbohydrates have been shown to prolong gastric emptying (GE) resulting in an overall slower rate of digestion (Sandhu et al., 1987; Darwiche, 2003).

The effect of a Wheat Bran Extract that closely resembles wheat bran-derived AXOS preparation on gastrointestinal transit and digestion was assessed in two studies performed on healthy human volunteers. The results of these studies show that overall AXOS has no impact on transit or on digestion of proteins or lipids. The first study (Cloetens et al., 2008a) involved 12 subjects receiving 5 pancake-based test meals containing radio-labeled markers for measuring gastrointestinal motility together with different amounts of AXOS (either 0, 0.2, 0.7, 2.2, or 4.9 g), with 1 week intervals between each of the 5 test meals. The AXOS added to the test meals had an average DP of 15 and an A/X ratio of 0.26, and was isolated from wheat bran in a similar way as Wheat Bran Extract. None of the tested doses of up to 4.9 g AXOS had any significant effect on GE, OCTT or oro-anal transit time (OATT) (Cloetens et al., 2008a). These experiments were performed with an early batch of Wheat Bran Extract. Subsequently the production method has further optimized resulting in a lower average degree of polymerization. Given the similarity, the results of these investigations are applicable.

In the second study (Cloetens et al., 2008b), 10 subjects received 4 test meals, each separated by a 1 week interval: a pancake meal without AXOS, a pancake meal with 2.2 g AXOS (the same as the Wheat Bran Extract that closely resembles wheat bran-derived AXOS preparation as described in the above mentioned study), a white bread with chocolate paste meal without AXOS, and a white bread with chocolate paste meal with 2.2 g AXOS. The meals further contained a radio-labeled marker for measuring GE and OCTT by breath tests. AXOS had no effect on either GE or on OCTT of the pancake meal, nor any effect on OCTT from the white bread with chocolate meal. AXOS administration caused a statistically significant acceleration of the GE of the white bread with chocolate meal. However, the authors argued that this observation could be explained by a type I statistical error associated with the high intra-individual day-to-day variability of GE. The apparent lack of impact on gastrointestinal motility is consistent with the fact that AXOS from wheat bran prepared by enzymatic extraction has a low intrinsic viscosity. The intrinsic viscosity of the AXOS preparation used in the study by Cloetens et al. (2008b) amounts to 0.52 dL/g which is higher than that of oligofructose (0.04 dL/g), but clearly lower than that of native high molecular weight arabinoxylans (2.81-4.23 dL/g) (Cloetens et al., 2008b). The viscosity of Wheat Bran Extract preparations was 0.12 dL/g.

The absence of any clear effects of Wheat Bran Extract on the OTTC is consistent with its relatively low osmotic potential. The water activity of a Wheat Bran Extract solution (Aw 0.976 at 50% w/w) was shown to be markedly higher than that of a lactulose solution (Aw 0.936 at 50% w/w)[6]. Furthermore, the water activity of Wheat Bran Extract was shown to be intermediate between that of FOS[7] (Aw 0.963 at 50% w/w) and inulin[8] (Aw 0.995 at 50% w/w), which are both widely used prebiotic food ingredients.

In the above mentioned study by Cloetens et al. (2008b), the effect of Wheat Bran Extract that closely resembles wheat bran-derived AXOS preparation on protein and lipid digestion was also investigated. The pancake test meals contained ^{13}C-labelled leucine incorporated in egg white and yolk protein digestion was measured through the kinetics of $^{13}CO_2$ appearance in breath tests by isotope ratio mass spectrometry. The chocolate paste in the white bread with chocolate paste test meals contained ^{13}C-labelled triglycerides, and lipid digestion was likewise assessed through breath tests. The investigators concluded from these experiments that AXOS has no overall impact on the digestion of proteins or lipids (Cloetens et al., 2008b).

2.2.2.2. Nutrient Bioavailability

In a randomized placebo-controlled cross-over trial involving twenty healthy volunteers, Cloetens et al. (2009a) assessed the effects of intake of AXOS on plasma levels of minerals and vitamins. The study involved two 3 week intake periods with a four week wash out period in between. During the intake periods the volunteers consumed either 13.9 g/day of Wheat Bran Extract or 13.9 g/day of maltodextrins, both dissolved in orange juice. During the study, the subjects were allowed to eat their usual diets but were asked to have a regular eating pattern (3 meals/day) and to avoid food products containing probiotics and/or prebiotics. Blood samples were collected before and after the 3-week Wheat Bran Extract and placebo intake periods. The plasma levels of sodium, calcium, magnesium, iron, chloride, bicarbonate remained unchanged after intake for 3 weeks of either 13.9 g/day of Wheat Bran Extract or placebo. There was a slight but statistically significant decrease in plasma potassium levels after Wheat Bran Extract intake prior to baseline, but the potassium level after Wheat Bran Extract intake was almost the same as before the placebo intake (Table 5). The potassium level was within the normal range and therefore the observed change is considered not to be biologically relevant.

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[6] Based on the water activity measurement of 50% solutions of Wheat Bran Extract, FOS, lactulose and inulin.
[7] Actilight, Syral - Beghin Meiji, France
[8] Fluka, Buchs, Switzerland, catalogue number 57610

Cloetens et al. (2009a) also measured levels of vitamin A, as representative of lipophilic vitamins levels, and vitamin B_9 (folate), as representative of hydrophilic vitamins, in blood plasma collected before and after the 3-week Wheat Bran Extract and placebo intake period. No statistically significant changes were observed after either Wheat Bran Extract or placebo intake (Table 5). The investigators concluded that a three week administration of 13.9 g/day of Wheat Bran Extract (corresponding to 10 g/day of AXOS) did not affect plasma vitamin and mineral levels. The lack of effect of Wheat Bran Extract on mineral and vitamin status is in line with the conclusion from numerous studies that consumption of increased levels of dietary fiber at recommended levels has no adverse effect on the vitamin and mineral absorption in humans (Kelsay, 1990; Gordon et al., 1995; Gorman and Bowman, 1993; Rossander et al., 1992).

It is known that wheat bran and other fiber-rich food products with a high phytic acid content can reduce the bioavailability of minerals such as iron, calcium, and zinc (Sandstead, 1992). Wheat Bran Extract has a very low phytic acid content (below the detection limit of 0.1%) and therefore no phytic acid related effects on mineral bioavailability would be expected.

In addition, the scientific literature suggests that the predictable effects on mineral and vitamin bioavailability of Wheat Bran Extract are beneficial rather than adverse. Lopez et al. (1999) reported that addition of arabinoxylan (8%) to a rat diet enhanced the uptake of calcium and magnesium in the caecum and improved the magnesium balance in rats. Furthermore, it is well documented that prebiotics, in particular FOS and inulin, stimulate the uptake of dietary calcium and magnesium (Weaver, 2005; Scholz-Ahrens et al., 2007) and that this increased absorption occurs primarily at the level of the colon as a result of enhanced SCFA production (Abrams et al., 2007). It has also been suggested that prebiotics stimulate the gastrointestinal production of vitamins. Indeed, Thoma et al. (2003) demonstrated that plasma vitamin B_{12} concentrations are significantly increased in rats after administration of FOS. The underlying mechanisms for the stimulation of vitamins by prebiotics have not yet been fully elucidated, but could be related to the stimulated capacity of certain *Bifidobacteria* to biosynthesize vitamins (Pompei et al., 2007).

Table 5: Influence of Wheat Bran Extract and placebo on plasma mineral and vitamin levels (n=20, median and IQR, Wilcoxon test, p=0.05). Wheat Bran Extract was given at 13.9 g/day during 3 weeks.

	before WBE	after 3w WBE	before placebo	after 3w placebo
K (mmol/l)	3 98 (3 80-4 24)	3 75 (3 51-3 96) p=0 013	3 78 (3 66-3 98)	3 86 (3 65-4 01)
Na (mmol/l)	139 6 (138 9-141 1)	140 1 (138 7-141 4)	140 4 (138 5-141 4)	139 9 (138 2-141 6)
Ca (mg/dl)	9 10 (8 93-9.40)	9.30 (9.06-9.53)	9.26 (8 97-9.39)	9.26 (9 07-9.55)
Mg (mg/dl)	2 07 (1.99-2.14)	2.07 (2.03-2.22)	2 00 (1 94-2.10)	2 06 (2 00-2 11)
Fe (µg/dl)	84 50 (58 25-122 0)	93 00 (78 25-123.0)	80.50 (61.25-112 3)	93 5 (70.25-115 5)
Cl (mmol/l)	105 3 (103 9-106 1)	105 2 (103 0-106 1)	105 3 (104 3-106 6)	105.9 (103 8-107 3)
Bicarbonate (mmol/l)	24 25 (23 48-25 33)	24 70 (23 50-27 25)	23 55 (22 40-25 78)	23 95 (22 65-25 28)
Anion gap (mEq/l)	14 35 (13 40-15 35)	13.20 (12.30-14.98)	14.40 (13.05-15.40)	13.90 (12.78-15 63)
Vitamin A (µg/l)	607 0 (525.3-746 0)	645 5 (607 3-752 3)	605 0 (570 8-703 0)	700 0 (582 0-732.5)
Vitamin B_9 (µg/l)	8.95 (6 13-9 90)	8 90 (7 08-10 58)	9 60 (7.50-11 98)	9.55 (7 76-10 93)

WBE = Wheat Bran Extract; w = weeks; Adapted from Cloetens et al. (2009a)

2.2.3. Metabolism

The metabolic fate of the carbohydrate moiety of AXOS and of the β-glucans fully resembles that of other prebiotics and other non-digestible/fermentable carbohydrates such as inulin, FOS, galactooligosaccharides, resistant starch, polydextrose, cyclodextrins, and lactulose, which also remain largely undigested in the upper gastrointestinal tract and are fermented in the colon to gases and SCFA (Macfarlane et al., 2006). The metabolism of AXOS and β-glucans has been extensively studied both in *in vitro* and *in vivo* experiments. As indicated earlier, the carbohydrate moiety of these oligo/polysaccharides are resistant to digestion in the upper part of the gastrointestinal tract down to the distal ileum (Sanchez et al., 2009; Okazaki et al., 1991; McBurney, 1991, Queenan et al., 2007). However, in the colon they are fermented by the resident microbiota resulting in the formation of H_2 and CO_2 and SCFA (Kabel et al., 2002; Grootaert el al., 2007). The SCFA produced in the colon lumen are absorbed through the colon epithelial cells (McNeil et al., 1978; Ruppin et al., 1980; Wong et al., 2006) and are metabolized (particularly butyrate) to CO_2 by cellular oxidation and thereby represent a major energy source for the colon mucosa (Roediger, 1982). Among the SCFA, propionate is transported to the liver where it can be used for gluconeogenesis, while acetate is transported to various tissues that use it as a direct energy source (Wong et al., 2006).

AXOS from cereal bran also contains ester-bound ferulic acid. The metabolism of free ferulic acid (FA) and the FA moiety in feruloylated arabinoxylan has been studied in rats (Zhao et al., 2003). Free FA is rapidly absorbed from the gastrointestinal tract in rats, with a recovery in the urine of 72% either as FA or as one of the liver formed FA conjugates such as FA-glucuronide, FA-sulphate, and FA-sulfoglucuronide (Zhao et al., 2003, 2005). The absorption of FA that is ester-linked to arabinoxylans was found to be markedly lower than that of free FA, as it depends on the release of FA through the action of esterases produced by the intestinal microbiota (Adam et al., 2002). In rats fed a diet containing 26% wheat bran (corresponding to an intake of ester-linked FA of 4.04 mg/kg bw), FA conjugates (mainly in the form of FA-sulfoglucuronide) were detected in the plasma at a level of about 1 μM, and urinary excretion of FA conjugates (mainly in the form of FA-sulfate or FA-sulfoglucuronide) corresponded to only about 2.3% of the ingested amount of FA (Rondini et al., 2004). The lower bioavailability of arabinoxylan-linked FA was confirmed in several studies examining the metabolism of cereal-derived ferulic acid in humans (Bourne et al., 2000; Kern et al., 2003; Harder et al., 2004). The FA excreted in the urine of healthy volunteers over a 24 hours period amounted to only 3.1% of the total FA ingested through wheat bran (Kern et al., 2003), whereas it amounted to 28% from ingestion of rye bran (Harder et al., 2004). It can therefore be concluded that arabinoxylan-linked FA is mainly eliminated via the feces, with a minor fraction being absorbed. Following absorption, the FA is metabolized mainly by conjugation to form FA-glucuronide, FA-sulfate, and FA-sulfoglucuronide, which are then excreted via the urine.

Any healthy diet rich in plant-derived fiber that comprises sources of both fermentable fiber and ester-linked FA leads to generation, absorption and excretion of the same metabolites (H_2, CO_2, SCFA, FA and conjugated FA) as those produced upon digestion of Wheat Bran Extract. Therefore, the metabolism of Wheat Bran Extract does not raise safety concerns and no systemic toxicity is expected following ingestion of Wheat Bran Extract.

2.3. Toxicological Information

2.3.1. Studies of Wheat Bran

Given the long history of safe use of wheat bran in humans and analogous data from animal consumption, no formal toxicological evaluation of wheat bran has been performed to date. However, wheat bran has been tested in numerous intervention studies in animals, at dietary levels up to 25% (Jenab and Thompson, 1998), and no effects indicative of potential toxicity have been observed (Barbolt and Abraham, 1980; Reddy et al., 1981; Cheng et al., 1987; Alabaster et al., 1995; Jenab and Thompson, 1998; Lopez et al., 2000; Higuchi et al., 2007). In addition, several human intervention studies have been performed with wheat bran (Reddy et al., 1989; Reddy et al., 1992; Chen et al., 1998; Harder et al., 2004; Muir et al., 2004; Costabile et al., 2007). These studies did not report any adverse effects due to dietary inclusion of wheat bran. The only report of any adverse finding was in the case of a 53 year old woman with chronic constipation, who was also taking an antidepressant, where intestinal obstruction was reported following the consumption of 160 to 200 g of unprocessed bran per day (Kang and Doe, 1979). Recently, toxicological effects of Wheat Bran Extract in humans and animals have been investigated in well designed studies.

2.3.2. Short-term Toxicity Study

In a dose-range finding study, the short-term toxicity potential of Wheat Bran Extract was assessed in rats in a 14-day unpublished pilot study in rats (Francois et al. 2010; Kubaszky, 2009a). The objective of this study was to establish palatability, tolerance and suitable dose levels for a complete 90-day toxicity study. This range finding study was performed under non-GLP conditions according to range finding recommendations for 90-day studies described in OECD Guidelines.

The test involved 3 groups of ten Wistar rats (5/sex/group). The controls were fed a basal semi-synthetic diet AIN93G (Reeves, 1997), whereas the low and high dose groups received the AIN93G semi-synthetic diet fully balanced to contain 5 and 10% Wheat Bran Extract, respectively. The Wheat Bran Extract-supplemented diets were prepared by replacing part of the pre-gelatinized corn starch, the main component of the AIN93G semi synthetic diet, with Wheat Bran Extract. The Wheat Bran Extract used in this study was a representative sample of Batch A (Table 2). The results of this study showed that Wheat Bran Extract administered to male and female rats for 14 consecutive days was palatable, well tolerated, and devoid of overt toxicity up to the maximum practicable dietary inclusion level of 10%, corresponding to a mean intake of 6919 mg/kg bw/day. There was a statistically significant dose-related increase in caecal content weights in both male and female rats fed the Wheat Bran Extract-supplemented diets which is not considered an adverse effect but a physiological response seen in rats fed similar fermentable non-digestible oligosaccharides (Younes et al., 1995; Campbell et al., 1997; Hsu et al., 2004; Boyle et al., 2008).

Although no adverse effects were observed in the 10% dose group, a high dose level of 7.5% Wheat Bran Extract was selected as appropriate for the subsequent 90-day subchronic toxicity test, taking into account rodent-specific physiological adaptation to high doses of Wheat Bran Extract in the diet, such as caecal enlargement and recognizing that even at 7.5%, the absolute dosage of Wheat Bran Extract would amount to approximately 5000 mg/kg body weight/day, i.e. approximately 50-fold the median Wheat Bran Extract dose anticipated for a 60 kg person.

2.3.3. Subchronic Toxicity Study

The subchronic toxicity of Wheat Bran Extract was evaluated in rats in a full GLP 90-day study in accordance with the study plan and the OECD 408 Protocol described in the OECD Guidelines for Testing of Chemicals (Section 4: Health Effects. Repeated Dose 90-day Oral Toxicity Study in Rodents; No.: 408, 21st September 1998. OECD, Paris) (Francois et al., 2010; Kubaszky, 2009b- unpublished report). The test involved 6 groups of Crl:(WI) BR Wistar rats (10/sex/group). Three control groups were included. Control group 1 was fed the basal AIN93G semi-synthetic diet (Reeves, 1997), control group 2 was fed the AIN93G diet supplemented with 7.5% inulin (widely used as non-digestible fiber), and control group 3 was fed AIN93G supplemented with 7.5% bran (the source of Wheat Bran Extract). Wheat Bran Extract was administered at doses of 0.3% (group 4), 1.5% (group 5), and 7.5% (group 6) in the AIN93G diet for at least 90 consecutive days. The inulin, wheat bran and Wheat Bran Extract diets were prepared by substituting a corresponding amount of the starch in the AIN93G diet, while maintaining all other constituents constant in order to optimize intergroup comparability. The mean intake of Wheat Bran Extract over the treatment period for males and females respectively was 166 or 178 mg/kg/day (0.3% Wheat Bran Extract, group 4), 836 or 917 mg/kg/day (1.5% Wheat Bran Extract group 5), and 4138 or 4571 mg/kg/day (7.5% Wheat Bran Extract group 6), with a mean intake for the combined sexes at the high dose-level of 4354 mg/kg/day.

The feeding of diets containing 0.3%, 1.5% and 7.5% Wheat Bran Extract to rats for at least 90 days had no adverse effects in terms of clinical signs, behavior, general physical condition, reaction to stimuli, grip strength or motor activity. Mean body weight, body weight gain, food consumption and food conversion efficiency were also unaffected by the treatment (Francois et al., 2010). Water intake was increased slightly in the male Wheat Bran Extract-treated groups probably as a consequence of the high non-digestible carbohydrate intake considering that even higher water consumption was observed in the wheat bran-treated group. Hematology, urinalysis, ophthalmoscopy, and macroscopic examination of organs/tissues revealed no treatment-related effects. Organ weight analysis showed increased absolute and relative caecal weights in the inulin control group and all the Wheat Bran Extract-treated groups, as was also observed in the 14-day study (see Section 2.3.2.) and as commonly seen in rodents with diets containing high concentrations of fermentable fiber (Younes et al., 1995; Campbell et al., 1997; Hsu et al., 2004; Boyle et al., 2008). Blood clinical chemistry of the Wheat Bran Extract-treated groups was similar to that of the control high fiber diet group, containing either inulin (control group 2) or wheat bran (control group 3). Compared to control group 1, the Wheat Bran Extract groups, particularly at the upper dose levels, showed slightly higher values for calcium, potassium and phosphorous, and lower values for AST and alkaline phosphatase, and bile acids. The increased levels of calcium, potassium and phosphorus are consistent with earlier observations that the intake of fermentable non-digestible carbohydrates, such as soluble fibers, promotes the bioavailability of these electrolytes from the gut (Demigne et al., 1989; Scholz Ahrens et al., 2007; Scholz-Ahrens and Schrezenmeir, 2007). Further, the observed reduction in blood serum concentrations of the main liver marker enzymes taken together with unchanged serum total protein levels and reduced bile acids, indicates a potentially beneficial effect on liver homeostasis. These findings may be consistent with increased dietary fiber intake, as similar changes were seen in the control group 2 (7.5% inulin) and control group 3 (7.5% wheat bran). Histopathologic examination revealed minimal hypertrophy of renal cortical tubules in the high dose Wheat Bran Extract group, yet in the absence of any degenerative change, and seen in association with increased serum electrolytes, especially calcium, this effect was consistent with a physiologically adaptive response resulting from the presence of Wheat Bran Extract in the diet. In the absence of any adverse effects, the no-observed-adverse-effect-level (NOAEL) of Wheat Bran Extract under the conditions of this study was 7.5% in the diet, the highest dose tested, which is equivalent to a daily intake for the sexes combined of 4354 mg/kg bw/day.

2.3.4. Genotoxicity Studies

2.3.4.1. Mutagenicity

The mutagenic potential of Wheat Bran Extract was assessed using the Bacterial Reverse Mutation Assay, also known as the Ames test and was performed in accordance with the study plan, the ICH Guidance (S2A, 1995; S2B, 1997), EPA Health Effects Test Guidelines (OPPTS 870.5100, EPA 712-C-98-247 and EPA 712-C-96-247; 1998, 1996), and the OECD Guidelines for Testing of Chemicals (No. 471, 21 July 1997) (Francois et al., 2010- manuscript submitted to International Journal of Toxicology; Hargitai, 2009-unpublished report). Two independent experiments were carried out using histidine-requiring auxotrophic strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537), and the tryptophan-requiring auxotrophic strain of *Escherichia coli* (WP2*uvrA*). Strains TA100, TA1535 and WP2*uvrA*$^-$ are constructed to detect base-pair mutations, while strains TA98 and TA1537 allow detecting mutagens that cause base-pair substitutions. The experiments were performed either in the presence or the absence of metabolic activation by a post liver mitochondrial supernatant fraction (S9). The strain-specific positive control chemicals used were 4-nitro-1,2-phenylenediamine at 4 μg/plate for *S typhimurium* strain TA98, sodium azide at 2 μg/plate for strains TA100 and TA1535, 9-aminoacridine at 50 μg/plate for strain TA1537, methyl-methanesulfonate at 2 μl/plate for *E coli* strain WP2*uvrA*$^-$, 2-aminoanthracene at 2 μg/plate for all *S. typhimurium* strains and at 50 μg/plate for *E coli* strain WP2*uvrA*$^-$. 2-Aminoanthracene requires metabolic activation to exert mutagenicity, whereas the other positive control chemicals are mutagenic with or without metabolic activation.

The bacterial cells were exposed to Wheat Bran Extract at concentrations up to 5000 μg/plate. The increase in the number of revertant colonies at the tested Wheat Bran Extract concentrations were minor, not dose-related, and within the historical control range, while the reference mutagens (positive controls) showed the expected increase in induced revertant colonies. The results of these experiments demonstrate that Wheat Bran Extract has no mutagenic activity on the bacterial tester strains under the test conditions used.

2.3.4.2. Clastogenicity

The potential clastogenicity of Wheat Bran Extract was assessed using a chromosome aberration assay in V79 Chinese Hamster lung cells (Francois et al., 2010- manuscript submitted to International Journal of Toxicology; Beres, 2009- unpublished report). This GLP study was performed in accordance with the study plan, the OECD Guidelines for Testing of Chemicals (No.: 473, 21 July 1997), EPA Health Effects Test Guidelines (OPPTS 870.5375; EPA 712-C-98-223, August 1998), and ICH Guidances (S2A, 1996; S2B, 1997). A preliminary study was performed in the presence and absence of the S9 metabolic activation system in order to determine cytotoxic concentrations of Wheat Bran Extract on cultured V79 Chinese Hamster lung cells. Thereafter, well-spread metaphase Chinese Hamster lung cells were exposed to different concentrations of Wheat Bran Extract ranging between 1250 and 5000 µg/ml (the latter being a cytotoxic concentration causing less than 50% survival of cultured lung cells). None of the experiments revealed biologically significant increases in the number of cells with structural chromosome aberrations, while in the positive control groups (ethylmethane sulphonate at 0.4 and 1.0 µl/ml and N-nitrosodimethylamine at 1.0 µl/ml) the chromosome aberrations were within the historical range. The results of these experiments demonstrate that Wheat Bran Extract was not clastogenic in this system, under the conditions employed.

2.4. Human Studies

2.4.1. Tolerance Studies

In four separate human intervention studies described below, the effects of Wheat Bran Extract and/or very similar products at doses ranging from 3 to 13.9 g/day for up to 28 days were investigated (Cloetens et al., 2009a; 2009b; 2009c; Cloetens, 2009; Francois and Broekaert, 2010a- unpublished report; Francois and Broekaert, 2010b- unpublished report). In all of these studies, the applied Wheat Bran Extract doses were well tolerated and no adverse effects were reported. Grasten et al. (2003) described the effects of the administration in a bread matrix of 13 g/day of a readily fermentable LMW arabinoxylan preparation (avDP 58) which had been isolated from a wheat flour wet processing stream to healthy volunteers for 19-22 days. No adverse effects were observed and, except for mild symptoms of increased flatulence, no differences were observed in fecal frequency or stool consistency.

2.4.1.1. Placebo-controlled Cross-over Tolerance Study of Wheat Bran Extract in Soft Drink with Healthy Adult Volunteers

In a randomized placebo-controlled cross-over clinical trial aimed at evaluating the prebiotic effects of Wheat Bran Extract in healthy adult volunteers, tolerance to orally administered Wheat Bran Extract was investigated (Francois and Broekaert, 2010a- unpublished report). In this study, 66 volunteers were recruited with all 63 volunteers completing the trial (36 of age 18-50 and 27 of age 51-83; 34 males, 31 females). Volunteers were randomly assigned to six groups. The study comprised a run-in period of 1 week, followed by three intake periods of three weeks each with a wash-out period of 2 weeks between each treatment period. During the intake periods, the volunteers consumed twice daily non-carbonated soft drinks providing either no Wheat Bran Extract (placebo), 2 x 1.5 g/day of Wheat Bran Extract, or 2 x 5.0 g/day of Wheat Bran Extract.

The volunteers were randomly assigned to 6 approximately equally sized groups differing in the sequence order of the three treatments over the three intake periods. The Wheat Bran Extract used in this study was a sample of Batch A (Table 2). At study run-in, and after each intake period, venous blood samples were collected and analyzed for full hematological and clinical chemistry parameters. Throughout the study the volunteers were asked to fill out a questionnaire recording the occurrence of any gastrointestinal symptoms as well as the degree of discomfort associated therewith. Moreover, any adverse events, which occurred during the study, were recorded.

Safety was assessed using the emergent adverse events and any changes in clinical blood parameters. Adverse events were categorized in 11 categories according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (v3.0) (CTCAE, 2006) prior to unblinding of the study. Comparison of the frequency of the adverse events revealed no statistically significant difference between the WBE treatments and placebo for any of the different adverse event categories. In a further analysis, the occurrence of adverse shifts was analyzed for each of the 47 clinical blood parameters. For a given subject a shift in a blood parameter was considered to be adverse when the blood parameter value after treatment was either (i) outside the normal range while the corresponding baseline value of that subject was within the normal range, (ii) below the normal range while the corresponding baseline value was above the normal range or (iii) above the normal range while the corresponding baseline value was below the normal range. For none of the 47 clinical blood parameters a statistically significant difference was observed when comparing the occurrence of adverse shifts between the three treatments (placebo, 3 g/day of Wheat Bran Extract, or 10 g/day of Wheat Bran Extract) for each of the parameters.

Gastrointestinal symptoms were monitored weekly throughout the study. The volunteers were asked to grade all of the 18 following symptoms: diarrhea, constipation, painful bowel movement, blood in stool, abdominal pain, bloating, abdominal cramps, abdominal stretching, borborygmy, flatulence, burping, acid regurgitation, retrosternal burning, nausea, vomiting, indigestion, difficulty swallowing, and hoarseness/sore throat. Occurrence frequency of the symptoms was graded on a 5-step scale ranging from never, occasionally, frequently, nearly always, to always. Distress severity of the symptoms was graded on a 5-step scale ranging from no (0), minimal (1), mild (2), moderate (3) to severe (4). Statistical analysis of the per protocol population data indicated that Wheat Bran Extract intake had either no or a slight beneficial effect on either distress severity or occurrence frequency of the surveyed gastrointestinal symptoms, with the exception of flatulence. Compared to the placebo treatment, intake of Wheat Bran Extract caused a shift of the distress severity scores for flatulence from the non-disturbing category towards the categories of minimally disturbing and mildly disturbing. However, no increases were seen in the scores of the more severe flatulence disturbance categories. Further, the survey showed a trend toward a decreased perceived constipation at 10 g Wheat Bran Extract /day and lower occurrence of abdominal pain at 3 g Wheat Bran Extract /day; the latter only in the 18 to 50 yrs age subgroup.

During the run-in period and the last week of each intake period, defecation frequency as well as consistency of the stool according to the Bristol Stool Form Scale from 1 to 7 (Riegler and Esposito, 2001) were recorded daily. Statistical analysis of the data from the per protocol population revealed no statistical difference in either average defecation frequency or stool consistency ($p > 0.1$). Overall, the data indicate that Wheat Bran Extract is well tolerated at levels up to 10 g/day by adults from both the 18-50 years and ≥ 51 years age subgroups.

2.4.1.2. Placebo-controlled Cross-over Tolerance Study of Wheat Bran Extract in a Soft Drink with Children

The tolerance of Wheat Bran Extract was investigated using a randomized placebo-controlled cross-over clinical trial evaluating the prebiotic effects of Wheat Bran Extract in healthy volunteers of (ages between 8 and 12 years) (Francois and Broekaert, 2010b- unpublished report). Twenty-nine volunteers (18 boys, 11 girls) were recruited and all subjects completed the trial. The study comprised a run-in period of one week and two intake periods of three weeks each with a wash-out period of two weeks between each treatment period. During the intake periods the volunteers consumed twice daily non-carbonated soft drinks providing either no Wheat Bran Extract (placebo) or 2 x 2.5 g/day of Wheat Bran Extract. The subjects were randomly assigned to two approximately equally sized groups differing in the sequence order of both treatments. The Wheat Bran Extract used in this study was a sample of Batch A (Table 2). During the course of study, the volunteers were asked to fill out a questionnaire registering the occurrence of gastrointestinal symptoms as well as the degree of discomfort associated therewith.

Adverse events as described for the above study in adults (Francois and Broekaert, 2010a-unpublished report) were recorded. Statistical analysis of the frequency of the different adverse event categories revealed no difference between the two treatments ($p > 0.1$). Gastrointestinal symptoms were monitored daily during the run-in period and the last week of each intake period. The volunteers were asked to grade the following symptoms: abdominal pain/cramps, flatulence and urge to vomit using a 5-step scale ranging from no (0), minimal (1), mild (2), moderate (3) to severe (4). Statistical analysis of the data from the per protocol population indicated that there was no impact of Wheat Bran Extract intake on severity of any of the surveyed gastrointestinal symptoms ($p > 0.1$). During the run-in period and the last week of each intake period, defecation frequency as well as consistency of the stool according to the Bristol Stool Form Scale from 1 to 7 (Riegler and Esposito, 2001) were recorded daily. Statistical analysis of the data from the per protocol population indicated that there was no difference in either average defecation frequency or stool consistency ($p > 0.1$). Overall, the data indicate that Wheat Bran Extract at 5 g/day is well tolerated by children.

2.4.1.3. Placebo-controlled Cross-over Tolerance Study of Wheat Bran Extract in Orange Juice with Adults

In a randomized placebo-controlled cross-over study involving twenty healthy adult volunteers, Cloetens et al. (2009a) investigated the tolerance to Wheat Bran Extract-like AXOS preparation following oral intake by healthy adult human volunteers (14 women and 6 men; mean age 24±5 years, mean BMI 20.9±2.3 kg/m²). The study comprised two intake periods of three weeks with a wash-out period of four weeks between treatments. During the intake periods the volunteers either consumed 13.9 g maltodextrin/day (placebo) or 13.9 g/day of the Wheat Bran Extract-like preparation, corresponding to 10 g AXOS/day. Both the maltodextrin and the Wheat Bran Extract-like preparation were administered after being dissolved in orange juice. The AXOS in the

Wheat Bran Extract-like preparation had an average degree of polymerization of 6 and an A/X ratio of 0.26. With the exception of its protein concentration (3.5%), the AXOS preparation used in this study was within specifications as set for Wheat Bran Extract (Table 1). Exclusion criteria included severe gastrointestinal complaints, the intake of antibiotics or medication influencing gut transit and/or gut microbiota during the previous three months, a history of abdominal surgery and pregnancy or anticipated pregnancy at the time of inclusion. During the study, the subjects were allowed to eat their usual diets but were asked to have a regular eating pattern (3 meals/day). The intake of food substances containing probiotics and/or prebiotics was forbidden, and the consumption of foods containing prebiotics such as chicory, artichoke, garlic, onions, green banana, leek and soybeans was limited. At the time of inclusion, the subjects received information about pro- and prebiotics and the available food products containing pro- and/or prebiotics. The subjects were asked to carefully read the food labels of products that they would consume in order to check if pro- and/or prebiotics had been added.

Before and after each intake period venous blood samples were collected and analyzed for hematological and clinical chemistry parameters. Although limited variation was seen before and after the respective treatments for some of these blood parameters, all remained within the normal range, and none of the observed variations was considered to be biologically relevant. Furthermore, through completion of a questionnaire the occurrence of the following 7 gastrointestinal symptoms was monitored before and after each intake period: gastrointestinal discomfort which diminishes after defecation, gastrointestinal pain which diminishes after defecation, gastrointestinal discomfort concomitant with constipation or diarrhea, gastrointestinal pain concomitant with constipation or diarrhea, flatulence, cramps, and bloating. The severity of the symptoms was graded on a 4-step scale ranging from no (0), mild (1), moderate (2) to severe (3). A score for each symptom was obtained by calculating the mean and standard deviation. The total score was the sum of the 7 individual scores. No significant increase in the total score of gastrointestinal symptoms was observed either after the placebo or AXOS treatment. When compared to the score before AXOS intake, only the flatulence score was significantly increased. However, the average flatulence score was graded as mild.

2.4.1.4. Cross-over Study of Tolerance of Wheat Bran AXOS as Compared to Fructooligosaccharide-Enriched Inulin

In two recent randomized, cross-over trials involving healthy volunteers, Cloetens et al. (2010; also see Cloetens, 2009) investigated the tolerance of AXOS derived from wheat bran compared to that of fructooligosaccharide-enriched inulin (FOS-IN) following oral intake by healthy human volunteers. (First trial: 9 females and 4 males, mean age 25±9 years, mean BMI 22.9±2.6kg/m^2; Second trial: 9 females and 2 males, mean age 25±7 years, mean BMI 22.4±3.6kg/m^2) Both trials comprised two intake periods of two weeks with a wash-out period of three weeks between treatments. During the intake periods the volunteers either consumed AXOS or FOS-IN dissolved in water. The respective trials differed only with respect to the administered doses. In trial 1, the subjects consumed 2 x 2.5 g/day FOS-IN and 2 x 2.5 g/day AXOS (2 x 4.35 g/day of an AXOS preparation derived from wheat bran), whereas 2x 1.1 g/day FOS-IN and 2 x 1.1 g/day AXOS (2 x 1.95 g/day of an AXOS preparation derived from wheat bran) were administered in study 2. FOS-IN and AXOS were ingested every morning and evening along with the meal. The AXOS preparation used in the study had an AXOS content of 57.5% with an average degree of polymerization of 9 and an A/X ratio of 0.26, and was prepared by enzymatic extraction from wheat bran in a similar way as Wheat Bran Extract is prepared. The inclusion/exclusion criteria and the dietary restrictions were the same as described for the study mentioned above (Cloetens et al., 2009a).

The gastrointestinal symptoms were monitored before, after one week of treatment, and at the end of each intake period using the questionnaire mentioned above (see study in 2.3.1.3). All symptoms were graded on average as mild since the mean scores were always lower than one. In both trials, highest scores were found for flatulence and bloating. The scores obtained before the AXOS intake period were generally lower than those obtained before FOS-IN intake. After 1 week intake of 5.0 g AXOS, flatulence was scored significantly higher than before intake (p=0.046), but this score was similar to that before or after 1 week of FOS-IN intake. The total score was significantly increased after 1-week intake of AXOS (p=0.028) but, again, this score was similar to that before or after 1 week of FOS-IN intake. In trial 2, no statistically significant differences were observed in any of the gastrointestinal symptoms before or after intake of FOS-IN and AXOS. The highest total score was observed after 1 week intake of FOS-IN. Overall it was concluded that at the tested doses AXOS and FOS-IN were equally well tolerated.

2.4.2. Tolerance Studies Conclusion

Overall, the results from 4 human volunteer studies demonstrate that Wheat Bran Extract is well tolerated without adverse effects. A mild increase in flatulence following Wheat Bran Extract intake can be related to an increased gas production associated with the colonic fermentation of the AXOS. Increased flatulence has been previously observed following the intake of other non-digestible poly/oligosaccharides such as inulin and FOS (Bouhnik et al., 1999; Bouhnik et al., 2007; Kruse et al., 1999). Moreover, mild flatulence is generally considered to be an acceptable (depending on the exposure levels to dietary fiber) potential consequence of increased colonic fermentation of dietary fiber, as sometimes observed from the intake of vegetables.

2.4.3. Human Studies Evaluating the Tolerance of XOS and Wheat Flour Derived AXOS

In five separate human intervention studies, the effects of preparations comprising mainly of XOS at doses ranging from 1.3 to 3.9 g/day for up to 8 weeks have been investigated (Table 6). In all of these studies, the applied XOS doses were well tolerated and no adverse effects were reported. In addition, Grasten et al. (2003) described the effects of the administration (in bread) of 13.9 g/day of a readily fermentable LMW arabinoxylan preparation (avDP 58), isolated from a wheat flour wet processing stream, to healthy volunteers for19-22 days. No adverse effects were observed, except for mild symptoms of increased flatulence, and no differences were noted in fecal frequency or stool consistency.

Table 6. Overview of clinical trials performed with XOS and wheat flour derived AXOS.

Tested compound	Specification of test population	Specification of test compound	Source of test compound	Level in diet (num in parentheses ar corrected for AX purity)
XOS	9 healthy subjects, between 50 and 60 years of age	>70% DP2 and DP3	birch wood	5 g (3 9 g) per day, weeks
XOS	14 subjects with hepatic cirrhosis with mild hyperammonaemia	>65% DP2 and DP3	corn cobs	3 g (2 8 g) per day
XOS	13 eldery persons, average age 77	>80% DP2 and DP3	corn cobs	4 0 g (3 8 g) per da during 21 days
XOS	14 healthy young women, between 23 and 26 years	avDP not specified	corn cobs	1 4 g (1 3 g) or 2 8 (2 7 g) per day duri days
XOS	26 type-2 diabetes patients, 19 men and 7 women	>80% DP2 and DP3	corn cobs	4 g (3 8 g) per day during 8 weeks
AXOS	14 healthy subjects	avDP and A/X ratio not specified	wheat flour processing byproduct	15 g (purity not specified) in bread during 19-22 days

2.5. Allergenicity and Other Related Concerns

Wheat allergies are among the eight most common food allergies, primarily affecting children under the age of three[9]. It is an abnormal immune system reaction to one or more proteins found in wheat. In the USA, wheat-related food has been found to be responsible for 2.5% of the food allergy cases in children (Bock and Atkins, 1990). In France, wheat ranked as the eighth most frequent food allergen in children and the 12th in adults (Rance et al., 1998). Wheat allergy involves the triggering by wheat proteins of the production of specific immunoglobulin E (IgE), and is to be discriminated from celiac disease which involves gliadin-specific IgA and IgG antibodies and which is a hereditary autoimmune disease and not a true allergy. Wheat allergies can be triggered by several types of wheat proteins, including albumins, globulins, gliadins, and glutenin (Hirschenhuber et al., 2006). In a study involving 18 patients with a diagnosis of hypersensitivity reactions to wheat, it was found that there was a concordance of positive reactions in the skin prick test with wheat endosperm and wheat bran (Pourpak et al., 2005), suggesting that wheat bran is also a source of allergens.

While wheat may contain potential allergens, its processing in the production of Wheat Bran Extract results in a reduction of its protein content to typically less than 1% which is an order of magnitude lower than in wheat (about 10-15% protein) or wheat bran (about 14-18% protein). While this does not eliminate a risk for wheat atopics, the risk is certainly no greater and possibly lower than that naturally contained in the starting materials.

The consumption of wheat and wheat-derived products is known to cause an adverse reaction in persons with celiac disease[10] (gluten intolerance). As mentioned above, this condition involves gliadin-specific IgA and IgG antibodies. While undesirable, it is not expected that the accidental consumption of a Wheat Bran Extract-containing food product will cause noticeable discomfort for persons with celiac disease. Gluten analysis from 5 batches of Wheat Bran Extract revealed the presence of gluten ranging from 2200 to 5000 mg/kg. Wheat Bran Extract-containing products will typically comprise 3 g Wheat Bran Extract per serving contributing at most about 15 mg of gluten. This is at the lower end of the safe limit for gluten intake for people with celiac disease, which was estimated by Hirschenhuber et al. (2006) to lie between 10 and 100 mg per day. This estimation was based on the observations that a daily calculated intake of 30 mg/day or less did not seem to affect the mucosa, while observable mild mucosa alterations occurred at 100 mg/day (Hirschenhuber et al., 2006).

Individuals with wheat allergy or celiac disease manage their condition by avoiding the consumption of wheat and wheat-derived products and rely on the declaration on food labels for the presence of wheat or derivatives thereof in foods that they intend to consume. The Food Allergen Labeling Prtection Act of 2004 (FDA, 2004) mandates that the labels of foods containing major food allergens (milk, eggs, fish,

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[9] Asthma and Allergy Foundation of America- http://www.aafa.org/display.cfm?id=9&sub=30

[10] Celiac disease is a hereditary disorder of the immune system affecting approximately 1% of the population Intake of high amounts of gluten by persons with celiac disease leads to damage of the mucosa of the small intestine, resulting in malabsorption of nutrients and vitamins.

crustacean shellfish, peanuts, tree nuts, wheat, and soy) declare the allergen in plain language, either in the ingredient list or via:

- the word "Contains" followed by the name of the major food allergen – for example, "Contains milk, wheat" – *or*
- a parenthetical statement in the list of ingredients – for example, "albumin (egg)"

Accordingly, any food to which Wheat Bran Extract has been added will be clearly labeled so as to declare the presence of wheat in accordance with this regulation.

3. RISK ASSESSMENT

There is sufficient qualitative and quantitative scientific evidence, including human and animal data, to determine safety-in-use of Wheat Bran Extract. The available scientific evidence suggests that Wheat Bran Extract will be as safe as the wheat and wheat bran from which it is derived. Wheat Bran Extract is produced through enzymatic extraction of the hemicellulosic fiber fraction of wheat bran, and its production occurs in accordance with standard operating procedures, using starting materials and processing materials that meet appropriate food grade specifications. Following oral consumption, AXOS and β-glucans, the main components in Wheat Bran Extract, remain undigested in the upper gastrointestinal tract and are subjected to bacterial fermentation in the colon. This fermentation results in the formation of short chain fatty acids and the release of ferulic acid, which are benign and well metabolized digestion products that are also produced upon digestion of plant-derived fibers naturally present in a regular healthy diet rich in cereals, fruits, and vegetables.

The safety of Wheat Bran Extract is further confirmed by:

- The long history of safe dietary exposure to similar components such as LMW arabinoxylan and β-glucan through foods, such as beer and bread (LMW arabinoxylans), going back thousands of years.
- The absence of toxic effects in *in vitro* and *in vivo* toxicity studies at significant multiples of anticipated human exposure.
- Lack of adverse effects in human tolerance studies.

Conventional toxicological testing methods should not efficiently be applied to foods and food components consumed in significant amounts because the bulkiness of such foods precludes exaggerated dose testing. For example, use of very high amounts to be incorporated in the diet for animal feeding studies, up to the level that the optimal nutritional balance may be perturbed, thus making it difficult to discriminate true toxicological effects from nutritional imbalance or physiological adaptation effects. These difficulties often make the use of conventional safety factors inappropriate for hazard identification and risk assessment for many products intended for use as a food or a major food ingredient. Thus use of doses of a substance higher than 5% in diet (2500 mg/kg/day) as a 'macroingredient' in animal studies precludes an interpretable outcome (Borzelleca, 1992). Hence for substances whose recommended dose (effective use levels) in human is high, conventional testing methodologies i.e. testing at a dose higher than 100 fold of human intake levels is generally not recommended.

The safety assessment of Wheat Bran Extract is based on the totality of available evidence, particularly evidence regarding the fate of Wheat Bran Extract in the gut in human and animal studies. A variety of experimental studies with animals and human clinical trials and comparison with data from other similar dietary fibers further corroborate the safety. In clinical trials, administration of Wheat Bran Extract at doses of up to 13.9 g/day did not cause any significant adverse effects. In a subchronic rat study, Wheat Bran Extract had no adverse effects at dose levels up to 7.5% (4354 mg Wheat Bran Extract/kg bw/day), the highest dose tested. Compared to this safe dose in animals, the estimated daily intake of Wheat Bran Extract from the intended uses of the extract at the 90th percentile of 10.1 g/person (217 mg/kg bw/day) is approximately 20-fold lower. The estimated daily intake, if ingested daily over a lifetime, is considered safe.

4. SUMMARY

Fugeia NV has developed a process to manufacture standardized Wheat Bran Extract by enzymatic depolymerization and extraction of the hemicellulosic fiber components from wheat bran. The main components of Wheat Bran Extract are arabinoxylan-derived oligosaccharides (AXOS) (>70%), next to β-glucans (10-14%) and minor amounts of other oligo/polysaccharides. The AXOS and β-glucans in Wheat Bran Extract are water soluble and have a lower degree of polymerization than the insoluble arabinoxylan and β-glucan of wheat bran. However, similar to native wheat arabinoxylan, Wheat Bran Extract AXOS are substituted with ferulic acid and glucuronic acid.

From a nutritional point of view, Wheat Bran Extract contains about 70% of dietary fiber making it an excellent ingredient for increasing the fiber content of food products, in particular processed or refined foods, lacking in dietary fiber. Furthermore, Wheat Bran Extract has been shown to have prebiotic properties as evidenced following its ingestion by increases in colonic Bifidobacteria, the production of short chain fatty acids and decreased protein fermentation. Therefore, Wheat Bran Extract is expected to serve as an alternative for existing prebiotic fiber ingredients, such as inulin, oligofructose, polydextrose, and resistant maltodextrin.

Fugeia NV intends to use Wheat Bran Extract as a food ingredient in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods at use levels of 2.4 to 3.2 g Wheat Bran Extract *per* serving. The intended use of Wheat Bran Extract in the above mentioned food categories will result in an estimated daily intake for "users only" at the 90th percentile of 10.1 g/person (217 mg/kg body weight/day). Such exposure is considered safe based on the following elements:

- Wheat bran, the source of Wheat Bran Extract, has a very long history of safe use in humans and animals, and is recommended as part of a healthy human diet.
- Wheat Bran Extract contains less potential toxicants (mycotoxins, heavy metals and pesticides) than its source material wheat bran as a consequence of their elimination in the production process.
- AXOS or comparable low molecular weight entities are naturally present in food products with long histories of safe use, such as bread and beer, and are

formed in the human large intestine as breakdown products of arabinoxylans, the main dietary fiber of cereals.

- Soluble low molecular weight β-glucans are currently present in a wide variety of food products and are formed in the human large intestine as a breakdown product of β-glucan, a prominent dietary fiber of most cereals.
- Ferulic acid is ubiquitous in all vegetable sources in the human diet and is generally considered as a beneficial antioxidant.
- In *in vitro* studies Wheat Bran Extract is not genotoxic.
- No adverse effects of Wheat Bran Extract in rats were reported in a 90-day subchronic toxicity experiments at levels up to 4354 mg/kg bw/day or a maximum of 7.5% in the diet.
- Wheat Bran Extract was well tolerated by human volunteers at doses up to 13.9 g/day.

Although under the intended conditions of use Wheat Bran Extract is considered to be a safe food ingredient for the general population, the product should not be consumed by persons with gluten intolerance or wheat allergy. Therefore, food products supplemented with Wheat Bran Extract will be labeled as containing wheat allowing persons suffering from either of these conditions to avoid such foods.

On the basis of scientific procedures[11] and history of exposure from natural sources, the consumption of Wheat Bran Extract as an added food ingredient is considered safe at levels up to 10.1 g/day. The intended uses are compatible with current regulations, *i.e.*, Wheat Bran Extract will be used in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods at use levels ranging from 2.4 to 3.2 g Wheat Bran Extract *per* serving and it is produced according to current good manufacturing practices (cGMP).

[11] 21 CFR §170.3 Definitions (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

5. CONCLUSION

Based on a critical evaluation of the publicly available data summarized above, the Expert Panel members whose signatures appear below, have individually and collectively concluded that Wheat Bran Extract, meeting the specifications cited above, and when used as a food ingredient in selected food products (baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods) at levels of 2.4 to 3.2 g Wheat Bran Extract/serving (reference amounts customarily consumed, 21CFR 101.12) when not otherwise precluded by a Standard of Identity as described in this monograph and resulting in the 90th percentile all-user estimated intake of 10.1 g Wheat Bran Extract/person/day is Generally Recognized As Safe (GRAS).

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Wheat Bran Extract, when used as described, is GRAS based on scientific procedures.

Signatures

(b) (6) [redacted]

Stanley M. Tarka, Jr., Ph.D. — 03 March 2010 — Date

(b) (6) [redacted]

John A. Thomas, Ph.D., F.A.T.S., D.A.T.S. — 5 March 2010 — Date

(b) (6) [redacted]

Professor Gerard Pascal — 16 March 2010 — Date

(b) (6) [redacted]

Madhusudan G. Soni, Ph.D., F.A.C.N. — 10 March 2010 — Date

6. REFERENCES

Adam, A., Crespy, V., Levrat-Verny, M., Leenhardt, F., Leuillet, M., Demigne, C., Remesy, C. The bioavailability of ferulic acid is governed primarily by the food matrix rather than its metabolism in intestine and liver in rats. J. Nutr. 2002, 132: 1962-1968.

Adams, T.B., Doull, J., Goodman, J.I., Munro, I.C., Newberne, P., Portoghese, P.S., Smith, R.L., Wagner, B.M., Weil, C.S., Woods L.A., Ford, R.A. The FEMA GRAS assessment of furfural used as a flavour ingredient. Food Chem. Toxicol. 1997, 35: 739-751.

Abrams, S.A., Hawthorne, K.M., Aliu, O., Hicks, P.D., Chen, Z., Griffin, I.J. An inulin-type fructan enhances calcium absorption primarily via an effect on colonic absorption in humans. J Nutr. 2007, 137:2208-2212.

Alabaster, O., Tang, Z.C., Frost, A., Shivapurkar, N. Effect of beta-carotene and wheat bran fiber on colonic aberrant crypt and tumour formation in rats exposed to azoxymethane and high dietary fat. Carcinogenesis. 1995, 16:127–132.

Andersson, A.A.M., Elfverson, C., Andersson, R., Regner, S., Aman, P. Chemical physical characteristics of different barley samples. J. Sci. Food Agric. 1999, 79: 979-986.

Bach-Knudsen, K.E., Hansen I. Gastrointestinal implications in pigs of wheat and oat fractions. Br. J. Nutr. 1991, 65: 217-232.

Barbolt, T.A., Abraham, R. Dose response, sex difference and the effect of bran in dimethylhydrazine induced intestinal tumourigenesis in rats. Toxicol. Appl. Pharmacol. 1980, 55:417–422.

Bataillon, M., Mathaly, P., Cardinali, A.P.N., Duchiron, F. Extraction and purification of arabinoxylan from destarched wheat bran in a pilot scale. Industrial Crops and Products. 1998. 8: 37-43.

Belitz, H.-D., Grosch, W., Schieberle, P. Food Chemistry. 3rd Edition. Springer-Verlag, Berlin, Germany, 2004.

Beres, E. 2009. Wheat Bran Extract: In vitro mammalian chromosome aberration test. LAB Research Ltd., STUDY CODE: 08/743-020C.

Bock, A.S., Atkins, F.M. Patterns of food hypersensitivity during sixteen years of double-blind placebo-controlled food challenges. J. Pediatr. 1990, 117: 561–567.

Bone, E., Tamm, A., Hill, M. Production of urinary phenols by gut bacteria and their possible role in causation of large bowel cancer. Am. J. Clin. Nutr. 1976, 29: 1448-1454.

Borzelleca, J.F. Macronutrient substitutes: safety evaluation. Regul. Toxicol. Pharmacol. 1992, 16: 253-264.

Bouhnik, Y., Raskine, L., Champion, K., Andrieux, C., Penven, S., Jacobs, H., Simoneau, G. Prolonged administration of low-dose inulin stimulates the growth of bifidobacteria in humans. Nutr. Res. 2007, 27, 187-193.

Bouhnik Y, Vahedi K, Achour L, Attar A, Salfati Y, Pochart P, Marteau P, Flourié B, Bornet F & Rambaud JC (1999) Short-chain fructo-oligosaccharide administration dose-dependently increases faecal bifidobacteria in healthy humans. J Nutr 129, 113-116.

Bourne, L. Paganga, G., Baxter, D., Hughes, P., Rice-Evans, C. Absorption of ferulic acid from low-alcohol beer. Free Radic. Res. 2000, 32: 273-280.

Boyle, F.G., Wrenn, J.M., Marsh, B.B., Anderson, W.I., Angelosanto, F.A., McCartney, A.L., Lien, E.L. Safety evaluation of oligofructose: 13 week rat study and in vitro mutagenicity. Food Chem. Toxicol. 2008, 46: 3132-3139.

Broekaert, W.F., Courtin, C.M., Verbeke, K., Van de Wiele, T., Verstraete, W., Delcour, J.A. Prebiotic and other health-related effects of cereal-derived arabinoxylans and (arabino)xylooligosaccharides. Crit. Rev. Food Sci. Technol. 2010 (In press).

Campbell, J.M., Fahey, G.C., Wolf, B.W. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats, J. Nutr. 1997. 127:130-136.

Carvalheiro, F. Duarte, L.C., Lopes, S., Parajo, J.C., Pereira, H., Gırio, F.M. Evaluation of the detoxification of brewery's spent grain hydrolysate for xylitol production by *Debaryomyces hansenii* CCMI 941. Process Biochem. 2005, 40:1215–1223.

Chassard, C., Goumy, V., Leclerc, M., Del'homme, C., Bernalier-Donadille, A. Characterization of the xylan-degrading microbial community from human faeces. FEMS Microbiol. Ecol. 2007, 61:121-131.

Chen, H.L., Haack, V.S., Janecky, C.W., Vollendorf, N.W., Marlett, J.A. Mechanisms by which wheat bran and oat bran increase stool weight in humans. Am. J. Clin. Nutr. 1998, 68: 711-719.

Cheng, B.O., Trimble, R.P., Illman, R.J., Stone, B.A., Topping, D.L. Comparative effects of dietary wheat bran and its morphological components (aleurone and pericarp-seed coat) on volatile fatty acid concentrations in the rat. Br. J. Nutr. 1987, 57:69-76.

Chung, Y.-C., Hsu, C.-K., Ko, C.-Y., Chan, Y.-C. Dietary intake of xylooligosaccharides improves the intestinal microbiota, fecal moisture, and pH value in the elderly. Nutr. Res. 2007, 27: 756-761.

Cloetens, L., Broekaert, W.F., Courtin, C.M., Delcour, J.A., Rutgeerts, P., Verbeke, K. The supplementation of 10 g/day arabinoxylo-oligosaccharides, a newly proposed prebiotic, to the diet of healthy subjects is well tolerated. Acta Gastro-Enterolgica Belgica 2009b, 72:N08.

Cloetens, L., Broekaert, W. F., Delaedt, Y., Ollevier, F., Courtin, C. M., Delcour, J. A., Rutgeerts, P., Verbeke, K. Tolerance of arabinoxylan-oligosaccharides and their prebiotic activity in healthy subjects: a randomized placebo-controlled, cross-over study. Br. J. Nutr. 2009a(Epub Dec 10), 103:703-713.

Cloetens, L., Delaedt, Y., Broekaert, W.F., Courtin, C.M., Ollevier, F., Delcour, J.A., Rutgeerts, P., Verbeke, K. The increased level of bifidobacteria and a beneficially modulated colonic metabolic activity suggest the prebiotic potential of arabinoxylo-oligosaccharides in healthy subjects. Acta Gastro-Enterolgica Belgica 2009c; 72:N09.

Cloetens, L., De Preter, V., Swennen, K.., Broekaert, W.F., Courtin, C.M., Delcour, J.A., Rutgeerts, P., Verbeke, K.. Dose-response effect of arabinoxylo-oligosaccharides on gastrointestinal motility and on colonic bacterial metabolism in healthy volunteers. J. Am. Coll. Nutr. 2008a, 27:512-518.

Cloetens, L., Swennen, K.., De Preter, V., Broekaert, W.F., Courtin, C.M., Delcour, J.A., Rutgeerts, P., Verbeke, K.. Effect of arabinoxylo-oligosaccharides on proximal gastrointestinal motility and digestion in healthy volunteers. e-SPEN the European e-Journal of Clinical Nutrition and Metabolism. 2008b, 3: e220-e225.

Cloetens, L., *In vivo* evaluation of the gastro-intestinal effects of arabinoxylan-oligosaccharides in healthy humans. Ph.D. thesis. 2009. Katholieke Universiteit Leuven, Leuven, Belgium. The complete thesis is available online at: https://lirias.kuleuven.be/handle/1979/2636

Cloetens, L., Van Duppen, J., Delaedt, Y., Broekaert, W. F., Ollevier, F., Courtin, C. M., Delcour, J. A., Rutgeerts, P., Verbeke, K. Low doses of arabinoxylo-oligosaccharides induce similar effects on colonic bacterial metabolism and the composition of faecal microbiota in healthy human subjects as fructo-oligosaccharide eniched inulin. 2010 (manuscript submitted for publication based on Ph.D. thesis work, see Cloetens 2009).

Costabile, A., Klinder, A., Fava, F., Napolitano, A., Fogliano, V., Leonard, C., Gibson, GR., Tuohy, K.M. Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. Br. J. Nutr. 2007, Aug 29:1-11.

Courtin, C.M. and Delcour, J.A. Arabinoxylans and endoxylanases in wheat flour bread-making. J. Cereal Sci. 2002, 35: 225-243.

Courtin, C.M., Broekaert, W.F., Aerts, G., Van Craeyveld, V., Delcour, J.A. Occurrence of arabinoxylo-oligosaccharides and arabinogalactan peptides in beer. J. Am. Soc. Brew. Chem. 2009a. 67: 112-117.

Courtin, C.M., Swennen, K., Verjans, P., Delcour J.A. Heat and pH stability of prebiotic arabinoxylooligosaccharides, xylooligosaccharides and fructooligosaccharides. Food Chem. 2009b, 112: 831-837.

Courtin, C.M., Van den Broeck, H., Delcour, J.A. Determination of reducing end sugar residues in oligo- and polysaccharides by gas-liquid chromatography. J. Chromatogr. A 2000, 866: 97-104.

Crittenden, R., Karppinen, S., Ojanen, S., Tenkanen, M.,Fagerstrom, R., Matto, J., et al. In vitro fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. Journal of the Science of Food and Agriculture, 2002, 82: 781-789.

CTCAE, 2006. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Cancer Therapy Evaluation program. Report available online at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf (website visited on February 10, 2010).

Cui, W., Wood, P.J., Blackwell, B., Nikiforuk, J. Physicochemical properties and structural characterization by two-dimensional NMR spectroscopy of wheat β-D-glucan. Comparison with other cereal β-D-glucans. Carbohydrate Pol. 2000. 41:249-258.

Darwiche, G., Björgell, O., Almer, L. The addition of locust bean gum but not water delayed the gastric emptying rate of a nutrient semisolid meal in healthy volunteers. BMC Gastroenterology 2003, 6: 3-12.

Demigne, C., Levrat, M.A., Remesy, C. Effects of feeding fermentable carbohydrates on the cecal concentrations of minerals and their fluxes between the cecum and blood plasma in the rat. J. Nutr. 1989, 119: 1625-1630.

Eeckhaut, V., Van Immerseel, F., Dewulf, J., Pasmans, F., Haesebrouck, F., Ducatelle, R., Courtin, C.M., Delcour, J.A., Broekaert, W.F. Arabinoxylooligosaccharides from wheat bran inhibit Salmonella colonization in broiler chickens. Poultry Sci. 2008, 87: 2329-2334.

Englyst, H.N., Cummings, J.H. Digestion of the polysaccharides of some cereal foods in the human small intestine. Am. J. Clin. Nutr. 1985, 42:778-787.

Englyst, K.N., Englyst, H.N., Carbohydrate bioavailability. Brit. J. Nutri. 2005, 94: 1-11.

FAO/WHO. Joint FAO/WHO expert consultation. Carbohydrates in human nutrition. 1988. FAO Food and Nutrition paper 66. Rome.

FCC (2008). Food Chemical Codex. United States Pharmacopeial Convention. Rockville, MD, USA.

FDA, 2004. Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282, Title II). Document available online at: http://www.fda.gov/Food/LabelingNutrition/FoodAllergensLabeling/GuidanceComplianceRegulatoryInformation/ucm106187.htm (website visited on February 11, 2010).

Femia, A.P, Salvadori, M., Broekaert, W.F., François, I.E.J.A., Delcour, J.A., Courtin, C.M., Caderni, G. Arabinoxylan oligosaccharides (AXOS) reduce preneoplastic lesions in the colon of rats treated with 1,2-dimethylhydrazine (DMH). Eur. J. Nutr. 2010, 49: 127-132.

Francois, I.E.J.A., Broekaert, W.F. 2010a. Clinical study interim report: *In vivo* evaluation of the physiological effects of Wheat Bran Extract (WBE) in healthy human volunteers (adults). Unpublished study Reference: ML5282-adults.

Francois, I.E.J.A., Broekaert, W.F. 2010b. Clinical study interim report: *In vivo* evaluation of the physiological effects of Wheat Bran Extract (WBE) in healthy human volunteers (juniors). Unpublished study Reference: ML5282-juniors.

Francois, I.E.J.A., Lescroart, O., Veraverbeke, W., Kubaszky, R., Hargitai, J., Esdaile, D.J., Beres, E., Soni, M.G., Cockburn, A., Broekaert, W.F., Safety assessment of a Wheat Bran Extract, containing arabinoxylan-oligosaccharides: Mutagenicity, clastogenicity and 90-day rat feeding studies. International Journal of Toxicology (In Press).

Gebruers, K., Dornez, E., Boros, D., Fras, A., Dynkowska, W., Bedo, Z., Rakszegi, M., Delcour, J.A., Courtin, C.M. Variation in the content of dietary fiber and components thereof in wheats in the HEALTHGRAIN Diversity Screen. J Agric. Food Chem. 2008, 56: 9740-9749.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., Roberfroid, M. B., Dietary modulation of the human colonic microbiota: updating the concept of prebiotics, Nutr. Res. Rev. 2004, 17: 259-275.

Glitso, L. V., Gruppen, H., Schols, H. A., Hojsgaard, S., Sandstrom, B., Knudsen, K. E. B. Degradation of rye arabinoxylans in the large intestine of pigs. J. Sci. Food Agric. 1999, 79: 961-969.

Gordon, D.T., Stoops, D., Ratliff, V. Dietary fiber and mineral nutrition. In: "Dietary Fiber in Health and Disease", Kritchevsky D and Bonfield C (eds.). Eagan Press, St. Paul, Minnesota, U.S.A. 1995, p. 267-293.

Gorman, M.A., Bowman, C. Position of the American Dietetic Association: Health implications of dietary fiber. J. Am. Dietetic Assoc. 1993, 93: 1446-1447.

Grasten, S., Liukkonen, K.-H., Chrevatidis, A., El-Nezami, H., Poutanen, K., Mykkanen, H. Effects of wheat pentosan and inulin on the metabolic activity of fecal microbiota and on bowel function in healthy humans. Nutr. Res. 2003, 23: 1503-1514.

Gray, J. Dietary fibre. Definition, analysis, physiology & health. In: ILSI Europe Concise Monograph Series, ILSI Europe, 2006.

Grootaert, C., Delcour, J.A., Courtin, C. M., Broekaert, W.F., Verstraete, W., Van de Wiele, T. Microbial metabolism and prebiotic potency of arabinoxylan oligosaccharides in the human intestine. Trends Food Sci. Technol. 2007, 18: 64-71.

Harder, H., Tetens, I., Let, M.B., Meyer, A.S. Rye bran bread intake elevates urinary excretion of ferulic acid in humans, but does not affect the susceptibility of LDL to oxidation ex vivo. Eur. J. Nutr. 2004, 43: 230-236.

Hargitai, J. 2009. Wheat Bran Extract: *In vitro* Toxicity Assay using the Bacterial Reverse Mutation Assay. LAB Research Ltd., STUDY CODE: 08/743-007M.

Harris, D. The origins and spread of agriculture and pastoralism in Eurasia. (Washington, D.C.: Smithsonian Institution Press). 1996.

Hashimoto, S., Shogren, M.D., and Pomeranz, Y. Cereal pentosans: their estimation and significance. I. Pentosans in wheat and milled wheat products. Cereal Chem. 1987, 64: 30-34.

Henry, R.J. Pentosan and (1→3),(1→4)-β-glucan concentrations in endosperm and wholegrain of wheat, barley, oats and rye. J. Cereal Sci. 1987, 6: 253-258.

Higuchi, M., Kobayashi, S., Kawasaki, N., Hamaoka, K., Watabiki, S., Orino, K., Watanabe, K. Protective effects of wheat bran against diquat toxicity in male Fischer-344 rats. Biosci. Biotechnol. Biochem. 2007, 71:1621-1625.

Hirschenhuber, C., Crevel, R., Jarry, B., Mäki, M., Moneret-Vautrin, A., Romano, A., Troncone, R., Ward, R. Review article: safe amounts of gluten for patients with wheat allergy or coeliac disease. Aliment. Pharmacol. Ther. 2006. 23:559–575.

Holgate, A.M., Read, N.W. Relationship between small bowel transit and absorption of a solid meal: influence of metoclopramide, magnesium sulphate and lactulose. Dig. Dis. Sci. 1983, 28:812-9.

Hollmann, J., Lindhauer, M.G. Pilot-scale isolation of glucuronoarabinoxylans from wheat bran. Carbo. Poly. 2005. 59: 225-230.

Holloway, W.D., Tasman-Jones, C., Bell E. The hemicellulose component of dietary fiber. Am. J. Clin. Nutr. 1980, 33:260-263.

Hong, B.H., Rubenthaler, G.L. and Allan, R.E. Wheat pentosans. I. Cultivar variation and relationship to kernel hardness. Cereal Chem. 1989, 66, 369-373.

Hsu, C.-K., Liao, J.-W., Chung, Y.-C., Hsieh, C.-P., Chan, Y.-C. Xylooligosaccharides and fructooligosaccharides affect the intestinal microbiota and precancerous colonic lesions development in rats. J. Nutr. 2004, 134:1523-1528.

Hughes, S.A., Shewry, P.R., Li, L., Gibson, G.R., Sanz, M.L., Rastall, R.A. *In vitro* fermentation by human fecal microflora of wheat arabinoxylans. J. Agric. Food Chem. 2007, 55:4589-4595.

IOM. Institute of Medicine. Dietary, Functional, and Total Fiber. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, D. C.: National Academies Press; 2002. 265-334.

JECFA. Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives. Furfural. 2004.

JECFA. General Specifications and Considerations for Enzyme Preparations Used in Food Processing. 2006. Document available online at: http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm (website visited on February 19, 2010).

Jenab, M., Thompson, L.U. The influence of phytic acid in wheat bran on early biomarkers of colon carcinogenesis. Carcinogen. 1998, 19:1087-1092.

Johnson, K. A. The production of secondary amines by human gut bacteria and its possible relevance to carcinogenesis. Med. Lab. Sci. 1977, 34:131-143.

Imaizumi, K., Nakatsu, Y., Sato, M., Sedernawati, Y., Sugano, M. Effects of xylooligosaccharides on blood glucose, serum and liver lipids and cecum short-chain fatty acids in diabetic rats. Agric. Biol. Chem. 1991, 55: 199-205.

Izydorczyk, M.S., Biliaderis, C.G. Cereal arabinoxylans: advances in structure and physicochemical properties. Carbo. Poly. 1995, 28: 33-48.

Izydorczyk, M.S., Dexter, J.E. Barley β-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products–a Review. Food res. Int. 2008, 41: 850-868.

Jaskari, J., Kontula, P., Siitonen, A., Jousimies-Somer, H., Mattila-Sandholm, T. and Poutanen, K. Oat beta-glucan and xylan hydrolysates as selective substrates for Bifidobacterium and Lactobacillus strains, Appl. Microbiol. Biotechnol. 1998, 49: 175-181.

Jenab, M., Thompson, L.U. The influence of phytic acid in wheat bran on early biomarkers of colon carcinogenesis. Carcinogenesis 1998, 19:1087-1092.

Kabel, M.A., Kortenoeven, L., Schols, H.A. Voragen, A.G.J. In vitro fermentability of differently substituted xylo-oligosaccharides. J. Agric. Food Chem. 2002, 50: 6205-6210.

Kajihara, M., Kato, S., Konishi M., Yamagishi, Y., Horie, Y., Ishii, H. Xylooligosaccharide decreases blood ammonia levels in patients with liver cirrhosis. Am. J. Gastroenterol. 2000, 95: 2514.

Kang, J.Y., Doe, W.F. Unprocessed bran causing intestinal obstruction. Br. Med. J. 1979, 1:1249–1250.

Kelsay J.L. Effects of fiber on vitamin bioavailability. In: "Dietary Fiber. Chemistry, Physiology, and Health Effects", Kritchevsky, D. et al. (eds), Plenum Press, New York. 1990, p. 129-135.

Kern, S.M., Bennett, R.N., Mellon, F.A., Kroon, P.A., Garcia-Conesa, M.T. Absorption of hydroxycinnamates in humans after high-bran cereal consumption. J. Agric. Food Chem. 2003, 51:6050-60555.

Kiso Y., Iino T., Kato, S. Remedies for hyperammonemia. 2001, US patent 6852707

Kruse, H.P., Kleessen, B., Blaut, M. Effects of inulin on faecal bifidobacteria in humans subjects. Br. J. Nutr. 1999, 82, 375-382.

Kubaszky, R. 2009a. Wheat Bran Extract: 14-day dose range finding toxicity/palatability study in rats. LAB Research Ltd., STUDY CODE: 08/743-101PE. pp. 1-127.

Kubaszky, R. 2009b. Wheat Bran Extract: A 90-Day Dietary Toxicity Study in Wistar Rats. LAB Research Ltd., STUDY CODE 08/743-101P. pp. 1-330.

Lairon, D., Bertrais, S., Vincent, S., Arnault, N., Galan, P., Boutron, M.-C., Hercberg, S. Dietary fibre intake and clinical indices in the French Supplementation en Vitamines et Minéraux AntioXydants (SU.VI.MAX) adult cohort. Proc. Nutr. Soc. 2003, 62: 11–15

Lang, R., Jebb, S.A. Who consumes whole grains, and how much? Proc. Nutr. Soc. 2003, 62:123-127.

Lopez, H. W., Vallery, F., Levrat-Verny, M.A., Coudray, C., Demigne, C., Remesy, C. Dietary phytic acid and wheat bran enhance mucosal phytase activity in rat small intestine. J. Nutr. 2000, 130:2020-2025.

Lopez, H.W., Levrat, M.A., Guy, C., Messager, A., Demigne, C., Remesy, C. Effects of soluble corn bran arabinoxylans on cecal digestion, lipid metabolism, and mineral balance. J. Nutr. Biochem. 1999, 10:500-509.

Macfarlane, S., Macfarlane, G.T. Proteolysis and amino acid fermentation. In: Human Colonic Bacteria: Role in Nutrition, Physiology and Pathology. Gibson GR Macfarlane GT (eds.) CRC Press, Boca Raton, FL.1995, pp. 75-100.

Malkki A. Physiological properties of dietary fibres as keys to physiological functions. Cereal Foods World, 2001, 46:196-199.

Maes, C., Delcour, J.A. Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran. J. Cereal Sci. 2002. 35: 315-326.

McBurney MI. Potential water-holding capacity and short-chain fatty acid production from purified fiber sources in a fecal incubation system. Nutrition, 1991, 7:421–424.

McCleary, B.V. Enzymatic modification of plant polysaccharides. Int. J. Biol. Macromol. 1986, 8: 349-354.

McNeil, N.I., Cummings, J.H., James, W.P.T. Short chain fatty acid absorption by the human large intestine. Gut 1978, 19: 819-822.

Moura, P., Barata, R., Carvalheiro, F., Girio, F., Loureiro-Dias, M.C., and Esteves, M.P. In vitro fermentation of xylo-oligosaccharides from corn cobs autohydrolysis by *Bifidobacterium* and *Lactobacillus* strains. LWT, 2007, 40:963-972.

Muir, J.G., Yeow, E.G., Keogh, J., Pizzey, C., Bird, A.R., Sharpe, K., O'Dea, K., Macrae, F.A. Combining wheat bran with resistant starch has more beneficial effects on fecal indexes than does wheat bran alone. Am. J. Clin. Nutr. 2004, 79:1020-1028.

Na, M.H., Kim, W.K. Effects of xylooligosaccharide intake on fecal Bifidobacteria, lactic acid and lipid metabolism in Korean young women. Korean J. Nutr. 2007, 40: 154-161.

Nystrom, L. , Lampi, A.M., Andersson, A.A., Kamal-Eldin, A., Gebruers, K., Courtin, C.M., Delcour, J.A., Li, L., Ward, J.L., Fraś, A., Boros, D., Rakszegi, M., Bedo, Z., Shewry, P.R., Piironen, V. Phytochemicals and dietary fiber components in rye varieties in the HEALTHGRAIN Diversity Screen. J Agric Food Chem. 2008, 56:9758-9766.

Okazaki, M., Fujikawa, S., Matsumoto, N. Effect of xylooligosaccharides on the growth of bifidobacteria. Bifidobacteria Microflora 1990, 9:77-86.

Okazaki, M., Koda, H., Izumi, R., Fujikawa, S., Matsumoto, N. *In vitro* digestibility and *in vivo* utilization of xylobiose, J. Jap. Soc. Nutr. Food Sci. 1991, 44: 41-44.

Osborne, T.B., Mendel, L.B. The nutritive value of wheat kernel and its milling products. J. Biol. Chem. 1919, 37:557-601.

Oscarsson, M., Andersson, R., Salomonsson, A.-C. and Åman, P. Chemical composition of barley samples focusing on dietary fibre components. J. Cereal Sci. 1996: 24, 161-170.

Palframan, R. J., Gibson, G. R. and Rastall, R. A. Carbohydrate preferences of Bifidobacterium species isolated from the human gut, Curr. Issues Intest. Microbiol. 2003, 4: 71-75.

Peterson, D.M., Wesenberg, D.M., Burrup, D.E. ß-Glucan content and its relationship to agronomic characteristics in elite oat germplasm. Crop Sci. 1995, 35:965-970.

Pompei A, Cordisco L, Amaretti A, Zanoni S, Matteuzzi D, Rossi M. Folate production by bifidobacteria as a potential probiotic property. Appl. Environ. Microbiol. 2007, 73: 179-185.

Pourpak, Z., Mesdaghi, M., Mansouri, M., Kazemnejad, A., Beiraghi Toosi, S., Farhoudi, A. Which cereal is a suitable substitute for wheat in children with wheat allergy? Pediatr. Allergy Immunol. 2005, 16: 262–266.

Queenan KM, Stewart ML, Smith KN, Thomas W, Fulcher RG, Slavin JL. Concentrated oat beta-glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. Nutr J. 2007, 6:6.

Ramakrishna, B. S., Roberts-Thomas, I. C., Pannall, P. R., Roediger, W.E.W. Impaired sulphation of phenol by the colonic mucosa in quiescent and active colitis. Gut. 1991, 32:46-49.

Rance, F., Kanny, G., Dutau, G., Moneret-Vautrin, D.A. Aspects cliniques de l'allergie alimentaire. Rev. Fr. Allergol. 1998, 38: 900–5.

Reddy, B. S., Engle, A., Simi, B., and Goldman, M. Effect of dietary fiber on colonic bacterial enzymes and bile acids in relation to colon cancer. Gastroenterol. 1992,102:1475–1482.

Reddy, B. S., Mori, H., Nicolais, M. Effect of dietary wheat bran and dehydrated citrus fiber on azoxymethane-induced intestinal carcinogenesis in Fischer 344 rats. J. Natl. Cancer Inst. 1981, 66: 553–557.

Reddy, B., Engle, A., Katsifis, S., Simi, B., Bartram, H-P., Perrino, P., Mahan, C. Biochemical epidemiology of colon cancer: effect of types of dietary fiber on fecal mutagens, acid, and neutral sterols in healthy subjects. Cancer Res. 1989, 49: 4629–4653.

Reeves, P.G. Components of the AIN-93 diets as improvements in the AIN-76A diet. J. Nutr. 1997, 127: 838S-841S.

Riegler, G., Esposito, I. Bristol scale stool form. A still valid help in medical practice and clinical research. Tech Coloproctol. 2001. 5: 163-164.

Roberfroid, M., Prebiotics: The concept revisited, J. Nutr. 2007, 137: 830s-837s.

Roediger WE. Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterol. 1982, 83: 424-429.

Rondini, L., Peyrat-Maillard, M.N., Marsset-Baglieri, A., Fromentin, G., Durand, P., Tome, D., Prost, M., Berset, C. Bound ferulic acid from bran is more bioavailable than the free compound in rat. J. Agric. Food Chem. 2004, 52:4338-4343.

Rossander, L., Sandberg, A.S., Sandstrom, B. The influence of dietary fiber on mineral absorption and utilization. In: "Dietary Fibre – A component of Food", Schweizer, T.F. and Edwards, Ch.A. (eds.), Springer-Verlag, London. 1992, p. 197-216.

Rouau, X., El-Hayek, M.-L. and Moreau, D. Effect of an enzyme preparation containing pentosanases on the bread-making quality of flours in relation to changes in pentosan properties. J. Cereal Sci. 1994, 19: 259-272.

Ruppin, H., Bar-Meir, S., Soergel, K.H., Wood, C.M., Schmitt, M.G. Absorption of short-chain fatty acids by the colon. Gastroenterol. 1980, 78: 1500-1507.

Rycroft CE, Jones MR, Gibson GR, Rastall RA. A comparative in vivo evaluation of the fermentation properties of prebiotic oligosaccharides. J Appl Microbiol 2001;91:878-87.

Sanchez, J.I., Marzorati, M., Grootaert, C., Baran, M., Van Craeyveld, V., Courtin, C.M., Broekaert, W.F., Delcour, J.A., Verstraete, W., Van de Wiele, T. Arabinoxylan-oligosaccharides (AXOS) affects the protein/carbohydrate fermentation balance and microbial population dynamics of the Simulator of Human Intestinal Microbial Ecosystem. Microbial Biotechnol. 2009, 2:101-113.

Sandberg A.-S., Claes Hasselblad, K. The effect of wheat bran on the absorption of minerals in the small intestine. Brit. J. Nutr. 1982, 48:185-191.

Sandberg A.-S., Andersson, H., Hallgren, B., Hasselblad, K., Isaksson, B., and Hulten, L. Experimental model for *in vivo* determination of dietary fibre and its effect on the absorption of nutrients in the small intestine. Br. J. Nutr. 1981, 45: 283-294.

Sandstead, H.H. Fiber, phytates, and mineral nutrition. Nutr. Rev. 1992, 50:30–31.

Sandhu, K.S., El Samahi, M.M., Mena, I., Dooley, C.P., Valenzuela, J.E. Effect of pectin on gastric emptying and gastroduodenal motility in normal subjects. Gastroenterology 1987, 92: 486-492.

Santos, A., San Mauro, M., Diaz, D.M. Prebiotics and their long-term influence on the microbial populations of the mouse bowel. Food Microbiol. 2006, 23:498-503.

Saulnier, L., Peneau, N., Thibault, J.-F. Variability in grain extract viscosity and water-soluble arabinoxylan content in wheat. J. Cereal Sci. 1995, 22: 259-264.

Scholz-Ahrens, K.E., Ade, P., Marten, B., Weber, P., Timm, W., Açil, Y., Gluer, C.C., Schrezenmeir, J. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J Nutr. 2007, 137:838S-846S.

Scholz-Ahrens, K.E., Schrezenmeir, J., Inulin and oligofructose and mineral metabolism: the evidence from animal trials. J Nutr. 2007, 137(11 Suppl):2513S-2523S.

Shue, W. H.-H., Lee, I.-Te, Chen, W., Chan Y.-C. Effects of xylooligosaccharides in type 2 diabetes melitus. J. Nutr. Sci. Vitaminol. (Tokyo). 2008, 54: 396-401.

Seal, C.J. Whole grains and CVD risk. Proc. Nutr. Soc. 2006, 65:24–34.

Slavin, J. Why whole grains are protective: biological mechanisms. Proceedings of the Nutrition Society. 2003, 62: 129–134.

Smiciklas-Wright, H., Mitchell, D.C., Mickle, S.J., Cook, A.J., Goldman, J.D. Foods Commonly Eaten in the United States: Quantities Consumed Per Eating Occasion and in a Day, 1994-1996. U.S. Department of Agriculture NFS Report No. 96-5, pp 252, 2002.

Swennen, K., Courtin, C.M., Lindemans, G.C.J.E., Delcour, J.A. Large-scale production and characterisation of wheat bran arabinoxylooligosaccharides. J. Sci. Food Agri. 2006. 86: 1722-1731.

Tanno, K., Willcox, G. How fast was wild wheat domesticated? Science. 2006. 311: 1886.

Thoma, C., Green, T., Ferguson, L. Citrus pectin and oligofructose improve folate status and lower serum total homocysteine in rats. Int. J. Vit. Nutr. Res. 2003, 73: 403-409.

Timm, D.A., Slavin, J.L., Dietary fiber and the relationship to chronic diseases. Am. J. Lifestyle Med. 2008. 2:233-240.

USDA. Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS) (On CD-ROM). U.S. Department of Agriculture; Riverdale, Maryland. 2005.

Van Craeyveld, V., Swennen, K., Dornez, E., Van de Wiele, T., Marzorati, M., Verstraete, W., Delaedt, Y., Onagbesan, O., Decuypere, E., Buyse, J., De Ketelaere, B., Broekaert, W.F., Delcour, J.A., Courtin, C.M. Structurally different wheat-derived arabinoxylooligosaccharides have different prebiotic and fermentation properties in rats. J. Nutr. 2008, 138: 2348–2355.

Van Haesendonck, I, P.H., Broekaert, W.F., Georis, J., Delcour, J., Courtin, C., Arnaut, F. Bread with increased arabinoxylo-oligosaccharide content. 2008, Publication No. WO08087167.

Van Laere, K. M. J., Voragen, C. H. L., Kroef, T., Van den Broek, L. A. M., Beldman, G., and Voragen, A. G. J. Purification and mode of action of two different arabinoxylan arabinofuranohydrolases from *Bifidobacterium adolescentis* DSM 20083. Appl. Microbiol. Biotechnol., 1999, 51: 606-613.

Visek, W.J. Diet and cell growth modulation by ammonia. Am. J. Clin. Nutr. 1978, 31, Supp 10: S216–S220.

Vietor, R.J., Angelino, S.A.G.F., Voragen, A.G.J. Structural features of arabinoxylans from barley and malt cell wall material. J. Cereal Sci. 1992: 15, 213-222.

Vinkx, C.J.A., Delcour, J.A. Rye (*Secale cereale* L.) arabinoxylans: a critical review. J. Cereal Sci. 1996, 24: 1-14.

Weaver, C.M. Inulin, oligofructose and bone health: experimental approaches and mechanisms. Br. J. Nutr. 2005, 93:S99-S103.

Wong, J.M., de Souza, R., Kendall, C.W., Emam, A., Jenkins, D.J. Colonic health: fermentation and short chain fatty acids. J. Clin. Gastroenterol. 2006, 40: 235-243.

Woodward, J. R., Fincher, G.B., Stone, B. A. Water-soluble (1-3),(1-4)-beta-D glucans from barley (*Hordeum vulgare*) endosperm. II. Fine structure. Carbohydrate Pol. 1982, 3:207–225.

Yamada, H., Itoh, K., Morishita, Y., Taniguchi, H. Structure and properties of oligosaccharides from wheat bran. Cereal Foods World 1993, 38: 490-492.

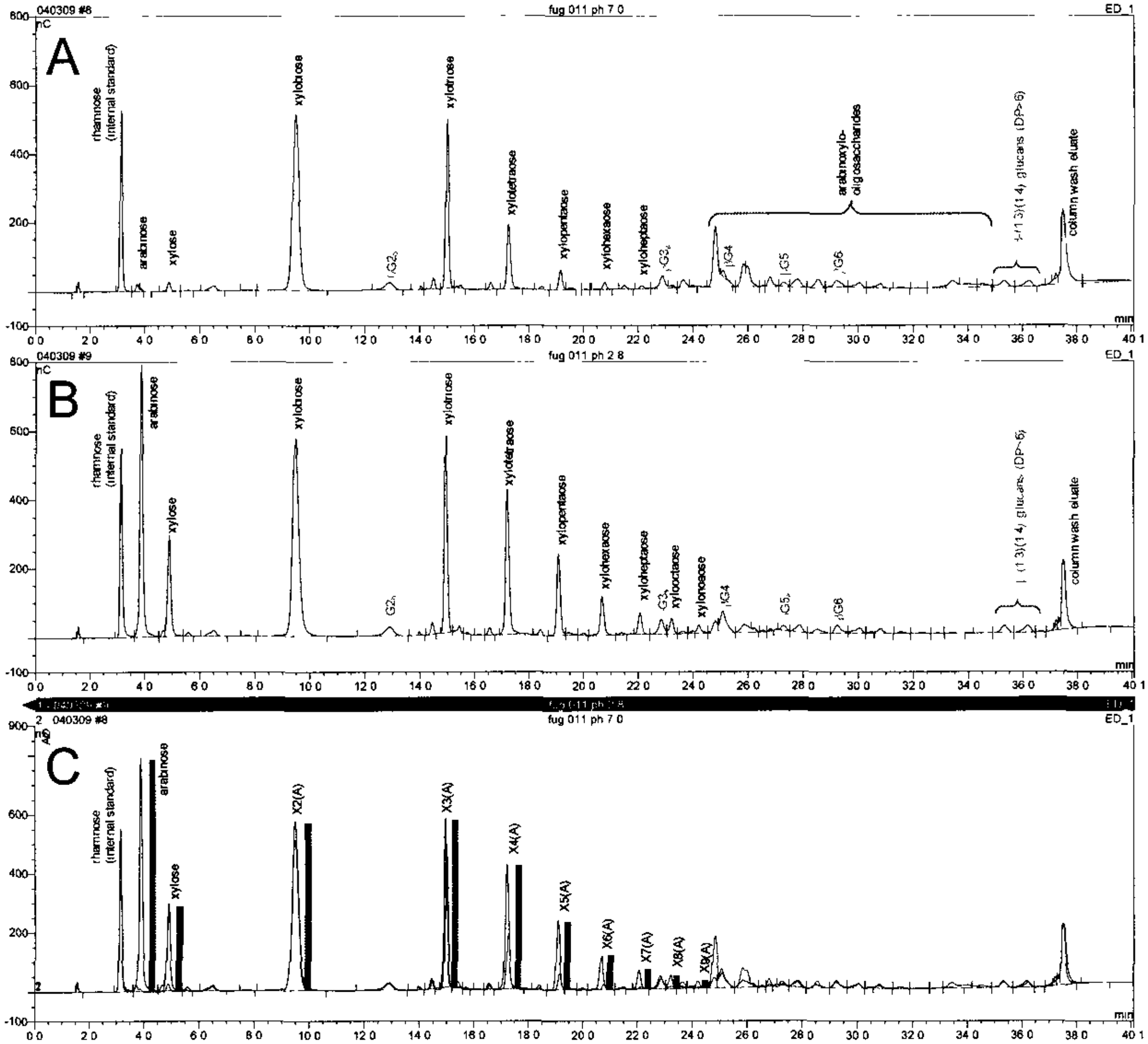
Younes, H., Garleb, K., Behr, S., Remesy, C., Demigne, C. Fermentable fibers or oligosaccharides reduce urinary nitrogen excretion by increasing urea disposal in the rat cecum. J. Nutr. 1995, 125:1010-1016.

Zhao, Z., Egashira, Y., Sanada, H. Ferulic acid sugar esters are recovered in rat plasma and urine mainly as the sulfoglucuronide of ferulic acid. J. Nutr. 2003, 133: 1355-1361.

Zhao, Z., Egashira, Y., Sanada, H. Phenolic antioxidants richly contained in corn bran are slightly bioavailable in rats. J. Agric. Food Chem. 2005, 53:5030-5035.

7. APPENDIX I

Representative HPAEC-PAD patterns of Wheat Bran Extract



HPAEC-PAD analysis of Wheat Bran Extract. (A) Pattern of untreated Wheat Bran Extract, with rhamnose added as an internal standard; (B) Pattern of 'de-arabinosylated' Wheat Bran Extract (through partial hydrolysis for 24h at 90°C at pH2.8), with rhamnose added as an internal standard;(C) Overlay of patterns of untreated Wheat Bran Extract (blue line) and de-arabinosylated Wheat Bran Extract (black line). In panel C, the peak heights of the untreated Wheat Bran Extract are indicated by blue bars at the right side of the peaks and represent the xylo-oligosaccharides, the difference in peak heights of untreated Wheat Bran Extract and de-arabinosylated Wheat Bran Extract are indicated by black bars at the right side of the peaks and represent the arabinoxylo-oligosaccharides. In panels A and B, the β-glucans are indicated in green: $\beta G2_b$ is cellobiose, $\beta G3_a$ is Glc-β1,3-Glc-β1,4-Glc, $\beta G4_a$ is Glc-β1,3-Glc-β1,4-Glc-β1,4-Glc, $\beta G5_a$ is Glc-β1,3-Glc-β1,4-Glc-β1,4-Glc-β1,4-Glc, and $\beta G6_a$ is Glc-β1,3-Glc-β1,4-Glc-β1,4-Glc-β1,4-Glc-β1,4-Glc.

It is important to note that the PAD response factors on dry weight basis of the different oligosaccharides decrease with increasing degree of polymerization.

8. APPENDIX II

ESTIMATED DAILY INTAKE OF WHEAT BRAN EXTRACT BY THE U.S. POPULATION FROM PROPOSED FOOD-USES

(Attached separately)

Estimated Daily Intake of Wheat Bran Extract (WBE) by the U.S. Population from Proposed Food-Uses

January 29, 2010

Estimated Daily Intake of Wheat Bran Extract (WBE) by the U.S. Population from Proposed Food-Uses

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Estimated Daily Intake of Wheat Bran Extract (WBE) by the U.S. Population from Proposed Food-Uses

1.0 INTRODUCTION

This document provides an assessment of the consumption of Wheat Bran Extract (WBE) by the United States (U S) population as proposed for use in baked goods and baking mixes; beverages and beverage bases, breakfast cereals; frozen dairy desserts; gelatins, puddings and fillings; grain products and pastas, jams and jellies, commercial; milk products, processed fruit and fruit juices, processed vegetables and vegetable juices, and snack foods

Estimates for the intake of WBE were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006, USDA, 2009) Calculations for the mean and 90th percentile all-person and all-user intakes, and percentage of consumers were performed for each of the individual proposed food-uses of WBE. Similar calculations were used to determine the estimated total intake of WBE from all proposed food-uses combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups

infants, ages 0 to 2;
children, ages 3 to 11,
female teenagers, ages 12 to 19;
male teenagers, ages 12 to 19,
female adults, ages 20 and up;
male adults, ages 20 and up, and
total population (all age and gender groups combined).

2.0 FOOD CONSUMPTION SURVEY DATA

2.1 Survey Description

National Health and Nutrition Examination Surveys (NHANES) for the years 2003-2004 are available for public use NHANES are conducted on a continual yearly basis, data from these surveys are released in 2-year cycles. Each year about 7,000 people from 15 different locations across the U S. are interviewed, and approximately 5,000 complete the health examination component of the survey Any combination of consecutive years of data collection is a nationally representative sample of the U.S population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Anderson, 1988) Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2003-2004 survey, these data were used to generate estimates for the current intake analysis.

The surveys provide the most appropriate data for evaluating food-use and food consumption patterns in the United States and contain 2 years of data on individuals selected *via* stratified multistage probability sampling of the U.S civilian non-institutionalized population

NHANES 2003-2004 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone within the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S. Small counties were combined to attain a minimum population size These PSUs were segmented and households were chosen within each segment One or more participants within a household were interviewed. Fifteen PSUs are visited each year. For NHANES 2003-2004, 12,761 individuals were selected for the sample, and 10,122 were interviewed (79 3%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2003-2004 collected socioeconomic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES 2003-2004 to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2009)

2.2 Statistical Methods

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of WBE by the U.S population Estimates for the daily intake of WBE represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2003-2004 data, these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S population All-person intake refers to the estimated intake of WBE averaged over all individuals surveyed, regardless of whether they potentially consumed food products containing WBE, and therefore includes "zero" consumers (those who reported no intake of food products containing WBE during the 2 survey days) All-user intake refers to the estimated intake of WBE by those individuals potentially consuming food products containing WBE, hence the "all-user" designation Individuals were considered users if they consumed 1 or more food products containing WBE on either Day 1 or Day 2 of the survey.

2.3 Statistical Reliability

Mean or percentile intake estimates based on small sample sizes or with high variability relative to the mean [assessed using the coefficient of variation (CV)] may be less statistically reliable than estimates based on adequate sample sizes or low variability relative to the mean (LSRO, 1995). Data presented herein for the estimated daily intake of WBE follow the guidelines proposed by the Human Nutrition Information Service/National Center for Health Statistics Analytic Working Group for evaluating the reliability of statistical estimates adopted in the "Third Report on Nutrition Monitoring in the United States", whereby an estimated mean may be unreliable if the CV is equal to or greater than 30% (LSRO, 1995) The CV is the ratio of the estimated standard error of the mean to the estimated mean, expressed as a percentage (LSRO, 1995). Therefore, for the estimated intakes of WBE presented herein, values were considered statistically unreliable if the CV was equal to or greater than 30% or the sample size is less than 30 respondents These values were not considered when assessing the relative contribution of specific food-uses to total WBE consumption and are marked with an asterisk.

3.0 FOOD USAGE DATA

The individual proposed food-uses and use-levels for WBE employed in the current intake analysis are summarized in Table 3-1. Food codes representative of each proposed food-use were chosen from the NHANES 2003-2004 (CDC, 2006; USDA, 2009). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2009). All food codes included in the current intake assessment are listed in Appendix C

Table 3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Wheat Bran Extract in the U.S.

Food Category	Proposed Food-Uses	Serving Size	Dose per Serving	Use-Levels (%)
Baked Goods and Baking Mixes	Biscuits	30 g	2 7 g	9 0
	Breads and Rolls (rye and reduced-calorie white bread)	80 g	2 4 g	3 0
	Breakfast Pastries (*i e* Danish)	30 g	2 7 g	9 0
	Cakes	55 g	3 g	5 45
	Cookies and Brownies	30 g	2 7 g	9 0
	Crackers (low sodium, regular, and sweet)	30 g	2 7 g	9 0
Beverages and Beverage Bases	Carbonated Beverages (diet or sugar free)	250 ml	1 5 ml	0 6

Table 3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Wheat Bran Extract in the U.S.				
Food Category	**Proposed Food-Uses**	**Serving Size**	**Dose per Serving**	**Use-Levels (%)**
	Meal Replacement Beverages (Soy based)	250 ml	3 ml	1 2
Breakfast Cereals	Ready-to-Eat Cereals	30 g	2 7 g	9 0
Frozen Dairy Desserts	Ice Cream and Novelties	100 g	2 4 g	2 4
	Frozen Yogurt	125 g	3 g	2 4
Gelatins, Puddings and Fillings	Custards and Puddings	100 g	2 4 g	2 4
Grain Products and Pastas	Meal Replacement, Granola and Cereal Bars	30 g	2 7 g	9 0
Jams and Jellies, Commercial	Jams, Jellies and Preserves	20 g	3 g	15 0
Milk Products	Flavored Milk and Milk Drinks	180 ml	1 08 ml	0 6
	Milk-based Meal Replacements	250 ml	3 ml	1 2
	Yogurt	125 g	3 g	2 4
Processed Fruit and Fruit Juices	Fruit Juices (Citrus and Non-Citrus)	250 ml	1 5 ml	0 6
Processed Vegetables and Vegetable Juices	Tomato Juice	250 ml	1 5 ml	0 6
Snack Foods	Pretzels	30 g	2 7 g	9 0

4.0 FOOD SURVEY RESULTS

Estimates for the total daily intakes of WBE from all proposed food-uses are provided in Tables 4 1-1 and 4 1-2 Estimates for the daily intake of WBE from individual proposed food-uses in the U S are summarized in Tables A-1 to A-7 and B-1 to B-7 of Appendices A and B, respectively Tables A-1 to A-7 provide estimates for the daily intake of WBE per person (g/day), whereas Tables B-1 to B-7 provide estimates for the daily intake of WBE on a per kilogram body weight basis (mg/kg body weight/day).

4.1 Estimated Daily Intake of Wheat Bran Extract from All Proposed Food-Uses

The estimated total intake of WBE from all proposed food-uses in the U.S. by population group is summarized in Table 4.1-1 Table 4 1-2 presents these data on a per kilogram body weight basis.

Approximately 84.1% of the total U.S population was identified as potential consumers of WBE from the proposed food-uses (6,951 actual users identified) Consumption of these types of foods by the total U S. population resulted in estimated mean all-person and all-user intakes of

WBE of 4.2 g/person/day (83 mg/kg body weight/day) and 5 0 g/person/day (97 mg/kg body weight/day), respectively (Tables 4.1-1 and 4.1-2) The 90th percentile all-person and all-user intakes of WBE from all proposed food-uses by the total population were 9 4 g/person/day (199 mg/kg body weight/day) and 10 1 g/person/day (217 mg/kg body weight/day), respectively.

Children reported the greatest percentage of users of the population groups at 96.4%, followed by female teenagers at 86.9% and female adults at 86 0% Infants reported the lowest percentage of users of any population group at 69.7% On an individual population basis, the greatest mean all-person intake of WBE on an absolute basis was determined in children, at 5.0 g/person/day (200 mg/kg body weight/day). The greatest mean all-user intake of WBE on an absolute basis was determined in male teenagers and male adults, at 5.4 g/person/day for both population groups (88 and 64 mg/kg body weight/day in male teenagers and male adults, respectively). Infants had the lowest mean all-person and all-user intakes of WBE on an absolute basis, with values of 3 0 and 3.8 g/person/day, respectively. On a body weight basis, mean all-person and all-user intakes of WBE were highest in infants, with intakes of 248 and 313 mg/kg body weight/day, respectively. The lowest mean all-person and all-user intakes on a per kilogram body weight basis were observed in male adults, with values of 51 and 64 mg/kg body weight/day, respectively (Table 4 1-2).

Table 4.1-1 Summary of the Estimated Daily Intake of Wheat Bran Extract from All Proposed Food-Uses in the U.S. by Population Group (2003-2004 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Infants	0 to 2	69 7	648	3 0	6 7	3 8	7 0
Children	3 to 11	96 4	1,241	5 0	9 4	5 2	9 5
Female Teenagers	12 to 19	86 9	862	4 0	8 9	4 6	9 8
Male Teenagers	12 to 19	84 1	840	4 6	10 1	5 4	11 0
Female Adults	20 and Up	86 0	1,831	4 1	9 1	4 7	9 6
Male Adults	20 and Up	79 3	1,529	4 3	10 4	5 4	11 4
Total Population	All Ages	84 1	6,951	4 2	9 4	5 0	10 1

When heavy consumers (90th percentile) were assessed, all-person and all-user intakes of WBE from all proposed food-uses were determined to be greatest in male adults (10.4 and 11 4 g/person/day, respectively). The lowest 90th percentile all-person and all-user intakes were in infants, with values of 6 7 and 7 0 g/person/day, respectively, on an absolute basis (Table 4.1-1) On a body weight basis, infants were determined to have the greatest all-person and all-user 90th percentile intakes of WBE, with values of 566 and 635 mg/kg body weight/day, respectively (Table 4 1-2). The lowest all-person and all-user 90th percentile intakes of WBE on a body weight basis were observed in female adults (121 and 131 mg/kg body weight/day, respectively).

Table 4.1-2 Summary of the Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from All Proposed Food-Uses in the U.S. by Population Group (2003-2004 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Infants	0 to 2	69 7	648	248	566	313	635
Children	3 to 11	96 4	1,241	200	426	206	431
Female Teenagers	12 to 19	86 9	862	72	164	83	169
Male Teenagers	12 to 19	84 1	840	75	165	88	185
Female Adults	20 and Up	86 0	1,831	57	121	66	131
Male Adults	20 and Up	79 3	1,529	51	128	64	139
Total Population	All Ages	84 1	6,951	83	199	97	217

4.1.1 All-Person Intakes from Individual Food Categories

Estimates for the mean and 90th percentile daily intakes of WBE from each individual proposed food-use are summarized in Tables A-1 to A-7 and B-1 to B-7 on a g/day and mg/kg body weight/day basis, respectively Tables A-7 and B-7 summarize the estimates for the mean all-person intakes of WBE by the total population (all ages) from each of the individual proposed food-uses on a g/person/day and mg/kg body weight/day basis, respectively The total U S population was identified as being significant consumers of fruit juice (46 7% users), ready-to-eat cereals (41.1% users), and crackers (21 2% users)

Consumption of ready-to-eat cereals provided the largest mean and 90th percentile all-person intakes of WBE, at 1 3 g/person/day (26 mg/kg body weight/day) and 4.3 g/person/day (80 mg/kg body weight/day), respectively In addition, high mean and 90th percentile all-person intakes of WBE resulted from the consumption of carbonated beverages (diet or sugar-free) (0 6 and 2 1 g/person/day, respectively) and fruit juice (0 6 and 1.7 g/person/day, respectively) On a body weight basis, mean and 90th percentile all-person intakes for fruit juice were 13 and 36 mg/kg body weight/day, and for carbonated beverages (diet or sugar-free) were 8 and 26 mg/kg body weight/day, respectively

Of the individual population groups, the highest mean and 90th percentile all-person intakes of WBE were determined to occur in infants consuming fruit juice, and in all other population groups consuming ready-to-eat cereals (Tables A-1 to A-6 and Tables B-1 to B-6) The highest mean all-person intake of WBE was reported in children consuming ready-to-eat cereals, at 2.0 g/person/day (75 mg/kg body weight/day), while the highest 90th percentile all-person intake of WBE was reported in male teenagers consuming ready-to-eat cereals, at 5 8 g/person/day

(97 mg/kg body weight/day) On a body weight basis, consumption of ready-to-eat cereals led to the highest mean all-person intake of WBE in children (75 mg/kg body weight/day) The highest 90th percentile intake of WBE on a body weight basis occurred in infants from the consumption of fruit juice (209 mg/kg body weight/day)

4.1.2 All-User Intakes from Individual Food Categories

Tables A-7 and B-7 also summarize the estimates for the mean all-user intakes of WBE by the total population (all ages) from each of the individual food-uses on a mg/person/day and mg/kg body weight/day basis, respectively. For all-user intakes, the contribution of each food-use to the overall intake is a function of both the estimated intake of WBE resulting from the consumption of the food, as well as the percentage of users identified as consumers of the food. For example, for the total population, the consumption of breakfast pastries resulted in an estimated mean all-user WBE intake of 4 3 g/person/day; however, only 196 users (2.4% of the total population) of breakfast pastries were identified and therefore, the contribution of this food-use to the mean all-user intake of WBE was not as important as the flavored milk and milk drinks (1,212 users representing 14 7% of the total population), for which the estimated mean all-user WBE intake was reported to be 1 4 g/person/day

Consumers of ready-to-eat cereals were identified as making the greatest contribution to the mean and 90th percentile all-user intakes of WBE at 3 4 and 6.4 g/person/day, respectively (68 and 139 mg/kg body weight/day, respectively). Of the other food-uses, the consumption of fruit juice, carbonated beverages (diet and sugar-free), and crackers also made significant contributions to the estimates for the mean (1 4, 3 4, and 1 4 g/person/day, respectively) and 90th percentile (2.8, 7 8, and 2 7 g/person/day, respectively) all-user intakes of WBE by the total population.

On an individual population group basis, the most significant contribution to the mean and 90th percentile all-user intakes of WBE were determined in infants consuming fruit juice, and the consumption of ready-to-eat cereals in all other population groups (Tables A-1 to A-6 and Tables B-1 to B-6) Children consuming ready-to-eat cereals made the largest contribution to the mean and 90th percentile all-user intakes of WBE at 3 0 and 6 0 g/person/day, respectively (112 and 231 mg/kg body weight/day, respectively) On a per kilogram body weight basis, infants consuming yogurt experienced the highest mean and 90th percentile all-user intakes of WBE at 143 and 274 mg/kg body weight/day, respectively

The estimated intakes of WBE were considered statistically unreliable if the CV was equal to or greater than 30% or the sample size was less than 30 individuals. Assessing the sample size for the intake estimate found that the intake values for cakes were unreliable in the total population. Assessing the CV and sample size for all-user intake estimates found the intakes for breads and rolls (rye and white breads), breakfast pastries (Danish), cookies and brownies, carbonated beverages (diet and sugar-free), meal replacement beverages (soy-based), ice cream and novelties, frozen yogurt, custards and puddings, meal replacement, granola and cereal bars, jams, jellies and preserves, milk-based meal replacement beverages and tomato juice to be statistically unreliable in infants. Furthermore, the cakes category contained no infant users thus there was no intake of WBE from this category The intake values for WBE from breads and rolls (rye and white breads), cookies and brownies, meal replacement beverages (soy-based), frozen yogurt, custards and puddings, milk-based meal replacement beverages, and tomato juice were statistically unreliable in children In female teenagers and male teenagers, the intake values for WBE were unreliable for the following food uses: breads and rolls (rye and white breads), cookies and brownies, meal replacement beverages (soy-based), frozen yogurt, custards and puddings, jams, jellies and preserves, milk-based meal replacement beverages, and tomato juice The intakes for breakfast pastries (Danish), meal replacement beverages (soy-based), and custards and puddings were unreliable in female adults, and breakfast pastries (Danish), cakes, meal replacement beverages (soy-based), custards and puddings, and milk-based meal replacement beverages were determined to be unreliable in male adults The statistical reliability of the all-person and all-user intakes of WBE among the individual population groups is indicated in Tables A-1 through A-6, and Tables B-1 through B-6 Due to the fact that intakes of WBE resulting from the intake of foods for which CV values are greater than 30% or for which fewer than 30 respondents were identified are not considered to be statistically reliable, these have been omitted from the discussion.

5.0 CONCLUSIONS

Consumption data and information pertaining to the individual proposed food-uses of WBE were used to estimate the all-person and all-user intakes of WBE for specific demographic groups and for the total U S population This type of intake methodology is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate the consumption of food products that are consumed relatively infrequently

In summary, on an all-user basis, the mean intake of WBE by the total U.S. population from all proposed food-uses was estimated to be 5 0 g/person/day or 97 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of WBE by the total U S. population from all proposed food-uses was estimated to be 10.1 g/person/day or 217 mg/kg body weight/day. Furthermore, the consumption of ready-to-eat cereals was determined to make the greatest impact on the mean and 90th percentile all-user intakes of WBE, with values of 3.4 g/person/day (68 mg/kg body weight/day) and 6.4 g/person/day (139 mg/kg body weight/day), respectively.

6.0 REFERENCES

Anderson SA, editor (1988). *Estimation of Exposure to Substances in the Food Supply* (Contract No. FDA 223-84-2059). Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB), Life Science Research Office (LSRO).

CDC (2006). *Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES)* Hyattsville (MD). Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf

CFR (2009). Part 170—Food additives. Section §170.3—Definitions In: *U.S. Code of Federal Regulations (CFR). Title 21. Food and Drugs (U S Food and Drug Administration).* Washington (DC) U.S. Food and Drug Administration (U.S FDA), U.S. Government Printing Office (GPO), pp 5-9. Available at: http://edocket.access.gpo.gov/cfr_2009/aprqtr/pdf/21cfr170.3.pdf.

LSRO (1995) *Third Report on Nutrition Monitoring in the United States* Prepared by Bethesda (MD) Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB) for the Interagency Board for Nutrition Monitoring and Related Research Washington (DC). U.S Government Printing Office, vol 1, pp. 19-31 & III-1 to III-10 and vol 2, pp VB-1 to VB-2

USDA (2000) *1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS)* (On CD-ROM, PB2000-500027) Riverdale (MD): U.S. Department of Agriculture (USDA).

USDA (2009). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2003-2004.* Riverdale (MD): U.S Department of Agriculture (USDA) Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release

APPENDIX A

Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Different Population Groups Within the United States (NHANES 2003-2004)

Table A-1 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Infants (Aged 0 to 2 Years) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Baked Goods and Baking Mixes						
Biscuits	4 0	37	< 0 1	na	0 8	1 4
Breads and Rolls (rye and white bread)	0 5	5	< 0 1*	na	0 3*	0 7*
Breakfast Pastries (i.e Danish)	1 4	13	< 0 1*	na	1 9*	4 7*
Cakes	0 0	0	na	na	na	na
Cookies and Brownies	1 5	14	< 0 1*	na	0 9*	2 6*
Crackers (low sodium, regular, and sweet)	35 6	331	0 5	1 4	1 2	2 6
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	1.6	15	< 0 1*	na	0 4*	0 7*
Meal Replacement Beverages (Soy based)	0.1	1	< 0 1*	na	1 5*	1 5*
Breakfast Cereals						
Ready-to-Eat Cereals	41 7	388	0 7	2 0	1 4	2 7
Frozen Dairy Desserts						
Ice Cream and Novelties	2 0	19	< 0 1*	na	0 8*	1 1*
Frozen Yogurt	1.0	9	< 0 1*	na	0 9*	1 4*
Gelatins, Puddings and Fillings						
Custards and Puddings	0 8	7	< 0 1*	na	1 6*	3 1*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	2 3	21	0 1*	na	1 6*	1 9*
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	2 8	26	0 1*	na	0 9*	2 0*

Table A-1 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Infants (Aged 0 to 2 Years) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (g)	90^{th} Percentile (g)	Mean (g)	90^{th} Percentile (g)
Milk Products						
Flavored Milk and Milk Drinks	8.2	76	0 1	0 4	1 1	2 7
Milk-based Meal Replacements	0 1	1	< 0 1*	na	0 4*	0 4*
Yogurt	12.9	120	0 3	1 4	1 7	2 9
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	50 6	471	0 9	2.5	1 6	3 3
Processed Vegetables and Vegetable Juices						
Tomato Juice	0 3	3	< 0 1*	na	0.4*	0 5*
Snack Foods						
Pretzels	4 7	44	0 1	na	0.8	1 9

na = not available
* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

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Table A-2	**Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Children (Aged 3 to 11 Years) Within the United States (2003-2004 NHANES Data)**					
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (g)	**90th Percentile (g)**	**Mean (g)**	**90th Percentile (g)**
Baked Goods and Baking Mixes						
Biscuits	6 8	87	0 2	na	2 0	3 3
Breads and Rolls (rye and white bread)	1 5	19	< 0 1*	na	0 6*	0 9*
Breakfast Pastries (i e Danish)	4 3	55	0 2	na	3 4	4 7
Cakes	0 1	1	< 0 1*	na	3 4*	3 4*
Cookies and Brownies	2 0	26	< 0 1*	na	1 1*	1 9*
Crackers (low sodium, regular, and sweet)	25 7	331	0 4	1 4	1 4	3 1
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	5 1	66	0 1	na	1 2	2.8
Meal Replacement Beverages (Soy based)	0 2	2	< 0 1*	na	1 9*	1 9*
Breakfast Cereals						
Ready-to-Eat Cereals	66 5	856	2.0	5.0	3.0	6 0
Frozen Dairy Desserts						
Ice Cream and Novelties	5 2	67	0.1	na	1 6	2 9
Frozen Yogurt	1 3	17	< 0 1*	na	1 2*	2.4*
Gelatins, Puddings and Fillings						
Custards and Puddings	0 8	10	< 0 1*	na	1 9*	2 7*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	4 1	53	0 2	na	2.0	3 9
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	5 7	73	0 1	na	1 7	5 6

Table A-2 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Children (Aged 3 to 11 Years) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (g)	**90th Percentile (g)**	**Mean (g)**	**90th Percentile (g)**
Milk Products						
Flavored Milk and Milk Drinks	32 6	420	0 5	1 5	1 3	2 2
Milk-based Meal Replacements	0 2	3	< 0 1*	na	1 4*	1 9*
Yogurt	12 1	156	0 3	1 4	2 1	2 9
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	66 1	851	0 8	2 1	1.3	2 7
Processed Vegetables and Vegetable Juices						
Tomato Juice	0 3	4	< 0 1*	na	0 8*	1 0*
Snack Foods						
Pretzels	7 9	102	0 2	0 3	2 2	4 8

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

Table A-3 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of *Total Users*	All-Person Consumption		All-User Consumption	
			Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Baked Goods and Baking Mixes						
Biscuits	7 2	71	0 2	na	2 8	5 7
Breads and Rolls (rye and white bread)	1 8	18	< 0 1*	na	0 7*	1.6*
Breakfast Pastries (i e Danish)	3 7	37	0.2	na	4 0	7 0
Cakes	0 1	1	< 0 1*	na	1 0*	1 0*
Cookies and Brownies	2 0	20	< 0.1*	na	1 1*	1 8*
Crackers (low sodium, regular, and sweet)	17 0	169	0 3	1.3	1 4	2 7
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	7 8	77	0 2	0 1	2.0	4.3
Meal Replacement Beverages (Soy based)	0 2	2	< 0 1*	na	0.8*	2 3*
Breakfast Cereals						
Ready-to-Eat Cereals	42 1	418	1 5	4 5	3 5	6.9
Frozen Dairy Desserts						
Ice Cream and Novelties	4 1	41	0 1	na	1 9	3 7
Frozen Yogurt	1 1	11	< 0 1*	na	1 8*	3.5*
Gelatins, Puddings and Fillings						
Custards and Puddings	0 2	2	< 0 1*	na	2 4*	2 5*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	5 6	56	0 2	na	2 5	3 3
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	2 3	23	0 1*	na	1 9*	4 0*

Table A-3 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (g)	**90th Percentile (g)**	**Mean (g)**	**90th Percentile (g)**
Milk Products						
Flavored Milk and Milk Drinks	20 0	198	0 2	0 8	1 1	2 1
Milk-based Meal Replacements	0 2	2	< 0.1*	na	2 6*	4 0*
Yogurt	6 6	65	0.2	na	3.1	4 9
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	46.3	459	0 6	2 0	1.5	2 9
Processed Vegetables and Vegetable Juices						
Tomato Juice	0 3	3	< 0 1*	na	1 7*	1 9*
Snack Foods						
Pretzels	6.1	61	0 2	na	2 3	5.2

na = not available
* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

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Table A-4 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (g)	**90th Percentile (g)**	**Mean (g)**	**90th Percentile (g)**
Baked Goods and Baking Mixes						
Biscuits	7.5	75	0.2	na	3.3	5.4
Breads and Rolls (rye and white bread)	1.3	13	< 0.1*	na	1.2*	1.7*
Breakfast Pastries (i.e. Danish)	5.1	51	0.4	na	5.1	9.4
Cakes	0.2	2	< 0.1*	na	3.5*	4.2*
Cookies and Brownies	2.0	20	0.1*	na	1.6*	3.9*
Crackers (low sodium, regular, and sweet)	10.8	108	0.2	na	1.7	2.8
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	4.8	48	0.2	na	3.2	5.4
Meal Replacement Beverages (Soy based)	0.1	1	< 0.1*	na	0.5*	0.5*
Breakfast Cereals						
Ready-to-Eat Cereals	43.8	438	1.9	5.8	4.3	7.3
Frozen Dairy Desserts						
Ice Cream and Novelties	4.5	45	0.1	na	1.8	4.5
Frozen Yogurt	0.4	4	< 0.1*	na	4.0*	8.8*
Gelatins, Puddings and Fillings						
Custards and Puddings	0.1	1	< 0.1*	na	0.8*	0.8*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	4.1	41	0.2	na	2.4	5.0
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	2.5	25	0.1*	na	2.0*	3.7*

000082

Table A-4 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Milk Products						
Flavored Milk and Milk Drinks	19 9	199	0 3	1 0	1 5	3 3
Milk-based Meal Replacements	0 2	2	< 0 1*	na	2.1*	2.1*
Yogurt	3 0	30	0 1	na	3 1	5 0
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	45 3	453	0 7	2.5	1 8	3 9
Processed Vegetables and Vegetable Juices						
Tomato Juice	0 4	4	< 0 1*	na	2.4*	2 9*
Snack Foods						
Pretzels	5 5	55	0 2	na	3 0	6 4

na = not available
* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

000083

Table A-5 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Baked Goods and Baking Mixes						
Biscuits	9.0	191	0 2	na	2 5	4 3
Breads and Rolls (rye and white bread)	6 9	146	0 1	na	0.8	1 6
Breakfast Pastries (i e. Danish)	1 0	22	0 1*	na	4 3*	4 7*
Cakes	0 6	12	< 0.1*	na	1 3*	2 5*
Cookies and Brownies	3 1	65	0.1	na	2.0	3.4
Crackers (low sodium, regular, and sweet)	22 8	485	0 3	1 1	1 2	2 5
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	21 0	448	0 8	2 9	3 2	6.7
Meal Replacement Beverages (Soy based)	0 7	14	< 0 1*	na	2 0*	4 0*
Breakfast Cereals						
Ready-to-Eat Cereals	33 2	707	1 0	3 5	3 0	5 4
Frozen Dairy Desserts						
Ice Cream and Novelties	6.2	131	0.1	na	1 7	3.1
Frozen Yogurt	2 1	45	< 0 1	na	2 3	4 8
Gelatins, Puddings and Fillings						
Custards and Puddings	0 7	15	< 0 1*	na	1 9*	3 5*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	5 4	114	0 2	na	2 5	5 9
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	6 8	144	0 1	na	1 6	3 2

Table A-5 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (g)	**90th Percentile (g)**	**Mean (g)**	**90th Percentile (g)**
Milk Products						
Flavored Milk and Milk Drinks	9 3	197	0 2	na	1 6	3 0
Milk-based Meal Replacements	1 5	31	< 0 1	na	3 0	5.4
Yogurt	8 9	190	0 2	na	2 5	4 1
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	42 6	906	0 5	1 5	1 2	2.4
Processed Vegetables and Vegetable Juices						
Tomato Juice	2 5	54	< 0 1	na	0 8	1 3
Snack Foods						
Pretzels	5 1	108	0 2	na	2 5	6 4

na = not available
* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

000085

Table A-6 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Baked Goods and Baking Mixes						
Biscuits	8 8	170	0 3	na	3 6	7.2
Breads and Rolls (rye and white bread)	6 7	129	0 1	na	0.9	1 6
Breakfast Pastries (i e Danish)	0 9	18	0.1*	na	5 8*	9 4*
Cakes	0 4	7	< 0 1*	na	3 1*	5 7*
Cookies and Brownies	2 6	50	0 1	na	2 2	5.8
Crackers (low sodium, regular, and sweet)	17 1	330	0 3	1 1	1 7	3 1
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	16 1	311	0 8	2 1	4 2	9.6
Meal Replacement Beverages (Soy based)	1 1	21	< 0.1*	na	2 9*	6.0*
Breakfast Cereals						
Ready-to-Eat Cereals	30 4	587	1 3	4 6	4 2	8 3
Frozen Dairy Desserts						
Ice Cream and Novelties	3 9	76	0 1	na	2 0	3 8
Frozen Yogurt	1 8	34	< 0 1	na	2.3	3 9
Gelatins, Puddings and Fillings						
Custards and Puddings	0 9	18	< 0 1*	na	1 7*	3 8*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	4 6	89	0 2	na	2 4	3 9
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	7.3	140	0 2	na	2 4	6 0

Table A-6 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (g)	**90th Percentile (g)**	**Mean (g)**	**90th Percentile (g)**
Milk Products						
Flavored Milk and Milk Drinks	6 3	122	0 1	na	1 6	3.0
Milk-based Meal Replacements	1 1	22	0 1*	na	4 8*	10 5*
Yogurt	4 5	87	0 1	na	2 5	4 8
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	37 4	722	0 5	1 6	1 5	3 1
Processed Vegetables and Vegetable Juices						
Tomato Juice	2 4	47	< 0 1	na	0 9	1 5
Snack Foods						
Pretzels	4 9	94	0 2	na	2 8	6 3

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

Table A-7 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Baked Goods and Baking Mixes						
Biscuits	7 6	631	0 2	na	2 8	5 5
Breads and Rolls (rye and white bread)	4 0	330	0 1	na	0 9	1.6
Breakfast Pastries (i e Danish)	2 4	196	0 1	na	4 3	8 2
Cakes	0 3	23	< 0 1*	na	1 7*	2 7*
Cookies and Brownies	2 4	195	0 1	na	1 9	3 4
Crackers (low sodium, regular, and sweet)	21 2	1,754	0 3	1 1	1 4	2 7
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	11 7	965	0 6	2 1	3 4	7 8
Meal Replacement Beverages (Soy based)	0 5	41	< 0 1	na	2 4	4 7
Breakfast Cereals						
Ready-to-Eat Cereals	41 1	3,394	1 3	4 3	3 4	6.4
Frozen Dairy Desserts						
Ice Cream and Novelties	4 6	379	0 1	na	1.7	3 4
Frozen Yogurt	1 5	120	< 0 1	na	2 2	4 2
Gelatins, Puddings and Fillings						
Custards and Puddings	0.6	53	< 0 1	na	1 9	3 5
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	4 5	374	0.2	na	2 4	3 9
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	5 2	431	0 1	na	1.9	4 5

000088

Table A-7 Estimated Daily Intake of Wheat Bran Extract from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Milk Products						
Flavored Milk and Milk Drinks	14 7	1,212	0 2	0 8	1 4	2 7
Milk-based Meal Replacements	0.7	61	< 0 1	na	3 7	7 7
Yogurt	7 8	648	0 2	na	2 4	4 4
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	46 7	3,862	0 6	1 7	1 4	2 8
Processed Vegetables and Vegetable Juices						
Tomato Juice	1 4	115	< 0 1	na	0.9	1 8
Snack Foods						
Pretzels	5 6	464	0 2	na	2 5	6 0

na = not available
* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

APPENDIX B

Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Different Population Groups Within the United States (NHANES 2003-2004)

Table B-1 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Infants (Aged 0 to 2 Years) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Baked Goods and Baking Mixes						
Biscuits	4.0	37	4	na	68	102
Breads and Rolls (rye and white bread)	0.5	5	< 1*	na	28*	54*
Breakfast Pastries (i.e. Danish)	1.4	13	3*	na	166*	363*
Cakes	0.0	0	na	na	na	na
Cookies and Brownies	1.5	14	1*	na	74*	221*
Crackers (low sodium, regular, and sweet)	35.6	331	44	148	98	214
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	1.6	15	< 1*	na	29*	54*
Meal Replacement Beverages (Soy based)	0.1	1	< 1*	na	128*	128*
Breakfast Cereals						
Ready-to-Eat Cereals	41.7	388	60	172	115	234
Frozen Dairy Desserts						
Ice Cream and Novelties	2.0	19	2*	na	67*	80*
Frozen Yogurt	1.0	9	1*	na	75*	119*
Gelatins, Puddings and Fillings						
Custards and Puddings	0.8	7	2*	na	162*	318*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	2.3	21	5*	na	146*	185*
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	2.8	26	5*	na	79*	156*

000091

Table B-1 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Infants (Aged 0 to 2 Years) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (mg/kg)	**90th Percentile (mg/kg)**	**Mean (mg/kg)**	**90th Percentile (mg/kg)**
Milk Products						
Flavored Milk and Milk Drinks	8 2	76	10	30	85	178
Milk-based Meal Replacements	0 1	1	< 1*	na	25*	25*
Yogurt	12 9	120	30	130	143	274
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	50 6	471	74	209	130	271
Processed Vegetables and Vegetable Juices						
Tomato Juice	0 3	3	< 1*	na	39*	42*
Snack Foods						
Pretzels	4 7	44	6	na	69	177

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

Table B-2 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Children (Aged 3 to 11 Years) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (mg/kg)	**90th Percentile (mg/kg)**	**Mean (mg/kg)**	**90th Percentile (mg/kg)**
Baked Goods and Baking Mixes						
Biscuits	6 8	87	7	na	81	134
Breads and Rolls (rye and white bread)	1.5	19	1*	na	23*	38*
Breakfast Pastries (i e Danish)	4 3	55	7	na	127	205
Cakes	0 1	1	< 1*	na	222*	222*
Cookies and Brownies	2 0	26	1*	na	39*	77*
Crackers (low sodium, regular, and sweet)	25 7	331	17	58	57	122
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	5 1	66	3	na	36	63
Meal Replacement Beverages (Soy based)	0 2	2	<1*	na	149*	158*
Breakfast Cereals						
Ready-to-Eat Cereals	66 5	856	75	182	112	231
Frozen Dairy Desserts						
Ice Cream and Novelties	5 2	67	3	na	55	116
Frozen Yogurt	1 3	17	< 1*	na	52*	89*
Gelatins, Puddings and Fillings						
Custards and Puddings	0 8	10	< 1*	na	60*	73*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	4 1	53	7	na	81	146
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	5 7	73	4	na	74	230

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Table B-2 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Children (Aged 3 to 11 Years) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Milk Products						
Flavored Milk and Milk Drinks	32 6	420	18	53	49	89
Milk-based Meal Replacements	0.2	3	< 1*	na	34*	33*
Yogurt	12 1	156	13	45	96	175
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	66 1	851	34	93	55	119
Processed Vegetables and Vegetable Juices						
Tomato Juice	0 3	4	< 1*	na	46*	58*
Snack Foods						
Pretzels	7 9	102	9	11	90	186

na = not available
* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

Table B-3 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Baked Goods and Baking Mixes						
Biscuits	7 2	71	3	na	43	83
Breads and Rolls (rye and white bread)	1 8	18	1*	na	15*	27*
Breakfast Pastries (i.e Danish)	3 7	37	4	na	80	146
Cakes	0 1	1	< 1*	na	15*	15*
Cookies and Brownies	2.0	20	< 1*	na	19*	37*
Crackers (low sodium, regular, and sweet)	17.0	169	6	20	27	63
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	7 8	77	3	2	31	56
Meal Replacement Beverages (Soy based)	0 2	2	< 1*	na	15*	41*
Breakfast Cereals						
Ready-to-Eat Cereals	42 1	418	28	80	66	128
Frozen Dairy Desserts						
Ice Cream and Novelties	4 1	41	2	na	34	80
Frozen Yogurt	1 1	11	< 1*	na	24*	51*
Gelatins, Puddings and Fillings						
Custards and Puddings	0 2	2	< 1*	na	39*	40*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	5 6	56	3	na	42	74
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	2.3	23	1*	na	32*	63*

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Table B-3 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (mg/kg)	**90th Percentile (mg/kg)**	**Mean (mg/kg)**	**90th Percentile (mg/kg)**
Milk Products						
Flavored Milk and Milk Drinks	20 0	198	4	16	21	45
Milk-based Meal Replacements	0 2	2	< 1*	na	30*	52*
Yogurt	6 6	65	4	na	54	96
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	46 3	459	10	33	26	52
Processed Vegetables and Vegetable Juices						
Tomato Juice	0 3	3	< 1*	na	33*	37*
Snack Foods						
Pretzels	6 1	61	3	na	42	88

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

Table B-4 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (mg/kg)	**90th Percentile (mg/kg)**	**Mean (mg/kg)**	**90th Percentile (mg/kg)**
Baked Goods and Baking Mixes						
Biscuits	7 5	75	3	na	53	86
Breads and Rolls (rye and white bread)	1 3	13	< 1*	na	16*	21*
Breakfast Pastries (i e Danish)	5 1	51	6	na	83	117
Cakes	0 2	2	< 1*	na	56*	60*
Cookies and Brownies	2 0	20	1*	na	26*	61*
Crackers (low sodium, regular, and sweet)	10 8	108	3	na	30	76
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	4 8	48	3	na	44	71
Meal Replacement Beverages (Soy based)	0 1	1	< 1*	na	13*	13*
Breakfast Cereals						
Ready-to-Eat Cereals	43.8	438	31	97	71	129
Frozen Dairy Desserts						
Ice Cream and Novelties	4 5	45	2	na	29	63
Frozen Yogurt	0.4	4	< 1*	na	75*	212*
Gelatins, Puddings and Fillings						
Custards and Puddings	0.1	1	< 1*	na	9*	9*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	4.1	41	3	na	45	89
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	2 5	25	1*	na	37*	46*

Table B-4 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Milk Products						
Flavored Milk and Milk Drinks	19 9	199	5	19	26	61
Milk-based Meal Replacements	0 2	2	< 1*	*na*	56*	59*
Yogurt	3 0	30	2	na	56	102
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	45 3	453	11	36	28	61
Processed Vegetables and Vegetable Juices						
Tomato Juice	0 4	4	< 1*	na	37*	45*
Snack Foods						
Pretzels	5 5	55	3	*na*	50	119

na = not available
* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

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Table B-5	**Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)**					

Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (mg/kg)	**90th Percentile (mg/kg)**	**Mean (mg/kg)**	**90th Percentile (mg/kg)**
Baked Goods and Baking Mixes						
Biscuits	9 0	191	3	na	34	66
Breads and Rolls (rye and white bread)	6 9	146	1	na	12	25
Breakfast Pastries (i e Danish)	1 0	22	1*	na	62*	80*
Cakes	0 6	12	< 1*	na	17*	24*
Cookies and Brownies	3 1	65	1	na	27	51
Crackers (low sodium, regular, and sweet)	22 8	485	4	14	17	32
Beverages and Beverage Bases						
Carbonated Beverages (diet or sugar-free)	21 0	448	11	39	42	88
Meal Replacement Beverages (Soy based)	0 7	14	< 1*	na	31*	68*
Breakfast Cereals						
Ready-to-Eat Cereals	33 2	707	15	51	44	78
Frozen Dairy Desserts						
Ice Cream and Novelties	6 2	131	2	na	23	42
Frozen Yogurt	2.1	45	1	na	33	79
Gelatins, Puddings and Fillings						
Custards and Puddings	0 7	15	< 1*	na	26*	48*
Grain Products and Pastas						
Meal Replacement, Granola and Cereal Bars	5 4	114	2	na	35	63
Jams and Jellies, Commercial						
Jams, Jellies and Preserves	6 8	144	2	na	25	56

Table B-5 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (mg/kg)	**90th Percentile (mg/kg)**	**Mean (mg/kg)**	**90th Percentile (mg/kg)**
Milk Products						
Flavored Milk and Milk Drinks	9.3	197	2	na	23	46
Milk-based Meal Replacements	1.5	31	1	na	38	74
Yogurt	8.9	190	3	na	35	61
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	42.6	906	6	21	17	37
Processed Vegetables and Vegetable Juices						
Tomato Juice	2.5	54	< 1	na	12	22
Snack Foods						
Pretzels	5.1	108	2	na	37	77

na = not available
* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2.3)

000100

Table B-6 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
<u>Baked Goods and Baking Mixes</u>						
Biscuits	8.8	170	3	na	44	83
Breads and Rolls (rye and white bread)	6.7	129	1	na	11	19
Breakfast Pastries (i.e. Danish)	0.9	18	1*	na	70*	114*
Cakes	0.4	7	< 1*	na	39*	78*
Cookies and Brownies	2.6	50	1	na	26	71
Crackers (low sodium, regular, and sweet)	17.1	330	3	11	20	37
<u>Beverages and Beverage Bases</u>						
Carbonated Beverages (diet or sugar-free)	16.1	311	8	26	45	100
Meal Replacement Beverages (Soy based)	1.1	21	< 1*	na	45*	99*
<u>Breakfast Cereals</u>						
Ready-to-Eat Cereals	30.4	587	16	57	50	99
<u>Frozen Dairy Desserts</u>						
Ice Cream and Novelties	3.9	76	1	na	23	40
Frozen Yogurt	1.8	34	< 1	na	26	41
<u>Gelatins, Puddings and Fillings</u>						
Custards and Puddings	0.9	18	< 1*	na	19*	36*
<u>Grain Products and Pastas</u>						
Meal Replacement, Granola and Cereal Bars	4.6	89	2	na	28	57
<u>Jams and Jellies, Commercial</u>						
Jams, Jellies and Preserves	7.3	140	2	na	29	66

Table B-6 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
			Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Milk Products						
Flavored Milk and Milk Drinks	6 3	122	1	na	19	36
Milk-based Meal Replacements	1.1	22	1*	na	63*	186*
Yogurt	4 5	87	1	na	30	55
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	37 4	722	6	21	18	38
Processed Vegetables and Vegetable Juices						
Tomato Juice	2 4	47	< 1	na	11	18
Snack Foods						
Pretzels	4 9	94	2	na	34	69

na = not available
* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

000102

Table B-7 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (mg/kg)	**90th Percentile (mg/kg)**	**Mean (mg/kg)**	**90th Percentile (mg/kg)**
<u>Baked Goods and Baking Mixes</u>						
Biscuits	7.6	631	4	na	45	84
Breads and Rolls (rye and white bread)	4.0	330	1	na	13	24
Breakfast Pastries (i.e. Danish)	2.4	196	2	na	91	151
Cakes	0.3	23	< 1*	na	25*	53*
Cookies and Brownies	2.4	195	1	na	28	67
Crackers (low sodium, regular, and sweet)	21.2	1,754	7	20	33	77
<u>Beverages and Beverage Bases</u>						
Carbonated Beverages (diet or sugar-free)	11.7	965	8	26	43	90
Meal Replacement Beverages (Soy based)	0.5	41	< 1	na	42	99
<u>Breakfast Cereals</u>						
Ready-to-Eat Cereals	41.1	3,394	26	80	68	139
<u>Frozen Dairy Desserts</u>						
Ice Cream and Novelties	4.6	379	2	na	29	64
Frozen Yogurt	1.5	120	< 1	na	33	58
<u>Gelatins, Puddings and Fillings</u>						
Custards and Puddings	0.6	53	< 1	na	36	73
<u>Grain Products and Pastas</u>						
Meal Replacement, Granola and Cereal Bars	4.5	374	3	na	43	95
<u>Jams and Jellies, Commercial</u>						
Jams, Jellies and Preserves	5.2	431	2	na	34	82

Table B-7 Estimated Daily per Kilogram Body Weight Intake of Wheat Bran Extract from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004 NHANES Data)						
Food-Use Category	**% Users**	**Actual # of Total Users**	**All-Person Consumption**		**All-User Consumption**	
			Mean (mg/kg)	**90th Percentile (mg/kg)**	**Mean (mg/kg)**	**90th Percentile (mg/kg)**
Milk Products						
Flavored Milk and Milk Drinks	14 7	1,212	4	12	34	65
Milk-based Meal Replacements	0 7	61	1	na	48	101
Yogurt	7 8	648	5	na	61	122
Processed Fruit and Fruit Juices						
Fruit Juices (Citrus and Non-citrus)	46 7	3,862	13	36	32	69
Processed Vegetables and Vegetable Juices						
Tomato Juice	1 4	115	< 1	na	13	33
Snack Foods						
Pretzels	5 6	464	3	na	48	105

na = not available
* Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30%, or for which fewer than 30 respondents were reported (see Section 2 3)

000104

APPENDIX C

Representative NHANES 2003-2004 Food Codes for All Proposed Food-Uses of Wheat Bran Extract in the United States

Representative NHANES 2003-2004 Food Codes for All Proposed Food-Uses of Wheat Bran Extract (WBE) in the United States

Baked Goods and Baking Mixes

Biscuits
[Wheat Bran Extract] = 9 0%

52101000	Biscuit, baking powder or buttermilk type, nfs
52101020	Biscuit dough, raw
52101030	Biscuit dough, fried
52101040	Crumpet
52101050	Crumpet, toasted
52101100	Biscuit, baking powder or buttermilk, from mix
52101150	Biscuit, baking pwdr/butter milk, refrig dough, lowfat
52102040	Biscuit, bak powder or buttermilk, from refrg dough
52103000	Biscuit, baking powder/buttermilk type, commercially baked
52104010	Biscuit, baking powder or buttermilk, homemade
52104040	Biscuit, whole wheat
52104100	Biscuit, cheese
52104200	Biscuit, cinnamon-raisin
52105100	Scones
52105110	Scones, whole wheat
52105200	Scone, with fruit

Breads and Rolls (rye and reduced-calorie white bread)
[Wheat Bran Extract] = 3 0%

51122000	Bread, reduced calorie/high fiber
51122010	Bread, reduced calorie/high fiber, toasted
51122050	Bread, reduced calorie/high fiber, Italian
51122060	Bread, reduced calorie/high fiber, Italian, toasted
51122100	Bread, reduced calorie/ high fiber, w/ fruit/nuts
51122110	Bread, reduced calorie/hi fiber, w/fruit/nuts, toast
51122300	Bread, white, special formula, added fiber
51122310	Bread, white, special formula, added fiber, toasted
51186010	Muffin, English (include sour dough)
51186020	Muffin, English, toasted
51186100	Muffin, English, w/ raisins
51186120	Muffin, English, w/ raisins, toasted
51186130	Muffin, English, cheese
51186140	Muffin, English, cheese, toasted
51186160	Muffin, English, w/ fruit other than raisins
51186180	Muffin, English, w/ fruit other than raisins, tstd
51187000	Melba toast
51187020	anisette toast
51401010	Bread, rye
51401020	Bread, rye, toasted
51401030	Bread, marble rye & pumpernickel
51401040	Bread, marble rye & pumpernickel, toasted
51401060	Bread, rye, reduced calorie/ high fiber (incl less)
51401070	Bread, rye, reduced calorie/ high fiber, toasted
51401200	Muffin, English, rye
51401210	Muffin, English, rye, toasted

51404010	Bread, pumpernickel
51404020	Bread, pumpernickel, toasted
51404500	Bagel, pumpernickel
51404510	Bagel, pumpernickel, toasted
51404550	Muffin, English, pumpernickel
51404560	Muffin, English, pumpernickel, toasted
51407010	Bread, black
51407020	Bread, black, toasted
51420000	Roll, rye
51421000	Roll, pumpernickel
51421100	Roll, pumpernickel, toasted

Breakfast Pastries (i.e.: Danish)
[Wheat Bran Extract] = 9 0%

53530000	Breakfast tart
53530010	Breakfast tart, lowfat

Cakes
[Wheat Bran Extract] = 5 45%

53104300	Carrot cake, diet
53104520	Cheesecake, diet
53104570	Cheesecake, diet, with fruit
53104650	Cheesecake, chocolate, reduced fat
53105500	Cake, choc, w/ icing, diet
53109210	Cake, cupcake, not choc, w/ icing, lowfat, no chol
53109270	Cupcake, choc, w/orw/o icing, fruit/cream fill, lowfat
53114150	Cake, lemon, low fat, ns as to icing
53114200	Cake, lemon, low fat, w/o icing
53114250	Cake, lemon, low fat, w/ icing
53115500	Cake, pineapple, fat free, w/ icing
53116280	Cake, pound, choc, fat free, no chol
53116380	Cake, pound, fat free, no chol
53116390	Cake, pound, reduced fat, no cholesterol
53120400	Cake, white, eggless, lowfat
53123500	Cake, shortcake, w/ whip topping & fruit, diet

Cookies and Brownies
[Wheat Bran Extract] = 9 0%

53202000	Cookie, almond
53203000	Cookie, applesauce
53203500	Cookie, biscotti
53204800	Brownie, diet, ns as to icing
53204830	Cookie, brownie, lowfat, w/ icing
53204840	Cookie, brownie, lowfat, w/o icing
53204850	Cookie, brownie, fat free, no chol, w/ icing
53204860	Cookie, brownie, fat free, w/o icing
53206030	Cookie, choc chip, reduced fat
53207050	Cookie, chocolate, w/ choc filling/coating, fat free
53209020	Cookie, chocolate sandwich, reduced fat
53220010	Cookie, fruit-filled bar, fat free
53220040	Cookie, fig bar, fat free
53233030	Cookie, oatmeal, fat free, w/ raisins(incl w/ dates)
53233040	Cookie, oatmeal, red fat, w/ raisins

53239010	Cookie, shortbread, reduced fat
53243050	Cookie, vanilla sandwich, reduced fat
53247050	Cookie, vanilla wafer, reduced fat
53260030	Cookie, dietetic, chocolate chip
53260150	Cookie, lemon wafer, lowfat
53260200	Cookie, dietetic, oatmeal w/ raisins
53260300	Cookie, dietetic, sandwich type
53260400	Cookie, dietetic, sugar or plain

Crackers (low sodium, regular, and sweet)
[Wheat Bran Extract] = 9 0%

54101010	Cracker, animal (incl arrowroot cookie)
54102010	Crackers, graham
54102020	Crackers, graham, chocolate covered
54102050	Crackers, oatmeal
54102060	Crackers, Cuban
54102070	Crackers, cuca
54102080	Crackers, graham, w/ raisins
54102100	Crackers, graham, lowfat
54102110	Crackers, graham, fat free
54102200	Crackers, graham, sandwich-type, with filling
54201010	Crackers, matzo, low sodium
54202010	Crackers, saltine, low sodium
54202050	Crackers, saltine, fat free, low sodium
54203010	Crackers, toast thins (rye/wheat/white), low sodium
54204010	Cracker, 100% whole wheat, lo sodium
54205010	Cracker, snack, low sodium
54205030	Cracker, cheese, low sodium
54205100	Cracker, snack, lowfat, low sodium
54206010	Puffed rice cake w/o salt
54207010	Crispbread, wheat, low sodium
54210010	Cracker, multigrain, salt-free
54222000	Crispbread, rye, low sodium
54301000	Cracker, snack
54301100	Cracker, snack, reduced fat
54301200	Cracker, snack, fat free
54304100	Cracker, cheese, reduced fat
54304500	Cracker, high fiber, no added fat
54305000	Crispbread, wheat, no added fat
54305500	Crispbread, wheat / rye, extra crispy
54307000	Crackers, matzo
54308000	Crackers, milk
54309000	Crackers, oat bran (include Nabisco oat thins)
54313000	Crackers, oyster
54319200	Puffed wheat cake (incl Quaker)
54322000	Crispbread, rye, no added fat
54325000	Crackers, saltines
54325050	Crackers, saltine, whole wheat
54334000	Crackers, toast thins
54336000	Cracker, water biscuit
54338000	Crackers, wheat
54338100	Crackers, wheat, reduced fat

Beverage and Beverage Bases

Carbonated Beverages (diet and sugar-free)
[Wheat Bran Extract] = 0 6%

92400100	Soft drink, nfs, sugar-free
92410250	Carbonated Water, sugar-free
92410320	Soft drink, cola-type, sugar-free
92410350	Soft drink, cola-type, decaffeinated, sugar-free
92410370	Soft drink, pepper-type, sugar-free
92410400	Soft drink, pepper-type, decaffeinated, sugar-free
92410420	Cream soda, sugar-free
92410560	Soft drink, fruit-flavored, w/ caffeine, sugar-free
92410620	Gingerale, sugar-free
92410720	Root beer, sugar-free
92410820	Chocolate-flavored soda, sugar-free
92411610	Cola w/ fruit or vanilla flavor, sugar-free
92411620	Cola w/ choc flavor, sugar free

Meal Replacement Beverages (soy-based)
[Wheat Bran Extract] = 1 2%

41440010	Meal replacement/supplement, liquid, hi protein
41440100	Meal replacement, liquid, soy base (isocal, osmolite)

Breakfast Cereals

Ready-to-Eat Cereals
[Wheat Bran Extract] = 9 0%

57100100	Cereal, ready-to-eat, nfs
57100400	Character cereals, TV or movie, General Mills
57100500	Character cereals, TV or movie, Kellogg's
57101000	all-bran cereal
57101020	all bran cereal w/ extra fiber
57102000	alpen cereal
57103000	alpha-bits cereal
57103020	alpha-bits w/ marshmallows cereal
57103050	amaranth flakes cereal
57103100	apple cinnamon cheerios
57103500	apple cinnamon squares mini-wheats cereal, Kellogg's
57104000	apple jacks cereal
57106050	Banana nut crunch cereal (post)
57106100	Basic 4 (rte cereal)
57106250	Berry berry kix
57106260	Berry Burst Cheerios
57106530	Blueberry morning, post
57107000	Booberry cereal
57110000	all-bran bran buds cereal, Kellogg's (formerly bran buds)
57111000	Bran chex cereal
57117000	Cap'n crunch cereal
57117500	Cap'n crunch's Christmas crunch cereal
57119000	Cap'n crunch's crunch berries cereal
57120000	Cap'n crunch's peanut butter crunch cereal
57123000	Cheerios
57124000	Chex cereal, nfs

57124200	Chocolate flavored frosted puffed corn cereal
57124500	Cinnamon grahams cereal, general mills
57125000	Cinnamon toast crunch cereal
57125900	Honey nut clusters cereal
57126000	Cocoa krispies cereal
57126500	Cocoa blasts cereal, Quaker
57127000	Cocoa pebbles cereal
57128000	Cocoa puffs cereal
57128880	Common sense oat bran cereal, plain
57130000	Cookie-crisp cereal (include all flavors)
57131000	Crunchy corn bran cereal, Quaker
57132000	Corn chex cereal
57134000	Corn flakes, nfs (include store brands)
57134090	Corn flakes, low sodium
57135000	Corn flakes, Kellogg
57137000	Corn puffs cereal
57138000	Total corn flakes
57139000	Count chocula cereal
57143000	Cracklin' oat bran cereal
57144000	Crisp crunch cereal
57148000	Crispix cereal
57148500	Crispy brown rice cereal
57148600	Harmony cereal, General Mills
57151000	Crispy rice cereal
57152000	Crispy wheats'n raisins cereal
57201800	Disney cereals, Kellogg's
57206000	Familia cereal
57206700	Fiber one cereal
57206800	Fiber 7 flakes cereal, health valley
57207000	Bran flakes cereal, nfs (formerly 40% bran flakes, nfs)
57208000	Complete wheat bran flakes, Kellogg's (form 40% bran flakes)
57209000	Natural bran flakes cereal, post
57211000	Frankenberry cereal
57212100	French toast crunch cereal, general mills
57213000	Froot loops cereal
57213850	Frosted cheerios cereal
57214000	Frosted mini-wheats cereal (incl all flavors)
57214100	Frosted wheat bites
57215000	Frosty o's cereal
57216000	Frosted rice cereal, nfs
57218000	Frosted rice krispies cereal
57219000	Fruit & fibre cereal, nfs
57221000	Fruit & fibre cereal, w/ dates, raisins, & walnuts
57221650	Fruit Harvest cereal, Kellogg's
57221700	Fruit rings, nfs (include store brands)
57221800	Fruit whirls cereal
57223000	Fruity pebbles cereal
57224000	Golden grahams cereal
57227000	Granola, nfs
57228000	Granola, homemade
57229000	Granola, lowfat, Kellogg's
57229500	Granola w/ raisins, lowfat, Kellogg's
57230000	Grape-nuts cereal
57231000	Grape-nut flakes
57231200	Great grains, raisin, date, & pecan, whole grain cereal, post
57231250	Great grains double pecan whole grain cereal, post

57232100	Healthy choice almond crunch cereal w/ raisins
57237100	Honey bunches of oats cereal
57237300	Honey bunches of oats w/ almonds, post
57238000	Honeycomb cereal, plain
57239000	Honeycomb cereal, strawberry
57239100	Honey crunch corn flakes cereal, Kellogg's
57240100	Honey nut chex cereal
57241000	Honey nut cheerios
57241200	Honey nut shredded wheat cereal, post
57243000	Honey smacks cereal
57243870	Jenny o's
57244000	Just right cereal
57245000	Just right fruit & nut cereal (w/ raisins, dates, nuts)
57250000	Pokemon, Kellogg's
57301100	Kaboom cereal
57301500	Kashi, puffed
57302100	King vitaman cereal
57303100	Kix cereal
57304100	Life cereal (plain & cinnamon)
57305100	Lucky charms cereal
57305150	Frosted oat cereal w/ marshmallows
57305170	Malt-o-meal coco-roos cereal
57305180	Malt-o-meal corn bursts cereal
57305200	Malt-o-meal crispy rice cereal
57305210	Malt-O-Meal Frosted Flakes
57305500	Malt-o-meal honey & nut toasty o's cereal
57305600	Malt-o-meal marshmallow mateys cereal
57306100	Malt-o-meal puffed rice cereal
57306120	Malto-o-meal puffed wheat cereal
57306500	Malt-o-meal golden puffs cereal (formerly sugar puffs)
57306700	Malt-o-meal toasted oat cereal
57306800	Malt-o-meal tootie fruities (rte cereal)
57307150	Marshmallow safari cereal, Quaker
57307500	Millet, puffed (cereal)
57308150	Mueslix cereal, nfs
57308190	Muesli with raisins, dates, and almonds
57308300	Multi bran chex
57308400	Multi grain cheerios
57308900	Natural muesli, jenny's cuisine
57309100	Nature valley granola, w/ fruit & nuts
57311700	Nu system cuisine toasted grain circles cereal
57316200	Nutty nuggets (ralston)
57316300	Oat bran flakes, health valley
57316410	apple cinnamon oatmeal crisp cereal (oatmeal crisp w/ apples
57316450	Oatmeal crisp w/ almonds cereal
57316500	Oatmeal raisin crisp cereal
57316710	Oh's, honey graham cereal
57316750	Oh's, fruitangy cereal
57318000	100% bran cereal
57319000	100% natural cereal, plain, Quaker
57319500	Sun country 100% natural granola, with almonds
57320500	100 % natural cereal, w/ oats, honey & raisins, Quaker
57321500	100% natural wholegrain cereal w/ raisins, lowfat, Quaker
57321700	Optimum, Nature's Path
57321800	Optimum Slim, Nature's Path
57322500	Oreo o's cereal, post

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57323000	Sweet crunch cereal, Quaker (formerly popeye)
57323050	Sweet puffs cereal, Quaker
57325000	Product 19 cereal
57327450	Quaker oat bran cereal
57327500	Quaker oatmeal squares cereal (formerly Quaker oat squares)
57328000	Quisp cereal
57329000	Raisin bran cereal, nfs
57330000	Raisin bran cereal, Kellogg
57331000	Raisin bran cereal, post
57332050	Raisin bran, total
57332100	Raisin nut bran cereal
57335500	Raisin squares mini-wheats cereal (formerly raisin squares)
57335550	Reese's peanut butter puffs cereal
57336000	Rice chex cereal
57337000	Rice flakes, nfs
57339000	Rice krispies cereal
57339500	Rice krispies treats cereal (Kellogg's)
57340000	Puffed rice cereal
57340700	Scooby Doo Cinnamon Marshmallow Cereal, Kellogg's
57341000	Shredded wheat 'n bran cereal
57341200	Smart Start, Kellogg's
57344000	Special k cereal
57346500	Toasted oatmeal, honey nut (Quaker)
57347000	Corn pops cereal
57347500	Strawberry squares mini-wheats cereal(strawberry squares)
57348000	Frosted corn flakes, nfs
57349000	Frosted flakes, Kellogg
57355000	Golden crisp cereal
57401100	Toasted oat cereal
57403100	Toasties, post
57404100	Malt-o-meal toasty o's cereal
57404200	Malt-O-Meal Apple and Cinnamon Toasty O's
57406100	Total cereal
57407100	Trix cereal
57408100	Uncle sam's hi fiber cereal
57409100	Waffle crisp cereal, post
57410000	Weetabix whole wheat cereal
57411000	Wheat chex cereal
57412000	Wheat germ cereal, plain
57413000	Wheat germ cereal, w/ sugar & honey
57416000	Puffed wheat cereal, plain
57416010	Wheat, puffed, presweetened w/ sugar
57417000	Shredded wheat, 100%
57418000	Wheaties cereal

Frozen Dairy Desserts

Ice Cream and Novelties
[Wheat Bran Extract] = 2 4%

13120400	Ice cream bar/stick w/ fruit
13130100	Light ice cream, nfs (formerly ice milk)
13130300	Light ice cream, not chocolate (formerly ice milk)
13130310	Light ice cream, chocolate (formerly ice milk)
13130320	Light ice cream, no sugar added, NS as to flavor
13130330	Light ice cream, no sugar added, flavors other than chocolate

13130340	Light ice cream, no sugar added, chocolate
13130590	Light ice cream, soft serve, ns flavor (formerly ice milk)
13130600	Light ice cream, soft serve, not choc (formerly ice milk)
13130610	Light ice cream, soft serve choc (tastee frz, dairy queen)
13130620	Light ice cream, soft serve cone, not choc (dairy queen)
13130630	Light ice cream, soft serve cone, choc (formerly ice milk)
13130640	Light ice cream, soft serve cone, ns flav(formerly ice milk)
13135000	Light ice cream, sandwich (dairy queen) (formerly ice milk)
13140100	Light ice cream, bar/stick, choc-coated (formerly ice milk)
13140110	Light ice cream, bar, choc covered, w/nuts (formerly ice milk)
13140450	Light ice cream, cone, nfs (formerly ice milk)
13140500	Light ice cream, cone, not chocolate (formerly ice milk)
13140550	Light ice cream, cone, chocolate (formerly ice milk)
13140600	Light ice cream, sundae, soft serve, choc/fudge top (ice milk)
13140630	Light ice cream, sundae, soft serve, fruit topping (ice milk)
13140650	Light ice cream, sundae, soft serve, not fruit/choc topping
13140660	Light ice cream, sundae, choc / fudge top (w/o whip cream)
13140670	Light ice cream, sundae, fruit top (w/o whip cream) (ice milk)
13140680	Light ice cream, sundae, no fruit/choc top (w/o whip cream)
13140700	Light ice cream, creamsicle or dreamsicle (formerly ice milk)
13140900	Light ice cream, fudgesicle (formerly ice milk)
13160150	Milk dessert, froz, nonfat, w/ low cal sweetener chocolate
13160160	Milk dessert, froz, nonfat, w/ low cal sweetener, not choc
13160400	Milk dessert, froz, milk-fat free, not choc
13160410	Milk dessert, froz, milk-fat free, choc
13160420	Fat free ice cream, NS as to flavor
13161000	Milk dessert bar, frozen, made from lowfat milk
13161500	*Milk dessert sandwich bar, frozen, dietary*
13161520	Milk dessert sandwich bar, frz, w/low-cal sweet, lofat
13161600	Milk des bar, frozen, lowfat milk & lo cal sweetener
13161630	Light ice cream, bar/stick, w/ low-cal sweetener, choc coat

Frozen Yogurt
[Wheat Bran Extract] = 2.4%

11459990	Yogurt, frozen, ns as to flavor, ns to type of milk
11460000	Yogurt, frozen, not chocolate, type of milk ns
11460100	Yogurt, frozen, chocolate, type of milk ns
11460150	Yogurt, frozen, ns as to flavor, lowfat milk
11460160	Yogurt, frozen, chocolate, lowfat milk
11460170	Yogurt, frozen, not chocolate, lowfat milk
11460190	Yogurt, frozen, ns as to flavor, nonfat milk
11460200	Yogurt, frozen, chocolate, nonfat milk
11460250	Yogurt, frozen, not chocolate, w/ sorbet/sorbet-coated
11460300	Yogurt, frozen, not chocolate, nonfat milk
11460400	Yogurt, frz, chocolate, nonfat milk, w/ low-cal sweet
11460410	Yogurt, frz, not choc, nonfat milk, w/ low-cal sweet
11460420	Yogurt, frozen, ns as to flavor, whole milk
11460430	Yogurt, frozen, chocolate, whole milk
11460440	Yogurt, frozen, not chocolate, whole milk
11461000	Yogurt, frozen, chocolate-coated
11461100	Yogurt, frozen, carob-coated
11461200	Yogurt, frozen, sandwich
11461250	Yogurt, frozen, cone, chocolate
11461260	Yogurt, frozen, cone, not chocolate

11461270	Yogurt, frozen, cone, not chocolate, lowfat milk
11461280	Yogurt, froz, cone, chocolate, lowfat milk

__Gelatins, Puddings and Fillings__

Custards and Puddings
[Wheat Bran Extract] = 2 4%

13210190	Pudding, Mexican bread (capirotada), lower fat
13210250	Pudding, choc, rte, lo cal, w/ art swtner, ns dry/can
13210290	Pudding, not choc, rte, lo cal, w/ art swtner
13210500	Pudding, tapioca, made from home recipe, made w/ milk
13210520	Pudding, tapioca, made from dry mix, made w/ milk
13210530	Pudding, tapioca, chocolate, made w/ milk
13220210	Pudding, not choc, from dry, low cal, artificial sweet, w/milk
13220220	Pudding, choc, from dry, low cal, artificial sweet, milk added
13220230	Pudding, can, choc, reduced fat (incl jell-o light)
13220235	Pudding, can, choc, fat free
13220240	Pudding, canned, not choc, reduced fat
13220245	Pudding, canned, not choc, fat free
13230120	Pudding, canned, low cal, w/artificial swtnr, not chocolate
13230140	Pudding, canned, lo cal, w/ art swtner, chocolate
13230510	Pudding, canned, tapioca, fat free

__Grain Products and Pastas__

Meal Replacement, Granola and Cereal Bars
[Wheat Bran Extract] = 9 0%

53540000	Breakfast bar, nfs
53540200	Breakfast bar, cereal crust w/ fruit filling, lowfat
53540250	Breakfast bar, cereal crust w/ fruit filling, fat free
53540500	Breakfast bar, date, w/ yogurt coating
53540600	Milk 'n Cereal bar
53541200	Meal replacement bar (incl slim fast bar)
53542100	Granola bar w/ oats, sugar, raisins, coconut
53542200	Granola bar, oats, fruit, nuts, lowfat
53542210	Granola bar, nonfat
53543100	Granola bar w/ peanuts, oats, sugar, wheat germ
53544200	Granola bar, chocolate-coated
53544210	Granola bar, w/ coconut, chocolate-coated
53544220	Granola bar w/ nuts, chocolate-coated
53544250	Granola bar, coated w/ nonchocolate coating
53544300	Granola bar, high fiber, yogurt coating, not chocolate
53544400	Granola bars, w/ rice cereal
53544450	Powerbar (fortified high energy bar)

__Jams and Jellies, Commercial__

Jams, Jellies and Preserves
[Wheat Bran Extract] = 15 0%

91402000	Jam, preserves, all flavors
91403000	Fruit butter, all flavors (include apple butter)
91404000	Marmalade, all flavors
91405000	Jelly, dietetic, all flavors, sweetened w/ artificial sweeten

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91405500	Jelly, reduced sugar, all flavors
91406000	Jam, marmalades, artificially sweetened
91406500	Jam preserves, marmalades, sweet w/ fruit juice conc
91406600	Jams, preserves, marmalades, low sugar (all flavors)

Milk Products

Flavored Milk and Milk Drinks
[Wheat Bran Extract] = 0 6%

11511000	Milk, chocolate, nfs
11511100	Milk, chocolate, whole milk based
11511200	Milk, chocolate, reduced fat milk based
11511300	Milk, chocolate, skim milk based
11511400	Milk, chocolate, lowfat milk-based
11512000	Cocoa, hot chocolate, not from dry mix, w/whole milk
11512500	Hot chocolate drink w/ evap milk, Puerto Rican
11513000	Cocoa & sugar mixture, milk added, ns type milk
11513100	Cocoa & sugar mixture, whole milk added
11513150	Cocoa & sugar mix, red fat milk added
11513200	Cocoa & sugar mixture, lowfat milk added
11513300	Cocoa & sugar mixture, skim milk added
11513400	Chocolate syrup milk added, ns as to type of milk
11513500	Chocolate syrup, whole milk added
11513550	Chocolate syrup, red fat milk added
11513600	Chocolate syrup, lowfat milk added
11513700	Chocolate syrup, skim milk added
11514100	Cocoa, sugar, & dry milk mixture, water added
11514300	Cocoa w/ nf dry milk, lo cal sweetener, water added
11514500	Cocoa w/ whey, lo cal sweetnr, fortifd, water added
11515100	Cocoa & sugar w/ milk, fortified, Puerto Rican
11515400	Cocoa w/ nfdm, low calorie, hi calcium, water added
11516000	Cocoa, whey, lo cal sweetner mix, lowfat milk added
11518000	Milk bev w/ nf dry mlk, lo cal sweet, water, chocolate
11518050	Milk bev w/nf dry milk, lo cal sweet, water, not chocolate
11518100	Milk bev w/nfd milk, lo cal sweet, hi calcium, chocolate
11520000	Milk, malted, unfortified, flavor ns
11521000	Milk, malted, unfortified, chocolate flavor
11521010	Milk, malted, unfortified, choc, made w/ skim milk
11522000	Milk, malted, unfortified, natural flavor
11525000	Milk, malted, fortified, natural flavor (incl ovaltine)
11526000	Milk, malted, fortified, chocolate (incl ovaltine)
11527000	Milk, malted, fortified, (incl ovaltine)
11531000	Eggnog, made w/ whole milk (include eggnog, nfs)
11531500	Eggnog, made w/ 2% reduced fat milk
11541000	Milk shake, ns as to flavor or type
11541100	Milk shake, homemade/ fountain-type, ns as to flavor
11541110	Milk shake, homemade or fountain-type, chocolate
11541120	Milk shake, homemade/fountain-type, not chocolate
11541400	Milk shake with malt (incl malted milk w/ice cream)
11541500	Milk shake, made w/ skim milk, chocolate
11541510	Milk shake, made w/ skim milk, not chocolate
11542000	Carry-out milk shake, ns as to flavor
11542100	Carry-out milk shake, chocolate
11542200	Carry-out milk shake, not chocolate
11551050	Milk fruit drink (incl licuado)

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11552200	Milk-based fruit drink (incl orange julius)
11553000	Fruit smoothie drink, w/ fruit and dairy products
11553100	Fruit smoothie drink, nfs
11560000	Choc-flavored drink, whey-& milk-based (incl yoo-hoo)
11560020	Milk drink, whey & milk-base, not choc (incl yoo-hoo)

Milk-based Meal Replacement Beverages
[Wheat Bran Extract] = 1 2%

11611000	Instant breakfast, fluid, canned
11612000	Instant breakfast, powder, milk added
11613000	Instant bfast, pwdr, swt w/ lo cal swt, milk added
11621000	Diet beverage, liquid, canned
11622010	Diet beverage, pwdr, reconst w/skim (incl carnation)
11623000	Meal supplement / replacement, prepared, rtd
11631000	High calorie bev, canned or powdered, reconstituted
11641000	Meal replacement, milk based, high protein, liquid
11651010	Meal replacement, cambridge, reconst, all flavors

Yogurt
[Wheat Bran Extract] = 2 4%

11410000	Yogurt, ns as to type of milk/flavor
11411010	Yogurt, plain, ns as to type of milk
11411100	Yogurt, plain, whole milk
11411200	Yogurt, plain, lowfat milk
11411300	Yogurt, plain, nonfat milk
11420000	Yogurt, vanilla, lemon, coffee, ns as to milk type
11421000	Yogurt, vanilla, lemon, coffee, whole milk
11422000	Yogurt, vanilla, lemon, coffee, lowfat milk
11423000	Yogurt, vanilla, lemon, coffee, nonfat milk
11424000	Yogurt, vanilla, lemon, coffee, nonfat milk, low cal sweet
11425000	Yogurt, chocolate, ns as to type of milk
11426000	Yogurt, chocolate, whole milk
11427000	Yogurt, chocolate, nonfat milk
11430000	Yogurt, fruit variety, ns as to milk type
11431000	Yogurt, fruit variety, whole milk
11432000	Yogurt, fruit variety, lowfat milk
11432500	Yogurt, fruit variety, lowfat milk, sweetened with low-calorie sweetener
11433000	Yogurt, fruit variety, nonfat milk
11433500	Yogurt, fruited, nonfat milk, low cal sweetener
11444000	Yogurt, fruit & nuts, ns as to type of milk
11445000	Yogurt, fruit & nuts, lowfat milk

<u>Processed Fruit and Fruit Juices</u>

Fruit Juices (citrus and non-citrus)
[Wheat Bran Extract] = 0 6%

61200500	acerola juice
61201010	Grapefruit juice, freshly squeezed
61201020	Grapefruit juice, unsweetened, ns as to form
61201220	Grapefruit juice, canned, bottled, carton, unsweet
61201620	Grapefruit juice, frozen, unsweetened (reconst)
61204000	Lemon juice, ns as to form
61204010	Lemon juice, fresh

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61204200	Lemon juice, canned or bottled
61204600	Lemon juice, frozen
61207000	Lime juice, ns as to form
61207010	Lime juice, fresh
61207200	Lime juice, canned or bottled
61207600	Lime juice, frozen
61210000	Orange juice, nfs
61210010	Orange juice, freshly squeezed
61210220	Orange juice, canned/bottled/carton, unsweetened
61210250	Orange juice, w/ calcium, can/bottle/carton, unsweetened
61210620	Orange juice, frozen, unsweetened, reconst w/ water
61210720	Orange juice, frozen, unsweetened, not reconstituted
61210820	Orange juice, froz, w/,calcium added, recon w/water
61213000	Tangerine juice, nfs
61213220	Tangerine juice, canned, unsweetened
61213230	Tangerine juice, canned, w/ sugar
61213620	Tangerine juice, frozen, unsweet, reconst w/ water
61214000	Grape-tangerine-lemon juice
61216000	Grapefruit & orange juice, nfs
61216010	Grapefruit & orange juice, fresh
61216220	Grapefruit & orange juice, canned, unsweetened
61216230	Grapefruit & orange juice, canned, w/ sugar
61216620	Grapefruit & orange juice, frozen, (reconstituted)
61219000	Orange & banana juice
61219100	Pineapple-orange-banana juice
61219150	Orange-white grape-peach juice
61219650	apricot-orange juice
61222000	Pineapple-grapefruit juice, nfs
61222200	Pineapple-grapefruit juice, canned, ns sweetened
61222220	Pineapple-grapefruit juice, canned, unsweetened
61222230	Pineapple-grapefruit juice, canned, w/ sugar
61222600	Pineapple-grapefruit juice, frozen, reconst w/water
61225000	Pineapple-orange juice, nfs
61225200	Pineapple-orange juice, canned, ns as to sweetener
61225220	Pineapple-orange juice, canned, unsweetened
61225230	Pineapple-orange juice, canned, w/ sugar
61225600	Pineapple-orange juice, frozen, reconst w/ water
61226000	Strawberry-banana-orange juice
64100100	Fruit juice, nfs (include mixed fruit juices)
64100110	Fruit juice blend, 100% juice, w/ vitamin c
64100120	Ambrosia juice (incl Knudsen's)
64101010	Apple cider (include cider, nfs)
64104010	Apple juice
64104150	Apple-cherry juice
64104200	Apple-pear juice
64104450	Apple-raspberry juice
64104500	Apple-grape juice
64104550	Apple-grape-raspberry juice
64104600	Blackberry juice (incl boysenberry juice)
64105400	Cranberry juice, unsweetened
64105500	Cranberry-white grape juice mixture, unsweetened
64116020	Grape juice, unsweetened
64120010	Papaya juice
64121000	Passion fruit juice
64122030	Peach juice, w/ sugar
64124020	Pineapple juice, unsweetened

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64124200	Pineapple-apple-guava juice, w/ added vitamin c
64125000	Pineapple juice-non-citrus juice blend, unsweetened
64132010	Prune juice, ns as to added sweetener
64132020	Prune juice, unsweetened
64132030	Prune juice, w/ sugar
64132500	Strawberry juice
64133100	Watermelon juice
64134000	Fruit smoothie drink, w/ fruit only

<u>**Processed Vegetables and Vegetable Juices**</u>

Tomato Juice
[Wheat Bran Extract] = 0 6%

74301100	Tomato juice
74301150	Tomato juice, low sodium
74302000	Tomato juice cocktail
74303000	Tomato & vegetable juice, mostly tomato (incl v-8)
74303100	Tomato & vegetable juice, mostly tomato, low sodium
74304000	Tomato juice w/ clam or beef juice

<u>**Snack Foods**</u>

Pretzels
[Wheat Bran Extract] = 9 0%

54408000	Pretzels, nfs
54408010	Pretzels, hard
54408020	Pretzels, soft
54408030	Pretzels, hard, unsalted
54408050	Pretzel, oat bran, hard
54408070	Pretzel, hard, multigrain
54408200	Pretzel, hard, chocolate coated
54408250	Pretzel, yogurt covered

SUBMISSION END

AM

Mcmahon, Carrie

Subject: FW: GRN 343 - points of clarification

Attachments: Certif organisme producteu[redacted].pdf

From: Madhu Soni [mailto:SoniM@bellsouth.net]
Sent: Monday, November 15, 2010 9:30 AM
To: Mcmahon, Carrie
Cc: 'Ed Steele'; 'Cathryn Sacra'
Subject: RE: GRN 343 - points of clarification

Dear Dr. McMahon,

In order to clarify the points in your email regarding GRN 343, we have just received information/confirmation from the enzyme supplier. The supplier wants the information about the source of microorganism to be kept confidential. Based on this, Fugeia NV has concluded that the source microorganisms of both the enzymes (a) *Bacillus licheniformis* for alpha-endo-amylase and (b) *Trichoderma longibrachiatum* for xylanase, used in the manufacturing of Wheat Bran Extract are non-toxigenic and non-pathogenic. As regards the identity of the microorganism used in the preparation of xylanase, the supplier has confirmed that it is *Trichoderma longibrachiatum.* The supplier has provided a certificate to this effect (please see attached) and requested to keep this information confidential.

Hope this answers your queries.

Best regards

Madhu

Madhu G. Soni, PhD, FACN
EAS Consulting Group
Phone: 772-299-0746

Attachment: Ce[redacted]teur…

CERTIFICAT D'ORIGINE
CERTIFICATE OF SOURCE

ATTN: Mr Olivier LESCROART – FUNGEIA NV

Produit / *Product* : [redacted]

We hereby confirm that the product above, commercially available since 2008, is produced using the safe and non-toxigenic production strain Trichoderma longibrachiatum.

[redacted]

[redacted]
Directeur général /
General manager

[redacted]

AM

Mcmahon, Carrie

Subject: FW: GRN 343 - points of clarification

Attachments: [redacted] mentioning [redacted].pdf

From: Madhu Soni [mailto:SoniM@bellsouth.net]
Sent: Wednesday, November 17, 2010 4:24 PM
To: Mcmahon, Carrie
Cc: 'Ed Steele'; 'Cathryn Sacra'
Subject: RE: GRN 343 - points of clarification

Dr. McMahon,

Fugeia NV has confirmed with the enzyme supplier that the xylanase used in the production of Wheat Bran Extract is produced by a strain identified under the name *Trichoderma longibrachiatum* with accession number [redacted] The prefix [redacted] indicates that the strain is deposited at the BCCM/MUCL (Agro) Industrial Fungi & Yeasts Collection (http://bccm.belspo.be/about/mucl.php), which is a well-established certified depository organization. We are also providing (please see attached) an [redacted] w [redacted] specifically referring to [redacted] strain as a *Trichoderma longibrachiatum* (see first page bottom of first column in the attachment) confirming both the relevance of the depository organization as well as the identity of the strain within [redacted]. Please note that the enzyme producer has given the information on the strain under confidentiality. Please let me know if there is a need to make the accession number public. In that case we will need to request permission from the enzyme supplier.

Fugeia NV has concluded that *Trichoderma longibrachiatum* the source organism for the enzyme xylanase that is used in the manufacturing of Wheat Bran Extract is non-toxigenic and non-pathogenic.

Hope the above information, satisfactorily addresses your query.

Regards

Madhu

Pages 000123-000127 removed under Freedom of Information exemption 4.