

ORIGINAL SUBMISSION

December 29, 2010

Dr. Robert Martin
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835

Re: GRAS exemption claim for Xylooligosaccharide as an ingredient in foods

Dear Dr. Martin,

This is to notify you that Shandong Longlive Bio-technology Co., Ltd. (or Shandong Longlive, based in Shandong, China) claims that the use of the substance described below (xylooligosaccharide, XOS) is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Shandong Longlive has determined such use to be Generally Recognized As Safe (GRAS).

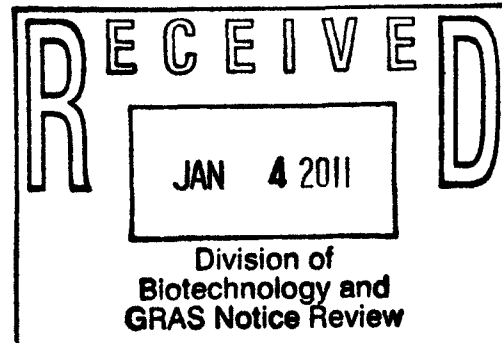
On behalf of Shandong Longlive, NutraSource (an independent consulting firm) assembled a panel of experts highly qualified by scientific training and experience to evaluate the safety of the intended use of XOS. The panel included Dr. Susan Cho at NutraSource (Clarksville, MD), Dr. June Zhou at the Veterans Administration Medical Center (Washington, D.C.), and Dr. George Fahey at the University of Illinois (Urbana, IL). Following independent critical evaluation of the available data and information, the panel has determined that the use of XOS (that is manufactured by Shandong Longlive, China) described in the enclosed notification is GRAS based on scientific procedures.

After reviewing the available data, the Expert Panel concluded in its December 2010 statement that the intended use of Shangdong Longlive's XOS (to be used as ingredient in baby and toddler foods, beverages and beverage bases, dairy product analogs, milk products, health foods, and general foods, at use levels of 0.095 to 1.14 g per serving), resulting in an estimated daily mean intake of 3.53 g XOS and 90th percentile daily intake of 8.08 g, is safe and GRAS for the general population.

This determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR section 170.36) as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

December 29, 2010

Dr. Robert Martin
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835



Re: GRAS exemption claim for Xylooligosaccharide as an ingredient in foods

Dear Dr. Martin,

This is to notify you that Shandong Longlive Bio-technology Co., Ltd. (or Shandong Longlive, based in Shandong, China) claims that the use of the substance described below (xylooligosaccharide, XOS) is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Shandong Longlive has determined such use to be Generally Recognized As Safe (GRAS).

On behalf of Shandong Longlive, NutraSource (an independent consulting firm) assembled a panel of experts highly qualified by scientific training and experience to evaluate the safety of the intended use of XOS. The panel included Dr. Susan Cho at NutraSource (Clarksville, MD), Dr. June Zhou at the Veterans Administration Medical Center (Washington, D.C.), and Dr. George Fahey at the University of Illinois (Urbana, IL). Following independent critical evaluation of the available data and information, the panel has determined that the use of XOS (that is manufactured by Shandong Longlive, China) described in the enclosed notification is GRAS based on scientific procedures.

After reviewing the available data, the Expert Panel concluded in its December 2010 statement that the intended use of Shangdong Longlive's XOS (to be used as ingredient in baby and toddler foods, beverages and beverage bases, dairy product analogs, milk products, health foods, and general foods, at use levels of 0.095 to 1.14 g per serving), resulting in an estimated daily mean intake of 3.53 g XOS and 90th percentile daily intake of 8.08 g, is safe and GRAS for the general population.

This determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR section 170.36) as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

Notifier's name and Address

Shandong Longlive Bio-technology Co., Ltd.

Attention: Alice Fu

Address: Room 806, D&D Fortune Center, No.182-6 Haier Road, Laoshan District, Qingdao, China

Phone number: 86-532-81926803

Fax number: 86-532-81926202

E mail address: alice@longlivegroup.com

Name of GRAS substance

Xylooligosaccharide (XOS) manufactured by Shangdong Longlive Bio-Technology Co., Ltd. (Shandong Longlive).

Product description

Xylooligosaccharide is a hydrolysis product of xylan (a type of dietary fiber), a common hemicellulose of cereal grains (corn cob, corn bran, rice bran, wheat bran, and psyllium) where it occurs in many different compositions and structures. Hemicelluloses and cellulose are considered as major dietary fiber components. The Shandong Longlive's manufacturing process involves a mild pretreatment of corncobs (see below), followed by enzymatic hydrolysis by endoxylanase, which has been commonly used in the production of xylose and xylitol for a long time. The 2007 FAO Technical Meeting Report on Prebiotics classified XOS, FOS, GOS, soya-oligosaccharides, and isomalto-oligosaccharides as prebiotics, defined as a food component that confers a health benefit on the host associated with modulation of the microbiota.

Applicable conditions of use of the notified substance

Xylooligosaccharide is expected to be used in baby and toddler foods (ready-to-eat [RTE] cereals for toddlers; cookies, crackers, and puffs, baby food; ready-to-serve [RTS] fruit-based baby/toddler food; fruit juices, baby food; RTS dinners, baby/toddler food), beverages and beverage bases (ready-to-drink [RTD] energy, sport, and isotonic beverages; carbonated and non-carbonated beverages, water, and beer; processed fruits, juice drinks, and punch; RTD non-milk based meal replacements and protein beverages), dairy product analogs (RTD soy beverages, chocolate milk, and flavored milk; frozen dairy desserts and mixes), milk products (RTD flavored milk and milk drinks; RTD milk-based meal replacements; yogurt, pudding, and jello), health foods (medicinal foods, chewing tablet, and capsule), and general foods (RTE cereals, cereal bars, granola bars, protein bars, and power bars; cookies, crackers, and puffs; chewing gum; chocolate, candy, confectionary and sweet). The proposed use levels of XOS are presented in Table 1.

Table 1. Proposed food application of XOS and maximum levels of use

Food Category	Proposed food uses	Serving size (g)	SD Longlive's XOS* concentration					XOS use levels, g/serving
			95P	70P	35P	20P	70L	
Baby and toddler foods	RTE cereals, toddler	20	0.4	0.54	1.08	1.9	0.77	0.38
	Cookies, crackers, and puffs, baby food	7	0.25	0.34	0.68	1.2	0.49	0.24
	RTS fruit-based baby/toddler food	60 (strained)	0.25	0.34	0.68	1.2	0.49	0.24
		110 (junior)	0.25	0.34	0.68	1.2	0.49	0.24
		125 (toddler)	0.4	0.54	1.08	1.9	0.77	0.38
	Fruit juices, baby food	125	0.25	0.34	0.68	1.2	0.49	0.24
Beverages and beverage bases	RTD energy, sport, and isotonic beverages	225	0.5	0.68	1.37	2.4	0.98	0.48
		225	0.5	0.68	1.37	2.4	0.98	0.48
	Processed fruits, juice drinks, and punch	244	0.3	0.41	0.83	1.45	0.59	0.29
	RTD non-milk based meal replacements and protein beverages	266	0.3	0.41	0.83	1.45	0.59	0.29
Dairy product analogs	RTD soy beverages, chocolate milk, and flavored milk	225	0.5	0.68	1.37	2.4	0.98	0.48
	Frozen dairy desserts and mixes	68	0.3	0.41	0.83	1.45	0.59	0.29
Milk products	RTD flavored milk and milk drinks	250	0.3	0.41	0.83	1.45	0.59	0.29
	RTD milk-based meal replacements	266	0.3	0.41	0.83	1.45	0.59	0.29
	Yogurt, pudding, and jello	225	0.5	0.68	1.37	2.4	0.98	0.48
Health foods	Medicinal foods	40	1.2	1.63	3.25	5.7	2.33	1.14
	Chewing tablet and capsule	2	1.2	1.63	3.25	5.7	2.33	1.14
General foods	RTE cereals, cereal bars, granola bars, protein bars, and power bars	40	0.3	0.41	0.83	1.45	0.59	0.29
	Cookies, crackers, and puffs	40	0.3	0.41	0.83	1.45	0.59	0.29
	Chewing gum	1 stick	0.1	0.14	0.27	0.48	0.19	0.095
	Chocolate, candy, confectionary and sweet	40	0.3	0.41	0.83	1.45	0.59	0.29

*95P=powder form of 95% XOS; 70P=70% XOS, powder; 35P=35% XOS, powder; 20P=20% XOS, powder; 70L=liquid form of 49% XOS; RTE=ready-to-eat; RTD=ready-to-drink; RTS=ready-to-serve.

Exposure estimates

Even if 100% of the product will be used under the intended use, the median intakes including XOS from all GRAS-proposed use categories by users of one or more foods is 1.06 g/d for male children aged 0-2 yr and 3.53 g/d for the entire population. The 90th percentile intakes including XOS from all GRAS-proposed use categories by users of one or more foods are 2.75 g/d (or 242 mg/kg BW/d) in infants and toddlers aged 0-2 yr, 3.96 g/d (or 152 mg/kg BW/d) for young children aged 3-11 yr, 6.62 g/d (or 103 mg/kg BW/d) in older children and teenagers aged 13-19 yr, and 8.83 g/d (or 112 mg/kg BW/d) in adults aged 20 and older. These levels are more than 12-35x below the NOAEL values that have been found from subacute toxicity studies in rats and chicks.

Basis of GRAS determination

Through scientific procedures.

Review and copying statement

The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of Shandong Longlive Bio-technology Co., Ltd. or NutraSource, Inc.

We enclose an original and two copies of this notification for your review. If you have any questions, please contact me.

Sincerely,

Susan Cho, Ph.D.
Chief Science Officer
NutraSource, Inc.
6309 Morning Dew Ct.
Clarksville, MD 21029
Phone: 410-531-3336 (O) or 301-875-6454 (C)
E mail: susanscho1@yahoo.com

Exposure estimates

Even if 100% of the product will be used under the intended use, the median intakes including XOS from all GRAS-proposed use categories by users of one or more foods is 1.06 g/d for male children aged 0-2 yr and 3.53 g/d for the entire population. The 90th percentile intakes including XOS from all GRAS-proposed use categories by users of one or more foods are 2.75 g/d (or 242 mg/kg BW/d) in infants and toddlers aged 0-2 yr, 3.96 g/d (or 152 mg/kg BW/d) for young children aged 3-11 yr, 6.62 g/d (or 103 mg/kg BW/d) in older children and teenagers aged 13-19 yr, and 8.83 g/d (or 112 mg/kg BW/d) in adults aged 20 and older. These levels are more than 12-35x below the NOAEL values that have been found from subacute toxicity studies in rats and chicks.

Basis of GRAS determination

Through scientific procedures.

Review and copying statement

The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of Shandong Longlive Bio-technology Co., Ltd. or NutraSource, Inc.

We enclose an original and two copies of this notification for your review. If you have any questions, please contact me.

Sincerely,

(b) (6)

Susan Cho, Ph.D.
Chief Science Officer
NutraSource, Inc.
6309 Morning Dew Ct.
Clarksville, MD 21029
Phone: 410-531-3336 (O) or 301-875-6454 (C)
E mail: susanscho1@yahoo.com

Executive Summary

The objective of this Generally Recognized as Safe (GRAS) determination is to summarize the available safety information on xylooligosaccharide (XOS) used as an ingredient in foods and beverages as a source of prebiotics.

We, the undersigned expert panel members, Susan Cho, Ph.D., June Zhou, Ph.D., and George Fahey, Ph.D., have individually and collectively critically evaluated the materials summarized in the XOS GRAS report, and conclude that XOS is safe and GRAS for its intended use in foods. There is broad-based and widely disseminated knowledge concerning the chemistry and physiological benefits of XOS. Xylooligosaccharide possesses prebiotic and antioxidant activities. Xylooligosaccharide is a non-digestible oligosaccharide (NDO) with a degree of polymerization (DP) units of 2-7.

Xylooligosaccharide and similar molecules (galactooligosaccharide [GOS] and fructooligosaccharide [FOS]) are naturally present in the diet. Xylooligosaccharide has a long history of safe use as a sweetener and a prebiotic ingredient in foods.

Xylooligosaccharide and most beta-linked carbohydrates are not digested by human pancreatic or brush border enzymes, and the compounds are not expected to be absorbed intact. Xylooligosaccharide reaches the large intestine where it is fermented by the colonic microflora to short-chain fatty acids (SCFA) that promote colon health. Published studies indicate that XOS is of low toxicity in animals and humans. The XOS described in this self-affirmation is prepared from corncob *via* hydrolysis by xylanase isolated from *Streptomyces olivaceoviridis*.

Intended food applications include baby and toddler foods (ready-to-eat [RTE] cereals for toddlers; cookies, crackers, and puffs, baby food; ready-to-serve [RTS] fruit-based baby/toddler food; fruit juices, baby food; RTS dinners, baby/toddler food), beverages and beverage bases (ready-to-drink [RTD] energy, sport, and isotonic beverages; carbonated and non-carbonated beverages, water, and beer; processed fruits, juice drinks, and punch; RTD non-milk based meal replacements and protein beverages), dairy product analogs (RTD soy beverages, chocolate milk, and flavored milk; frozen dairy desserts and mixes), milk products (RTD flavored milk and milk drinks; RTD milk-based meal replacements; yogurt, pudding, and jello), health foods (medicinal foods, chewing tablet, and capsule), and general foods (RTE cereals, cereal bars, granola bars, protein bars, and power bars; cookies, crackers, and puffs; chewing gum; chocolate, candy, confectionary and sweet).

Assuming all of food products will be used under the intended use (at use levels of between 0.095 and 1.14 g per serving), the 90th percentile daily consumption under proposed new food use of XOS would result in 2.75 g/d (or 242 mg/kg body weight (BW)/d) in infants and toddlers aged 0-2 yr, 3.96 g/d (or 152 mg/kg BW/d) for young children aged 3-11 yr, 6.62 g/d (or 103 mg/kg BW/d) in older children and teenagers aged 13-19 yr, and 8.83 g/d (or 112 mg/kg BW/d) in adults aged 20 and older. These levels represent more than 12-35x below the no-observed-adverse-effect level (NOAEL) values of over 3,000-4,000 mg/kg BW/d that have been found from subacute toxicity studies in rats. A chick study also demonstrated no adverse effects of XOS at 4% of the diet. Human clinical studies reported beneficial effects with no adverse effects of XOS.

Intakes of XOS (5.5-10 g/d) were well tolerated without adverse events for durations of up to 8 wk.

There are no indications of significant adverse effects related to XOS in the publicly available literature. The acute toxicity of XOS has been reported to be > 15 g/kg BW in rats. Subchronic toxicity studies administering XOS via diet were unremarkable; NOAEL determinations of 3,000-4,000 mg/kg BW/d in rats and 4% of the diet in chicks, the highest doses administered, have been reported. The mutagenicity and genotoxicity studies of XOS showed that XOS was not to be genotoxic or mutagenic. The only side effect reported in humans is gastrointestinal discomfort which is a transient symptom when consumed in large quantities. This type of gastrointestinal discomfort is a common phenomenon associated with high intakes of dietary fiber and that have no toxicological relevance to humans.

Therefore, not only is the proposed use of XOS safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS) according to Title 21 Code of Federal Regulations (21 CFR). The basis of the present proposed GRAS determination is through scientific procedures.

Susan Cho, Ph.D.
President, NutraSource, Inc., Clarksville, MD 21029

Signature: _____ Date: _____

June Zhou, Ph.D.
Deputy Director, VA Medical Center, Washington, D.C.

Signature: _____ Date: _____

George C. Fahey, Jr., Ph.D.
Professor Emeritus, University of Illinois, Urbana, IL 61801

Signature: _____ Date: _____

Intakes of XOS (5.5-10 g/d) were well tolerated without adverse events for durations of up to 8 wk.

There are no indications of significant adverse effects related to XOS in the publicly available literature. The acute toxicity of XOS has been reported to be > 15 g/kg BW in rats. Subchronic toxicity studies administering XOS via diet were unremarkable; NOAEL determinations of 3,000-4,000 mg/kg BW/d in rats and 4% of the diet in chicks, the highest doses administered, have been reported. The mutagenicity and genotoxicity studies of XOS showed that XOS was not to be genotoxic or mutagenic. The only side effect reported in humans is gastrointestinal discomfort which is a transient symptom when consumed in large quantities. This type of gastrointestinal discomfort is a common phenomenon associated with high intakes of dietary fiber and that have no toxicological relevance to humans.

Therefore, not only is the proposed use of XOS safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS) according to Title 21 Code of Federal Regulations (21 CFR). The basis of the present proposed GRAS determination is through scientific procedures.

Susan Cho, Ph.D.
President, NutraSource, Inc., Clarksville, MD 21029

Signature: _____ (b) (6) _____ Date: 12/24/2010

June Zhou, Ph.D.
Deputy Director, VA Medical Center, Washington, D.C.

Signature: _____ Date: _____

George C. Fahey, Jr., Ph.D.
Professor Emeritus, University of Illinois, Urbana, IL 61801

Signature: _____ (b) (6) _____ Date: 12/23/10

Intakes of XOS (5.5-10 g/d) were well tolerated without adverse events for durations of up to 8 wk.

There are no indications of significant adverse effects related to XOS in the publicly available literature. The acute toxicity of XOS has been reported to be > 15 g/kg BW in rats. Subchronic toxicity studies administering XOS via diet were unremarkable; NOAEL determinations of 3,000-4,000 mg/kg BW/d in rats and 4% of the diet in chicks, the highest doses administered, have been reported. The mutagenicity and genotoxicity studies of XOS showed that XOS was not to be genotoxic or mutagenic. The only side effect reported in humans is gastrointestinal discomfort which is a transient symptom when consumed in large quantities. This type of gastrointestinal discomfort is a common phenomenon associated with high intakes of dietary fiber and that have no toxicological relevance to humans.

Therefore, not only is the proposed use of XOS safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS) according to Title 21 Code of Federal Regulations (21 CFR). The basis of the present proposed GRAS determination is through scientific procedures.

Susan Cho, Ph.D.
President, NutraSource, Inc., Clarksville, MD 21029

Signature: _____ Date: _____

June Zhou, Ph.D.
Deputy Director, VA Medical Center, Washington, D.C.

(b) (6)
Signature: _____ Date: 12-28-2010

George C. Fahey, Jr., Ph.D.
Professor Emeritus, University of Illinois, Urbana, IL 61801

Signature: _____ Date: _____

I. Identity of Substance

I.A. Common or trade name: Xylooligosaccharide (XOS) manufactured by Shangdong Longlive BioTechnology (SD Longlive).

I.B. Standards of identity: We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity that is located in Title 21 of the Code of Federal Regulations.

I.C. Background

Xylooligosaccharide is a non-digestible oligosaccharide (NDO) composed of 2-7 xylose molecules bonded with β (1-4) glycosidic bonds. Xylooligosaccharide is naturally present in fruits, vegetables, bamboo, honey, and milk, and can be produced on an industrial scale by enzymatic hydrolysis of xylan. Xylan is the major component of plant hemicelluloses that are composed of xylose and arabinose backbones with side chains of galactose, glucose, and(or) mannose (Southgate, 1979).

Xylooligosaccharide has a sweet taste and is used as an alternative sweetener. Xylooligosaccharide also functions as a prebiotic by stimulating the growth of healthy microflora, such as bifidobacteria, in the gut (FAO, 2007; Grootaert et al., 2007; Kabel et al., 2002; Vazquez et al., 2000). Non-digestible oligosaccharides resist digestion and absorption in the human small intestine and are completely or partially fermented in the large intestine. Xylooligosaccharide is widely used in fields of medicine and in health products, foods, beverages, and feed (Moure et al., 2006; Vazquez et al., 2000). Xylooligosaccharide has acceptable organoleptic properties and does not exhibit toxicity or negative effects on human health (Vazquez et al., 2000).

SD Longlive's XOS is made from corncob. Corncob contains approximately 35% xylan (Aachary and Prapulla, 2009; Moure et al., 2006) and is an important by-product of the corn industry. Corncob is utilized to produce XOS, xylose, and xylitol (Aachary and Prapulla, 2009; Wang et al., 2010). A partial hydrolysis of xylan (polysaccharides) results in XOS, while a complete hydrolysis produces xylose, a monosaccharide.

Like other NDOs including fructooligosaccharide (FOS) and galactooligosaccharide (GOS), XOS escapes digestion in the upper gastrointestinal tract and is fermented in the lower gastrointestinal tract to short-chain fatty acids (SCFA; Alles et al., 1996, 1997; Campbell et al., 1997; Fleming et al., 1983a, 1983b, 1983c) and exerts prebiotic activities by promoting the growth of bifidobacteria (Table 1; Bounnik et al., 1997, 2004; Crittenden et al., 2002; Fujikawa et al., 1991; Moura et al., 2008; Park et al., 2002; Ryu et al., 2002; van Laerke et al., 2000; van Loo et al., 1999; Yazawa et al., 1978). The 2007 FAO Technical Meeting Report on Prebiotics classified XOS, FOS, GOS, soya-oligosaccharides, and isomalto-oligosaccharides (IMO) as prebiotics, defined as a food

component that confers a health benefit on the host associated with modulation of the microbiota (FAO, 2007).

Table 1. Classification of xylose-based carbohydrates and NDOs.

Item	Chemical classification	Digestibility in upper GI tract	Fermentability in the lower GI tract
Xylose-based carbohydrates			
Xylan	polysaccharide	Non-digestible	Yes
Xylooligosaccharide	oligosaccharide	Non-digestible	Yes
Xylose	monosaccharide	Mostly absorbed	Negligible
Other non-digestible oligosaccharides similar in nature to XOS			
Fructooligosaccharide	oligosaccharide	Non-digestible	Yes
Galactooligosaccharide	oligosaccharide	Non-digestible	Yes
Soya oligosaccharide	oligosaccharide	Non-digestible	Yes
Isomaltooligosaccharide	oligosaccharide	Non-digestible	Yes

I.D. Physicochemistry and structure of XOS

Molecular formula: $C_5^nH^{8n+2}O^{4n+1}$, where $n=2-7$.

Acid and thermal stability

XOS is stable after heating to 100°C under acid conditions (pH=2.5-8), which covers the

pH value of the vast majority of food systems (Courtin et al., 2009; Vazquez et al., 2000). In food processing, XOS shows advantages over inulin in terms of resistance to both acids and heat, allowing their utilization in low-pH juices and carbonated drinks (Modler et al., 1994; Vazquez et al., 2000).

I.E. Manufacturing Process

Xylooligosaccharide is produced from xylan-rich corncob by chemical methods, autohydrolysis, direct enzymatic hydrolysis of a susceptible substrate, or a combination of chemical and enzymatic treatments (Garrote et al., 2001; Ninawe et al., 2005; Pazur et al., 1957; Vazquez et al., 2000). However, enzymatic production of XOS is preferred in the food industry (Parajo et al., 2004; Pazur et al., 1957). Corncob is a heterogenous substrate sparingly soluble in water and the complete extraction of xylan is time-consuming. Thus, a mild pretreatment method (see below) is widely used to make the xylan available for enzymatic reaction (Jiang et al., 2005, 2006; Parajo et al., 2004).

The SD Longlive's manufacturing process involves a mild pretreatment of corncobs (see below), followed by enzymatic hydrolysis by endoxylanase, which has been commonly used in the production of xylose and xylitol for a long time.

Process description

1. Size mixing: mix corncob powder with water in a ratio of 1:6-10 and heat to 75 °C

or above.

2. Cooking/pretreatment: add glacial acetic acid (0.2-1.5% by weight of corncob) and hold the mixture at 155-180 °C under a pressure of 0.50-0.70 MPa for 30-120 min to break down the hemicelluloses present in corncob.
3. Hydrolysis: add xylanase (isolated from *Streptomyces olivaceoviridis*) and

incubate at 45-60°C, pH 5.0-6.0 (adjusted by 1 mol/L hydrochloric acid or 1 mol/L

sodium hydroxide) for 4-10 h to hydrolyze beta-1,4-xylosidic bonds in the beta-(1,4)-linked D-xylosyl backbone of xylan into XOS.

4. Separation of the slag: separate the liquid from hydrolysis products through the liquid slag separator.

5. Decoloring: add activated carbon (0.5-1.5% by weight) at 70-85°C for 25-40 min,

and then filter by plate and frame filter (equipment for filtration) to remove the pigment and other impurities.

6. Ion exchange at $\leq 45^{\circ}\text{C}$:

Cation column: Strongly acidic cation exchange resin, cross-linked polystyrene matrix, sulphonate functional group, Na^{+} counter-ion.

Anion column: Macroporous, weakly basic anion exchange resin, cross-linked polystyrene matrix, dimethyl-tertiary amine functional group, OH^{-} counter-ion.

7. Filtration: filter impurities through nanofilter membrane at 15-30°C.

8. Concentration: concentrate the liquid to 40%-75% at 60-80°C.
9. Adjust the saccharide content: prepare several different concentration liquids by adding some excipient.
10. Spray drying: spray dry with inlet temperature of 130-160°C and outlet temperature of 65-85°C.

Quality control process:

Process tanks and lines are cleaned with sodium hydroxide and hydrogen peroxide following standard procedures common to the dairy industry. All ion exchange resins used for chromatographic purification of the XOS and for demineralization comply with 21 C.F.R. § 173.25. Celite is cleared under 27 CFR § 24.243 (filtering aids). Similar uses of activated carbon are considered GRAS for purification and clarification of wine as per 27 CFR §24.246. All processing aids used in the manufacturing process are considered safe and suitable.

The immobilized enzyme preparation is sterilized every 3 d with a solution of food grade acetic acid, potassium sorbate, and sodium benzoate. The materials from enzyme sources are not included in the final product (rt-PCR and ELISA methods are used for verification).

I.F. Specifications

Tables 2 and 3 list specifications of XOS.

Table 2. Specifications of SD Longlive's XOS preparations

Item	XOS 95	XOS 70		XOS 35	XOS 20
	Powder	Powder	Liquid	Powder	Powder
Dry substance, %	≥95	≥95	70-75	≥94	≥94
Moisture, %	≤5.0	≤5.0	≤30.0	≤6.0	≤6.0
Ash, %	≤0.3	≤0.3	≤0.3	≤0.3	≤0.3
pH	3.5-6.5	3.5-6.5	3.5-6.5	3.5-6.5	3.5-6.5
XOS ₂₋₇ content, dry wt. basis	≥ 95%	≥ 70%	≥ 70%	≥ 35%	≥ 20%
XOS ₂₋₄ content, dry wt. basis	≥ 65%	≥ 50%	≥ 50%	/	/
Arsenic, ppm by wt.	≤0.3	≤0.3	≤0.3	≤0.3	≤0.3
Lead, ppm by wt.	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Copper, ppm by wt.	≤5	≤5	≤5	≤5	≤5

*95P=powder form of 95% XOS; 70P=70% XOS, powder; 35P=35% XOS, powder; 20P=20% XOS, powder; 70L=liquid form of 49% XOS.

Table 3. Microbiological specifications

Microorganism	Limit
Total bacterial count, cfu/g or ml	≤1000 cfu/g or ml
Coliform bacteria, MNP/100g or ml	≤30 or not detectable
Yeast, cfu/g or ml	≤25
Mold, cfu/g or ml	≤25
Pathogenic bacteria (salmonella, shigella, and golden yellow staphylococci)	Negative

Table 4. Sensory properties of XOS

Product	XOS liquid	XOS powder
Appearance	Transparent and sticky liquid, without visible impurities	Powder without visible impurities
Color	Yellowish	White or yellowish
Taste	Sweet taste without abnormal flavor	Sweet taste without abnormal flavor
Odor	No abnormal smell	No abnormal smell

I.F. Analytical methods

The following methods were used in the chemical and microbiological analysis: total plate count and yeast and mold count by the USP 2021 method; *E.coli* and salmonella by the USP 2022 method; lead by inductively coupled plasma mass spectrometry (ICP/MS) or atomic absorption (AA) spectrophotometry.

II. Natural occurrence and exposure to XOS.

II.A. Food sources of XOS

Xylooligosaccharide is a hydrolysis product of xylan (a type of dietary fiber), a common hemicellulose of cereal grains (corn cob, corn bran, rice bran, wheat bran, and psyllium) where it occurs in many different compositions and structures (Garcia et al., 2000). Hemicelluloses and cellulose are considered as major dietary fiber components. The xylan from corncobs has a chemical composition of 4-O-methyl-D-glucuronic acid, L-arabinose, and D-xylose in the proportion of 2:7:19, respectively (Ai et al., 2005; Collins et al., 2005; Garcia et al., 2000).

D-xylose (monosaccharide), a hydrolysis product of XOS and xylan, is found in the juice of fruits such as apples and peaches as a component of the polysaccharide, xylan (Hardinge et al., 1965). D-Xylose is a white crystal or crystalline powder with a mild and fresh sweet taste but without a smell. Its degree of sweetness is about 60–70% of that of sugar. D-xylose is used for browning and flavor improvement of foods. It is also used as a low-calorie sweetener (Suzuki et al., 1999). In Japan, D-xylose is widely used as a natural food additive in the fishery products and baking industries (MHLW, 1996a, 1996b; JETRO, 2004).

II.B. Intended use

Table 5 presents primary applications of XOS that include baby and toddler foods (RTE cereals for toddlers; cookies, crackers, and puffs, baby food; RTS fruit-based baby/toddler food; fruit juices, baby food; RTS dinners, baby/toddler food), beverages and beverage bases (RTD energy, sport, and isotonic beverages; carbonated and non-carbonated beverages, water, and beer; processed fruits, juice drinks, and punch; RTD non-milk based meal replacements and protein beverages), dairy product analogs (RTD soy beverages, chocolate milk, and flavored milk; frozen dairy desserts and mixes), milk products (RTD flavored milk and milk drinks; RTD milk-based meal replacements; yogurt, pudding, and jello), health foods (medicinal foods, chewing tablet, and capsule), and general foods (RTE cereals, cereal bars, granola bars, protein bars, and power bars; cookies, crackers, and puffs; chewing gum; chocolate, candy, confectionary and sweet), at use levels of 0.095 to 0.48 g per serving. Food codes representative of each proposed food use were chosen from the National Center for Health Statistics (NCHS) 2007-2008 National Health and Nutrition Examination Survey (NHANES) (CDC, 2010; USDA, 2010) and were grouped in food use categories according to Title 21, Section

s170.3 of the *Code of Federal Regulations* (U.S. FDA, 2008). XOS ingredients are not intended for use in meat or poultry-containing products.

Table 5. Intended use of XOS

Food Category	Proposed food uses	Serving size (g)	SD Longlive's XOS* concentration					XOS use levels, g/serving
			95P	70P	35P	20P	70L	
Baby and toddler foods	RTE cereals, toddler	20	0.4	0.54	1.08	1.9	0.77	0.38
	Cookies, crackers, and puffs, baby food	7	0.25	0.34	0.68	1.2	0.49	0.24
	RTS fruit-based baby/toddler food	60 (strained)	0.25	0.34	0.68	1.2	0.49	0.24
		110 (junior)	0.25	0.34	0.68	1.2	0.49	0.24
		125 (toddler)	0.4	0.54	1.08	1.9	0.77	0.38
	Fruit juices, baby food	125	0.25	0.34	0.68	1.2	0.49	0.24
Beverages and beverage bases	RTD energy, sport, and isotonic beverages	60 (strained)	0.25	0.34	0.68	1.2	0.49	0.24
		110 (junior)	0.25	0.34	0.68	1.2	0.49	0.24
	RTD dinners, baby/toddler food	170 (toddler)	0.4	0.54	1.08	1.9	0.77	0.38
Beverages and beverage bases	RTD energy, sport, and isotonic beverages	225	0.5	0.68	1.37	2.4	0.98	0.48
	Carbonated and non-carbonated beverages and water, and beer	225	0.5	0.68	1.37	2.4	0.98	0.48
	Processed fruits, juice drinks, and punch	244	0.3	0.41	0.83	1.45	0.59	0.29
	RTD non-milk based meal replacements and protein beverages	266	0.3	0.41	0.83	1.45	0.59	0.29
Dairy product analogs	RTD soy beverages, chocolate milk, and flavored milk	225	0.5	0.68	1.37	2.4	0.98	0.48
	Frozen dairy desserts and mixes	68	0.3	0.41	0.83	1.45	0.59	0.29
Milk products	RTD flavored milk and milk drinks	250	0.3	0.41	0.83	1.45	0.59	0.29
	RTD milk-based meal replacements	266	0.3	0.41	0.83	1.45	0.59	0.29
	Yogurt, pudding, and jello	225	0.5	0.68	1.37	2.4	0.98	0.48
Health foods	Medicinal foods	40	1.2	1.63	3.25	5.7	2.33	1.14
	Chewing tablet and capsule	2	1.2	1.63	3.25	5.7	2.33	1.14
General foods	RTE cereals, cereal bars, granola bars, protein bars, and power bars	40	0.3	0.41	0.83	1.45	0.59	0.29
	Cookies, crackers, and puffs	40	0.3	0.41	0.83	1.45	0.59	0.29
	Chewing gum	1 stick	0.1	0.14	0.27	0.48	0.19	0.095
	Chocolate, candy, confectionary and sweet	40	0.3	0.41	0.83	1.45	0.59	0.29

*95P=powder form of 95% XOS; 70P=70% XOS, powder; 35P=35% XOS, powder; 20P=20% XOS, powder; 70L=liquid form of 49% XOS; RTE=ready-to-eat; RTD=ready-to-drink; RTS=ready-to-serve.

II.C. Current consumer intake levels

Based on food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES; 2007-2008) compiled by the U.S. Department of Health and Human Services, National Center for Health Statistics and the Nutrition Coordinating Center, estimates of 2-d average intakes of dietary fiber were calculated from the food code list and the survey database of diet recalls. The usual intake estimation procedure requires multiple days of nutrient intake data for at least a representative subsample of the individuals in the sample in order to estimate variances. The NHANES provides the most current food consumption data available for the American population. The NHANES was conducted between 2007-2008 with non-institutionalized individuals in the U.S. In each of the two survey years, data were collected from a nationally representative sample of individuals of all ages. The food and dietary supplement record for each individual includes the gram weight and nutrient data for all foods consumed during the day of the recall. All estimates were generated with USDA sampling weights to adjust for differences in representation of subpopulations. However, the NHANES dataset and USDA food composition tables do not list XOS content in foods. Thus, it is not possible to estimate the current consumer intake levels.

II.D. Estimated Daily Intake of XOS from GRAS Uses

Using food intake data reported in the 2007-2008 NHANES, exposure levels to SD Longlive's XOS that will result from the intended uses were estimated (Table 6). The estimate is based on the assumption that 100% of the products are used under the intended use. This is a highly unlikely scenario since it is not possible to use all the food groups under the intended use. Also, wastage and other losses should be considered.

Even if 100% of the product will be used under the intended use, the median intakes including XOS from all GRAS proposed use categories by users of one or more foods is 1.06 g/d for male children aged 0-2 yr and 3.53 g/d for the entire population. The 90th percentile intakes including XOS from all GRAS-proposed use categories by users of one or more foods are 2.75 g/d (or 242 mg/kg BW/d) in infants and toddlers aged 0-2 yr, 3.96 g/d (or 152 mg/kg BW/d) for young children aged 3-11 yr, 6.62 g/d (or 103 mg/kg BW/d) in older children and teenagers aged 13-19 yr, and 8.83 g/d (or 112 mg/kg BW/d) in adults aged 20 and older. These levels are more than 12-35x below the NOAEL values that have been found from subacute toxicity studies in rats and chicks (Graham et al., 2004; Park et al., 2001; SD Longlive, 2010).

On an individual population basis, the greatest mean and 90th percentile all-user exposures were estimated to occur in male adults (aged over 20 yr) at 9.96 g/person/d (115 mg/kg BW/d). On a body weight basis, mean and 90th percentile all-user intakes of XOS were highest in infants, ages 0 to 2 yr, with intakes of 246 mg/kg BW/d.

Table 6a. XOS exposure estimates, g/d

Age, yr	Gender	Mean	SE	Pct 10	Pct 25	Pct 50	Pct 75	Pct 90
0-2	All	1.27	0.03	0.07	0.47	1.02	1.76	2.75
0-2	Male	1.34	0.05	0.08	0.51	1.06	1.90	2.95
0-2	Female	1.20	0.04	0.06	0.44	0.99	1.69	2.53
3-11	All	2.26	0.04	0.86	1.33	2.03	2.91	3.96
3-11	Male	2.37	0.05	0.89	1.36	2.09	3.13	4.20
3-11	Female	2.15	0.04	0.84	1.29	1.95	2.75	3.63
12-19	All	3.64	0.08	1.17	1.96	3.10	4.59	6.62
12-19	Male	4.12	0.11	1.36	2.18	3.43	5.09	7.67
12-19	Female	3.12	0.07	1.01	1.79	2.78	4.05	5.83
20+	All	4.83	0.08	1.61	2.62	4.19	6.27	8.83
20+	Male	5.48	0.11	1.93	3.07	4.75	7.10	9.96
20+	Female	4.25	0.07	1.40	2.35	3.73	5.55	7.60
0+	All	4.23	0.06	1.19	2.10	3.53	5.60	8.08

Pct=percentile; Pct 10=10th percentile.

Table 6b. XOS exposure estimates, mg/kg BW

Age, yr	Gender	Mean	SE	Pct 10	Pct 25	Pct 50	Pct 75	Pct 90
0-2	All	112.32	3.03	7.45	44.16	88.71	151.30	242.10
0-2	Male	116.65	3.99	8.04	46.29	89.07	158.77	244.05
0-2	Female	107.85	3.41	7.10	41.82	88.04	144.96	239.40
3-11	All	84.99	1.25	30.85	48.86	75.14	110.02	152.02
3-11	Male	88.01	1.81	32.07	51.93	78.76	113.71	155.31
3-11	Female	81.89	1.61	29.73	46.15	72.15	106.52	147.42
12-19	All	57.25	1.33	18.39	31.52	49.66	72.82	103.15
12-19	Male	61.79	1.84	20.78	33.44	51.30	76.72	113.21
12-19	Female	52.39	1.28	15.90	29.13	47.15	69.03	92.91
20+	All	61.59	0.93	20.58	33.57	52.72	79.46	112.41
20+	Male	63.86	1.13	22.44	34.96	54.80	82.68	115.42
20+	Female	59.52	0.99	18.94	32.35	51.02	76.43	108.53
0+	All	66.32	0.92	20.83	34.52	55.55	84.86	123.05

Pct=percentile; Pct 10=10th percentile.

III. Basis for GRAS determination

III.A. Current regulatory status

Currently, XOS is not listed as an approved food additive or a GRAS-affirmed substance in the U.S. The 2007 FAO Technical Meeting Report on Prebiotics classified XOS as a prebiotic, defined as a food component that confers a health benefit on the host associated with modulation of the microbiota (FAO, 2007). Xylooligosaccharide is commercially used as a food ingredient in Japan. The FOSHU ('Food for Specified Health Use') has been used in Japan since 1991 (Vazquez et al., 2000). FOSHU foods are expected to have a specific effect on health due to the relevant constituent(s) of the foods.

Other NDOs, such as FOS (GRN 44), GOS (GRN 236, 285, and 286) and IMO (GRN 246), already are listed as GRAS substances. Also, xylose and xylitol (xylose-based sugar alcohol) are listed as GRAS substances and are included in the *"Everything" Added to Food in the United States* (EAFUS) list. The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS. Many dietary fiber ingredients containing xylose backbones (i.e., xylans or arabinoxylans) such as psyllium, corn bran, rice bran, and wheat bran, are considered as GRAS ingredients by the US FDA (21 CFR, Part 184.1890, 182.8890, and 182.8892) when used in accordance with good manufacturing practice. FDA has approved a health claim for psyllium fiber and heart disease risk reduction.

In addition, XOS is a component of dietary fiber that is considered as an essential nutrient low in the American diet. The Institute of Medicine (IOM, 2002) and the USDA Dietary Guidelines Committee (2004, 2010) recommended increased consumption of dietary fiber for Americans of all ages.

III.B. Intended technical effects

Xylooligosaccharide can be used as an ingredient in foods and beverages as a prebiotic source.

III.C. Review of safety data

III.C.1. Metabolic fate of XOS

Digestion tests with salivary juice, gastric juice, pancreatic juice, and intestinal juice show that the digestive juices can not decompose XOS (Kunimasa and Shigeaki, 1991). For example, xylobiose (X2) cannot be hydrolyzed by saliva, pancreatin, gastric juice, or intestinal mucosa homogenate and ingested X2 is not excreted in feces or urine in the 24 h following oral administration. Joo et al. (1998) compared the digestibility of XOS, FOS and IMO by digestive tract juices and the effect of XOS on the absorption of bile acids. HPLC analysis showed no hydrolysis products of FOS, IOS, or XOS after 4 h of *in vitro* digestion (Joo et al., 1998). Also, supplementation of 4.0 g XOS/d for 3 wk decreased the fecal pH values (XOS group, 6.50 vs. control, 7.39, $p < 0.05$) in the elderly (Chung et al., 2007). These data suggest utilization of XOS by intestinal bacteria (Okazaki et al., 1991).

Colonic XOS fermentation leads to production of CO₂, H₂, SCFA (acetate, propionate, and butyrate) and lactate. These may be further metabolized to provide energy generation for the host. A number of health effects have been reported for SCFA, including improvement in bowel function, calcium absorption, lipid metabolism, and reduction of the risk of colon cancer (Scheppach et al., 2001).

The health effects of XOS are mainly related to their effects on the gastrointestinal flora. Xylooligosaccharide is a class of NDO prebiotic that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Gibson and Roberfroid, 1995; Moure et al., 2006). Prebiotics increase the numbers of bifidobacteria and decrease clostridia (Gibson and Roberfroid, 1995; Holzapfel and Schillinger, 2002; Rycroft et al., 2001).

Xylooligosaccharide has been demonstrated to be extensively utilized by several species of bifidobacteria. *In vitro* assays proved that *Bifidobacterium* spp. and *B. adolescentis* utilized both X2 and X3. XOS was readily utilized by *B. bifidum*, and the oral ingestion of XOS promoted the proliferation of *B. bifidum* in intestines (Okazaki et al., 1990; Oku et al., 2002; Par et al., 1992). However, XOS is used slightly or not at all by species of bacteroides, staphylococcus, *Escherichia coli*, and clostridium under *in vitro* conditions. Xylooligosaccharide was utilized by limited strains of lactobacilli (Kontula et al., 1998).

In vivo administration of XOS to human subjects promoted growth of bifidobacteria (Okazaki et al., 1990; Ryu et al., 2002). Bifidobacteria are associated with decreased illness and the suppression of potentially pathogenic and putrefactive bacteria (Camilleri, 2006; de Vrese et al., 2006; Hidaka et al., 1991) due to their specific utilization of oligo- and polysaccharides that are not utilized by other intestinal bacteria (Yazawa et al., 1978). Also, bifidobacteria produce substances that would be bactericidal to others including some clostridia.

Xylooligosaccharide also maintains fecal water content within the normal range (Dohnalek et al., 1998 a, 1998b; Jeong et al., 1998; Tateyama et al., 2005). Howard et al. (1995) reported that consumption of XOS supports a modest enhancement of cecal epithelial cell proliferation in mice and rats. The trophic effect of fermentable fiber on colonic epithelial cell proliferation has been attributed to SCFA production resulting from the anaerobic fermentation of structural oligo- and polysaccharides by bacteria (Sakata, 1987; Sakata and von Englehardt, 1983). Increased cecum weights following consumption of other indigestible carbohydrates (sorbitol, mannitol, xylitol, caramel, and polydextrose) in rodents is a well established phenomenon, and is not considered to have toxicological relevance to humans (WHO, 1987).

III.C.2. Safety studies

III.C.2.1. Preclinical Studies of XOS

Published studies indicate that XOS is of low toxicity to animals. The acute toxicity of XOS has been reported to be 10 g/kg BW in the rat (Park et al., 1999) and >20 g/kg BW in mice (SD Longlive, 2010). Table 7 summarizes the toxicity studies on XOS. A subacute toxicity of XOS in young rats demonstrated that the NOAEL of XOS was 3,000 mg/kg BW (Park et al., 2000) and 4% in the diet in chicks (Graham et al., 2004). Unpublished data from SD Longlive confirmed the previous findings; NOAEL was found to be 4,000 mg/kg BW. Other animal studies measuring various endpoints reported no

adverse effects of XOS (Gobinath et al., 2010; Howard et al., 1995; Hsu et al., 2004). In addition, various studies showed no mutagenic, teratogenic, or genotoxicity effects of XOS (Oh et al., 1999; SD Longlive 2010). Related compounds such as xylose, xylan, and fibers containing a xylose backbone were found to be safe (Fleming and Lee, 1983; Imazawa et al., 1999; Jiang et al., 1986; Kuroiwa et al., 1967; Marlett et al., 2002; Yen et al., 1992; Yoshino et al., 2006).

Table 7. Acute and subacute toxicity studies of XOS

Species	Length of the study	Measurement endpoints	Results	Reference
Rat	Single dose, observed 14 d	Acute toxicity	LD50=10 g/kg BW	Park et al., 1999
Mouse	Single dose, observed 14 d	Acute toxicity	LD50>20 g/kg BW	SD Longlive, 2010
Rat	13 wk	Subacute toxicity	NOAEL – 3,000 mg/kg BW/d; the highest dose administered	Park et al., 2000
Rat	30 d	Subacute toxicity	NOAEL – 4,000 mg/kg BW/d; the highest dose administered	SD Longlive, 2010
Chick	21 d	Subacute toxicity	4% in the diet in chicks; the highest dose administered	Graham et al., 2004

Comparison with GOS (Table 8)

The literature shows that GOS and XOS have similar toxicity patterns. Like GOS, XOS is known as a prebiotic carbohydrate ingredient that enhances growth of bifidobacteria and lactobacilli in the gastrointestinal tract (Holma et al., 2002; Okazaki et al., 1990, 1991). The acute toxicity of GOS has been reported to be 15 g/kg BW in rats (Matsumoto et al., 1993). Subchronic toxicity studies administering GOS via gavage or in the diet reported the NOAEL values of 2,000 and 5,000 mg/kg BW/d, the highest doses administered (Anthony et al., 2006). The mutagenicity/genotoxicity of GOS was evaluated in the bacterial reverse mutation test and a mammalian chromosome aberration test and an *in vivo* mouse micronucleus assay. It was concluded that GOS was not genotoxic or mutagenic (Yasutake et al., 2003). FDA had no question on the GOS GRAS notice (GRN 285 and 286).

Table 8. Comparison of toxicity studies with XOS and GOS

Species	Study	Results	Reference
XOS	Acute toxicity	LD ₅₀ >10 g/kg BW in rats and mice	Park et al., 1999 SD Longlive, 2010
GOS	Acute toxicity	LD 50=15 g/kg BW in rats	Matsumoto et al., 1993
XOS	Subacute toxicity	NOAEL>3,000 mg/kg BW/d in rats or 4% in the diet in chicks	Graham et al., 2004; Park et al., 2000; SD Longlive, 2010
GOS	Subacute toxicity	NOAEL=2,000-5,000 mg/kg BW/d	Anthony et al., 2006

III.C.2.1.1. Acute toxicity test in the rat (Park et al., 1999)

Park et al. (1999) reported that XOS had no toxic effects and that the LD₅₀ value of XOS was above 10 g/kg BW in Sprague Dawley® (SD) rats.

III.C.2.1.2. Acute toxicity test in the mouse (SD Longlive, 2010)

Twenty mice (10 males and 10 females) weighing 18-22 g were infused with XOS at the dosage of 20 g/kg BW twice daily and were observed at 14 d. There were no obvious toxicity symptoms either in both male or female mice and no death of animals at 14 d (Table 7). Thus, it was concluded that the LD₅₀ value of XOS in both male and female mice was greater than 20 g/kg BW.

III.C.2.1.3. Subacute toxicity in the rat (Table 7; Park et al., 2000)

Park et al. (2000) evaluated subacute toxicity of XOS in SD rats. Groups of 60 male and 60 female rats were orally administered with 0, 333, 1,000 or 3,000 mg/kg for 13 wk. Hematological values and histopathological findings were investigated at the end of 13 and 17 wk (i.e., at the end of 4 wk of recovery periods). No death or toxic effects were observed during the test periods. There were statistically significant changes in several criteria, but these change had no direct relationship to dosage. Clinical changes were general occurrence and no specific toxicity was related to XOS. Gross necropsy and histopathology revealed that no target organs were found in treated mice with XOS. No treatment-related changes in BWs were noted during the treatment period. Daily food intakes in the XOS-treated groups of both sexes were not significantly different from the control groups except transient changes noted on d 1 in males. According to the results, the NOAEL of XOS was estimated to be > 3,000 mg/kg.

Hematological values:

No treatment-related changes in hematological values (white blood cell [WBC], red blood cell [RBC], hematocrit [HCT], mean corpuscular hemoglobin ([MCH], mean corpuscular hemoglobin concentration [MCHC], and lymphocyte values) were noted during the treatment period. In males at 4 wk recovery, a significant decrease in MCH

values (19.8 ± 0.5 vs. 19.2 ± 0.4 pg; $P < 0.05$) were observed in the 3,000 mg/kg group. However, all of hematological values were within the physiologically normal ranges.

Serum biochemistry:

Serum analysis showed no treatment-related changes in serum concentrations of aspartate aminotransferase (AST or SGOT), alanine aminotransferase (ALT or SGPT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatine (CREA), glucose, total cholesterol (T-C), total bilirubin (T-BIL), total protein (TP), albumin (ALB), the ratio of albumin to globulin (A/G ratio), creatine phosphokinase (CPK), triglyceride (TG), calcium (Ca), inorganic phosphorus (IP), Na, K, and Cl, during the treatment period. The only exception was the phospholipid (PL-E) concentration (148.6 ± 28.1 vs. 123.8 ± 13.2 mg/dl; $P < 0.05$) noted in the 1,000 mg/kg group in males at 13 wk, but it was not considered as a treatment related change.

Gross and histopathological findings in male and female rats treated orally with XOS:

No abnormalities have been observed in brain, hypophysis, adrenal gland, liver, spleen, kidney, heart, testis, ovary, prostate/uterus, lung, thymus, thyroid gland, salivary gland, urinary bladder, seminal vesicle, epididymis, preputial gland, pancreas, skin, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, artery, cervical spinal cord, lumbar spinal cord, tongue, trachea, esophagus, sciatic nerve, muscle, femur, sternum, eyes, harderian gland, mesenteric lymph node, submandibular lymph node, or abdominal cavity.

Urine analysis:

Urine analysis showed no treatment-related changes in concentrations of glucose, bilirubin, ketone, protein, urobilinogen, nitrite, or specific gravity, pH, occult blood, and color during the treatment period.

III.C.2.1.4. Subacute toxicity study in the chick (Table 9; Graham et al., 2004)

Day-old chicks were fed diets containing 0, 0.4, 4,000 or 40,000 mg/kg XOS to 21 d old (18 chicks/diet). XOS did not influence chick growth, liver weight, gut length, or ileal digesta dry matter (Table 9). However, XOS decreased ileal lactic acid concentration and increased cecal butyric acid and SCFA. XOS was rapidly fermented in the ceca, and elevated plasma xylose levels. XOS also decreased overall cecal bacterial numbers but had little influence on the overall bacterial community profile. No adverse effects were noted in any test group. The NOAEL was found to be 4% in the diet.

Table 9. Effect of XOS on chick performance and gut parameters.

Dosage, % in diet	Control; 0	0.04	0.4	4.0
14 day live-weight, g	383	354	368	380
21 day live-weight, g	750	724	742	731
Gut length, cm	121	116	120	125
Ileal digesta dry matter, %	16.4	16.3	15.8	15.6
Ileal soluble xylose units, g/kg	0.3	0.5	1.7	10.4
Cecal soluble xylose units, g/kg	0.34	0.22	0.36	1.08
Plasma xylose, mg/l	0.5	1.0	2.2	13.2
Ileal lactic acid, mmol	45	33	31	25
Cecal total VFA, mmol	133	133	130	151
Cecal butyric acid, mmol	13.9	14.0	14.8	20.8
Cecal propionic acid, mmol	4.7	4.5	3.8	3.1
Cecal bacteria, 10 ¹¹ cells/g	1.99	1.94	1.80	1.54

From Graham et al., 2004

III.C.2.1.5. A 30 day feeding study in rats (Table 7; SD Longlive, 2010)

From this study, the NOAEL was determined to be 4,000 mg/kg BW/d. In this study, 80 weaning rats, weighing 50-60 g, were randomly assigned to 4 groups: control, 1,000, 2,000, or 4,000 mg/kg BW/d (10 male and 10 female rats per group). All animals showed normal growth activity. There were no significant differences in BW, food intake, or food availability between any test group and the control group. Hematological values and serum chemistry values, such as hemoglobin, red blood cell count, white blood cells, aminotransferase, BUN, creatine, cholesterol, nitroglycerine, blood sugar, total protein, and albumin were in the normal range and there were no significant differences among treatment groups and control groups. Also, there were no significant microscopic pathological changes in liver, spleen, kidney, stomach, duodenum, testis, or ovary in any treatment group compared with the control group.

III.C.2.1.6. Other animal studies showing no adverse effects of XOS

As shown in Table 10, other animal studies reported no adverse effects of XOS. Even 6-10% XOS in the diet did not show any adverse effects in rats.

Table 10. Other animal studies showing no adverse effects of XOS

Species	Dosage	Length of the study	Measurement endpoints	Reference
Weanling male mice	0.28 g XOS/d	14 d	Colonic crypt depth and epithelial cell proliferation and colonic microflora. BW gain.	Howard et al., 1995
Streptozotocin-induced diabetic Wistar rats	10% in diet	6 wk	Activity of antioxidant enzymes, fecal microflora	Gobinath et al., 2010
Male Sprague-Dawley rats, 1,2-dimethylhydrazine (DMH)-treated	6% in diet	35 d	Cecal microbiota, cecal pH, cecal weight, and serum lipid levels, and the number of aberrant crypt foci (ACF) in the colon	Hsu et al., 2004

Colonic health study in mice (Table 10)

Howard et al. (1995) evaluated the impact of supplementing soluble fiber (XOS, FOS, or gum arabic) to a semi-elemental diet on colonic epithelial cell proliferation and microflora. Consumption of XOS increased cecal crypt depth (XOS, 175.8 vs. control, 168.5 μm ; $P < 0.05$) and labeling index (XOS, 0.21 vs. control, 0.17; $P < 0.05$) relative to the other three treatments. Consumption of XOS and the control diet resulted in comparable cell density (number of cells in a vertical-half of the crypt), crypt depth, cell proliferation zone, and labeling index of cecum and distal colon. No adverse effects of XOS were reported.

Bifidogenic effect of XOS in streptozotocin-induced diabetic Wistar rats (Table 10)

The XOS obtained from alkali-pretreated corncob was supplemented at 10% (w/w) in the basal diet of streptozotocin-induced diabetic Wistar rats, while the control rats were fed with a basal diet for a period of 6 wk (Gobinath et al., 2010). Xylooligosaccharide supplementation exerted favorable influences on diabetic rats by significantly improving body weight (weight gain; XOS, -19.9 ± 10.2 vs. control, -37.3 ± 6.1 g; $P < 0.05$), reducing hyperglycemia and plasma cholesterol (XOS, 12146 ± 101 vs. control, 2295 ± 175 mg/l; $P < 0.05$), and increasing the activity of antioxidant enzymes (catalase and glutathione reductase) in the blood of diabetic rats. Supplementation of XOS and FOS resulted in a significant increase in bifidobacteria (\log_{10} CFU/g wet contents; XOS, 10.2 ± 0.12 vs. control, 8.89 ± 0.21 ; $P < 0.05$) and lactobacilli (\log_{10} CFU/g wet contents; XOS, 7.81 ± 0.23 vs. control, 7.45 ± 0.16 ; $P < 0.05$) in the cecum of normal rats. No adverse effects were reported.

Carcinogenicity test (Table 10)

Hsu et al. (2004) evaluated the effects of XOS and FOS on the alteration of cecal microbiota, cecal pH, cecal weight, and serum lipid concentrations, as well as their

inhibitory effect on pre-cancerous colon lesions in male SD rats. The rats were randomly assigned to 4 groups: control, treatment with 1,2-dimethylhydrazine (DMH, 15 mg/kg BW/wk for 2 wk), treatment with DMH + 60 g XOS/kg diet, and treatment with DMH + 60 g FOS/kg diet. Rats were fed the experimental diets for 35 d, beginning 1wk after the second dose of DMH. Both XOS and FOS markedly decreased the cecal pH (XOS, 6.08 ± 0.11 vs. FOS, 6.16 ± 0.07 vs. DMH-control, 6.53 ± 0.12 ; $P < 0.05$), and increased the total cecal weight (XOS, 18.5 ± 0.8 vs. FOS, 19.0 ± 0.1 vs. DMH-control, 14.6 ± 0.9 g; $P < 0.05$) and bifidobacteria population (\log_{10} CFU/g wet contents; XOS, 10.93 ± 0.07 vs. FOS, 10.09 ± 0.12 vs. DMH-control, 8.95 ± 0.26 ; $P < 0.05$). XOS had a greater effect on the bacterial population than did FOS. Moreover, both XOS and FOS markedly reduced the number of aberrant crypt foci in the colon of DMH-treated rats (number of 2 crypts/focus; XOS, 1.20 ± 0.33 vs. FOS, 3.10 ± 0.69 vs. DMH-control, 4.80 ± 1.00 ; $P < 0.05$; number of >4 crypts/focus; XOS, 0.30 ± 0.15 vs. FOS, 0.60 ± 0.27 vs. DMH-control, 2.80 ± 1.04 ; $P < 0.05$). These results suggest that dietary supplementation of NDOs, such as XOS and FOS, may be beneficial to gastrointestinal health, and that XOS is more effective than FOS. No adverse effects of XOS were reported.

III.C.2.1.7. Study with fish

Xu et al. (2009) investigated the effect of XOS on the growth performance and digestive enzyme activities of the allogynogenetic crucian carp, *Carassius auratus gibelii*. XOS was added to fish basal semi-purified diets at three concentrations: diet 1, 50 mg/kg; diet 2, 100 mg/kg; diet 3, 200 mg/kg, respectively. Twelve aquaria ($n = 20$) with three replicates for each treatment group (diets 1-3) and control without XOS were used. Weights of all collected carp from each aquarium were determined at the initial phase and at the end of the experiment, and carp survival also was determined by counting the individuals in each aquarium. After 45 d, there were significant differences in the relative rate of gain and daily weight gain of diets 1-3 compared with the control (relative rate of gain; XOS groups, 0.26-0.30 vs. control, 0.20, $P < 0.05$; daily weight gain, XOS groups, 0.101-0.131 vs. control, 0.076, $P < 0.05$). However, the survival rate was not affected. No adverse effects of XOS were reported.

III.C.2.1.8. Mutagenicity and genotoxicity tests of XOS

Table 11 shows that the Ames test, polychromatophilic normocyte micronucleus test of bone marrow in mice, sperm abnormality test in mice, and testis chromosome aberration test in mice showed no adverse effects of XOS.

Table 11. Summary of mutagenicity and genotoxicity studies showing no adverse effects of XOS

Test	Dosage of XOS	Ref
Ames test	5,000 ug/plate	Oh et al., 1999
Ames test	5,000 ug/plate	SD Longlive, 2010
Polychromatophilic normocyte micronucleus test of bone marrow (BM) in mice, 5 d	10.0 g/kg BW	SD Longlive, 2010
Sperm abnormality test in mice, 5 d	10.0 g/kg BW	SD Longlive, 2010
Testis chromosome aberration test in mice, 5 d	10.0 g/kg BW	SD Longlive, 2010

Bacterial reverse mutation assay of XOS (Oh et al., 1999)

To evaluate the bacterial reverse mutation of XOS, the *in vitro* Ames test using *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* (WP2 uvrA) with and without rat liver microsomal enzyme (S-9 fraction) was conducted. Xylooligosaccharide at a concentration up to 5,000 ug/plate did not cause bacterial reverse mutation.

Ames test (SD Longlive, 2010)

The Ames test with four strains of *Salmonella typhimurium* TA97, TA98, TA100, and TA102 with or without S-9 showed that XOS at five concentrations (250, 500, 1,000, 2,500, or 5,000 µg/plate) had no mutagenicity. The number of revertant colonies did not exceed two times that of spontaneous revertant colonies in all dosage groups in two experiments. No dose-response relationship was observed.

Polychromatophilic normocyte micronucleus test of bone marrow in mice (SD Longlive, 2010)

The polychromatophilic normocyte micronucleus test of bone marrow in mice with concentrations of 2.5, 5.0, and 10.0 g/kg BW showed no abnormalities. In this study, distilled water was given to the negative control group and cyclophosphamide (CTX, 40 mg/kg BW) to the positive control group. There were no significant differences in micronucleus rate between any test group and the negative control group.

Sperm abnormality test in mice (SD Longlive, 2010)

Twenty-five male mice, weighing 25-30 g were used in this study. The dosages of XOS were 2.5, 5.0, and 10.0 g/kg BW. Distilled water was given to the negative control group and CTX (40 mg/kg BW) to the positive control group. The testing material was given by gastric perfusion continuously for 5 d. The animals were sacrificed 35 d after the first time of gastric perfusion. There was no significant difference in sperm abnormality rate between testing groups of different doses and the negative control group ($P>0.05$). The data indicate that the sample did not cause abnormality of sperm in mice.

Testis chromosome aberration test in mice (SD Longlive, 2010)

Twenty-five male mice weighing 25-30 g were used in this study. The dosages of XOS were 2.5, 5.0, and 10.0 g/kg BW. Distilled water was given to the negative control group and CTX (40 mg/kg BW) to the positive control group. The testing material was given by gastric perfusion continuously for 5 d. The animals were sacrificed 13 d after the first time of gastric perfusion. There was no significant difference in chromosome aberration rate between test groups receiving different doses and the negative control group. The data indicate that the sample did not cause chromosome aberration of the testis in mice.

III.C.2.2. Preclinical studies of D-Xylose, a related compound

The oral and intravenous LD₅₀ of D-xylose have been found to be more than 23 g/kg and 11.3 g/kg, respectively, for mice (Umetsu, 1981). The NOAEL determined from a subchronic toxicity and a chronic toxicity studies was 5% in the diet.

III.C.2.2.1 Digestibility and metabolism of D-xylose

Schutte et al. (1991a) studied ileal digestibility and urinary excretion of D-xylose and associated effects (ileal and fecal digestibility of dry matter (DM), organic matter (OM), gross energy (GE) and nitrogen) in pigs. Castrated pigs were prepared with a post-valvular T-cecum cannula to measure ileal digestibility. Fecal digestibility was measured in non-cannulated pigs. D-Xylose was given at dietary inclusion levels of 100 and 200 g/kg (or 10% and 20%), and the control sugar, D-glucose, at a rate of 200 g/kg diet. Ileal digestibility of D-xylose as well as that of D-glucose was found to be close to 100%. The presence of D-xylose in the diet decreased ileal digesta pH (20% XOS, 6.0 vs. 10% XOS, 6.2 vs. control, 6.5; 20% vs. control, $P < 0.05$). In pigs fed the 100 g D-xylose/kg diet, 44.5% of the D-xylose intake appeared in the urine. This percentage increased significantly to 52.6% when pigs were fed the 200 g D-xylose/kg diet. Ileal and fecal digestibility of DM (ileal digestibility coefficient; 20% XOS, 81.9 vs. 10% XOS, 85.7 vs. control, 86.2; 20% vs. control, $P < 0.05$; fecal digestibility coefficient; 20% XOS, 92.2 vs. 10%, 95.5 vs. control, 94.9; 20% vs. control, $P < 0.05$) decreased significantly in pigs fed the 20% xylose diet. Also, a chick study reported that mean ileal digestibility of D-glucose and D-xylose was nearly 100% (Schutte et al., 1991b).

III.C.2.2.2. A subchronic toxicity study of D-xylose

Imazawa et al. (1999) conducted a 13-wk subchronic toxicity study of D-xylose that was performed in male and female F344 rats at dose levels of 0, 0.2, 0.6, 1.7, and 5% D-xylose in the CRF-1 powder diet. Rats were randomly allocated to 5 groups each consisting of 10 males and 10 females. Treated groups showed no changes in BW gain or food intake, and all animals survived until the end of the experiment. No clear dose-response effect was observed in the hematological data in either males or females given D-xylose. Serum biochemistry studies revealed decreases in AST in the 0.2 and 5% D-xylose group males and 0.2, 1.7, and 5% group females compared to the control value. However, the changes were not considered specific because of the lack of any

clear dose-response effect. In addition, no histopathological changes indicating obvious toxicity of D-xylose were observed in the livers of either sex treated with D-xylose. Based on these data, the NOAEL of D-xylose in F344 rats of both sexes was judged to be 5% or more in the diet.

A chick study evaluating nutritional effects of xylose demonstrated that xylose did not affect liver weight when 25, 50, or 75 g/kg of D-xylose was fed for 21 d (Schutte et al., 1992), although a slightly negative dose-dependent effect on weight gain and feed utilization was observed. Overall, no adverse effects were reported related to xylose consumption in rats and chicks.

III.C.2.2.3. Two year chronic toxicity/carcinogenicity study of xylose

Kuroiwa et al. (1967) conducted a 2 yr chronic toxicity/carcinogenicity study of D-xylose (purity 99%) using groups of 50 male and 50 female F344 rats at dietary doses of 0 (control), 2.5, and 5%. The doses were selected on the basis of results from a 13 wk subchronic toxicity study. There were no significant differences in clinical signs, mortality, or hematological findings between any test group and the control group. Decreases in absolute weight and increases in relative weight of the brain (absolute wt; 5%, 2.06 ± 0.05 vs. control, 2.09 ± 0.07 ; relative wt; 5%, 0.56 ± 0.07 vs. control, 0.52 ± 0.07 ; $P < 0.05$) in males, and decreases of absolute kidney weight (absolute wt; 5%, 1.57 ± 0.16 vs. control, 1.71 ± 0.41 ; relative wt; 5%, 0.68 ± 0.12 vs. control, 0.70 ± 0.20 ; $P < 0.05$) in females were observed in the 5% group, but there were no remarkable histopathological changes. A variety of tumors developed in all groups, including the controls, but all were histologically similar to those known to occur spontaneously in F344 rats. No statistically significant increase in the incidence of any type of neoplastic lesion was found for either sex in the treated groups. Thus, it was concluded that, under the present experimental conditions, D-xylose was not carcinogenic to F344 rats.

Although no data are available on mutagenicity of D-xylose, lack of mutagenic or antimutagenic activity of Maillard reaction products prepared with xylose and amino acids in the Ames test has been reported (Yen et al., 1992).

III.C.2.3. Preclinical studies of Xylan, a related compound

Several beneficial effects associated with xylans have been reported by many authors. For instance, inhibitory action on mutagenicity activity and heating seems to increase the detoxification ability of dietary fibers, antiphlogistic effects, and both mitogenic and comitogenic activities (Ebringerova and Hromadkova, 1997; Oliveira, 2009). No adverse effects were reported from any studies on xylan.

Fleming and Lee (1983) compared the effects of select purified fibers to those derived from cereals or legume seeds in a 9 wk rat study. Most diets were designed to contain approximately 10% dietary fiber and 10% protein. Cellulose, xylan, and raffinose had no influence on feed intake, weight gains, or feed efficiency ratios (FERs). No adverse effects of xylan were noted.

Jiang et al. (1986) determined the effects of purified cellulose and xylan on the apparent absorption and tissue concentration of zinc and copper in a 25 wk study with male weanling SD rats. The control group was fed a fiber-free diet and six other groups were fed a diet containing 3, 6, or 12% cellulose or xylan. After 26 wk of consuming the test diets, there were no significant differences between groups in regard to weight gain, feed intake, or feed efficiency. The average amounts of ingested cellulose and xylan that survived the passage of the intestinal tract were 86 and 18%, respectively. In contrast to cellulose, which significantly lowered apparent absorption of both zinc and copper, xylan did not exert a significant influence on apparent absorption and tissue concentrations of the minerals.

III.C.2.4. Human clinical trials

As shown in Table 12, human clinical studies reported beneficial effects with no adverse effects of XOS. The majority of studies on XOS were conducted in healthy adults, and typical intakes of XOS were between 1 and 10 g/person/d for periods of up to 3 wk. Intakes of XOS (5.5-10 g/d) were reported to be well tolerated without adverse events for durations of up to 8 wk (Chung et al., 2007; Iino et al., 1997; Kobayashi et al., 1991; Okazaki et al., 1990; Oku et al., 2002; Sheu et al., 2008; Tateyama et al., 2005). These dosages are comparable to the estimated XOS exposure under the proposed uses.

A sufficiently high, regular ingestion of XOS may cause diarrhea due to osmogenic retention of fluid in both the small and large intestines. This outcome disappears within a few days. Xylooligosaccharide intake has been found highly effective for the reduction of severe constipation in pregnant women without adverse effects (Tateyama et al., 2005). In addition, related compounds such as xylose, xylan, and fibers containing a xylose backbone were found to be safe (Cho et al., 2001; Cho and Clark, 2001; Cho et al., 2004; Holma et al., 2010; Marteau et al., 1994).

Table 12. Human clinical studies showing no adverse effects of XOS

Subject	Daily dosage, g	Duration, wk	Measurement endpoints	Reference
Elderly men and women	4	3	Serum hematological and biochemical variables, fecal microflora including bifidobacteria, fecal pH/moisture, and stool consistency	Chung et al., 2007
Men	0.4	2-4 wk	Stool consistency	Iino et al., 1997
Healthy women	2-10	single dose	Fecal microflora including bifidobacteria, fecal moisture, and stool consistency	Kobayashi et al., 1991
Healthy men	1-2	3	Fecal microflora including bifidobacteria	Okazaki et al., 1990
Healthy men	0.12 g/kg BW	single dose	Gastrointestinal tolerance	Oku et al., 2002
Type 2 diabetics	4	8	Blood sugar and lipid profiles	Sheu et al., 2008
Constipated pregnant women	4.2	4	Stool consistency	Tateyama et al., 2005

Comparison with GOS and other oligosaccharides

The majority of studies on GOS were conducted in healthy adults, and typical intakes of GOS were between 5 to 15 g/person/d for periods of between 1 to 3 wk. In three studies, intakes of GOS between 5.5 to 10 g were reported to be well tolerated without adverse events for durations of between 1 and 2.5 mo (Ito et al., 1990; Shadid et al., 2007; Vulevic et al., 2008). Among the studies that included tolerance endpoints, side-effects were limited to reports of flatulence when GOS was consumed on a repeat basis in quantities of between 10 and 15 g (Alles et al., 1999; Deguchi et al., 1997; Ito et al., 1990; Teuri et al., 1998). However, this effect was not consistently reported in all studies at these intakes (Bouhnik et al., 2004, 2007; Shadid et al., 2007; Teuri and Korpela, 1998). Similar observations of increased flatulence have been reported following the consumption of FOS (15 g) over a 7 d period (Alles et al., 1996). These gastrointestinal effects are expected in association with the consumption of indigestible carbohydrates in large quantities.

III.C.2.4.1. XOS and intestinal health

Like other fiber ingredients, XOS is known to maintain fecal water content within the normal range and to relieve constipation as well as diarrhea without having side effects. No adverse effects were reported related to the consumption of XOS at the daily dosage of 0.4-10 g (Chung et al., 2007; Iino et al., 1997; Kobayashi et al., 1991; Okazaki et al., 1990).

Tateyama et al. (2005) reported that administration of 4.2 g XOS daily for 4 wk increased the stool frequencies ($1.1 \pm 0.4/\text{wk}$ in the pre-treatment wk, and increased to $6.7 \pm 1.9/\text{wk}$ after 4 wk administration of XOS) in 30 constipated pregnant women. At the end of the study, 27 subjects could defecate spontaneously. The occurrence of very loose or very hard stools decreased and the stool consistency normalized.

Kobayashi et al. (1991) reported reduction of diarrhea with dosages of 2-10 g XOS/d.

Prebiotic effects of XOS were demonstrated in healthy men and elderly subjects. Chung et al. (2007) reported that supplementation of 4 g XOS/d for 3 wk increased the population of bifidobacteria and fecal moisture and decreased fecal pH. XOS supplementation had no effects on serum hematological and biochemical variables (Chung et al., 2007).

Administration of 1-2 g XOS daily for 3 wk increased the percentage of bifidobacteria relative to the total intestinal microflora in healthy men (Okazaki et al., 1990). No adverse effects were reported.

III.C.2.4.2. XOS and metabolic syndrome

Sheu et al. (2008) reported that dietary supplementation with 4 g XOS/d for 8 wk was effective in improving blood sugar (glucose, HbA1c, and fructosamine) and lipid (total cholesterol, low density lipoprotein [LDL] cholesterol, oxidized LDL, and apolipoprotein B) profiles in type 2 diabetes. However, Chung et al. (2007) reported no changes in blood lipid profiles. No adverse effects were reported in these studies.

III.C.2.5. Safety of Xylan and xylose-containing fiber in humans

Marthinsen and Fleming (1982) evaluated the abilities of dietary fibers to promote excretion of intestinal fermentation gases in five healthy men. Responses to feeding xylan, pectin, cellulose, and corn bran (0.5 g/kg BW/d) were compared to a fiber-free diet. The pectin- and xylan-containing diets generally resulted in more gas than did the cellulose- or corn bran-containing diets, indicating that xylan and pectin are more fermentable fibers than cellulose.

In addition, other fiber ingredients based on xylose backbones, such as wheat bran, rice bran, and psyllium, are known for their fecal bulking effects and gastrointestinal regularity improvement (Cho et al., 2001; Cho and Clark, 2001; Cho et al., 2004; Holma et al., 2010; Marteau et al., 1994) without having negative effects on mineral bioavailability (Cho et al., 2001b). These fibers are fermented by intestinal microflora to produce SCFAs that improve colonic health. However, fermentation in the large intestine can result in a minor side effect such as the formation of gases (including hydrogen, carbon dioxide and methane), which is often associated with flatulence and

intestinal discomfort. Intestinal discomfort can be a transient symptom since the human body is able to adapt to higher intakes of dietary fiber.

III.C.3. Allergy

No case report of allergy to XOS was identified in the literature.

III.C.4. Information pertaining to the safety of the bacterial enzymes

Xylanase from *Streptomyces olivaceoviridis* has been widely used in the manufacture of XOS in the food industry (Ai et al., 2005; Jiang et al., 2005, 2006). The manufacture of XOS involves a number of extensive purification steps (such as the activated carbon filtration, ion-exchange, and chromatography separation stages) where potential metabolic impurities produced during fermentation are expected to be removed. Additional unpublished safety studies supporting the fact that the xylanase is obtained from a non-pathogenic, non-toxicogenic microorganism were provided by SD Longlive, and are presented as additional corroborating safety data (Appendix).

In addition, a xylanase preparation from another microorganism (expressed in a self-cloned strain of *Bacillus subtilis*) has been proven safe for food processing as demonstrated in acute and subchronic oral toxicity studies in rats, mutagenicity, and chromosomal aberrations assays (Harbak and Thygesen, 2002).

IV. Conclusions

Documentation qualifying a substance as GRAS has been compiled where such documentation includes technical evidence and common knowledge of safety under the conditions of intended use, as recognized by qualified experts (the Expert Panel). Technical evidence of safety includes the chemical identity of the substance, the method of manufacture, analytical data on composition and specifications, estimates of dietary exposure, safety data from animal and human clinical studies, and nutritional benefits from animal and human clinical studies.

The information/data provided by SD Longlive in this report and supplemented by the publicly available toxicity data on XOS provide a sufficient basis for an assessment of the safety of XOS for the proposed use as an ingredient in food, when prepared according to appropriate specifications and used according to GMP. Key findings are summarized here:

1. XOS is well characterized and of consistent quality across lots and free from chemical and microbial contamination.
2. XOS has a long history of use in foods and beverages in the U.S.
3. The XOS manufacturing process has been safely used for many years in the food industry.

4. The safety and nutritional benefits of XOS are well established by human clinical trials and animal studies of XOS. There are no indications of significant adverse effects related to XOS consumption in the publicly available literature at the proposed use levels.
5. Increased consumption of dietary fiber has been recommended by the USDA Dietary Guidelines Committee and IOM.
6. Intended use of XOS as part of the proposed food use results in levels of exposure significantly below those associated with any adverse effects and provides a reasonable certainty of safety.

Therefore, not only is the proposed use of XOS safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS).

V. Discussion of information inconsistent with GRAS determination

We are not aware of information that would be inconsistent with a finding that the proposed use of XOS preparations in foods and beverages, meeting appropriate specifications and used according to GMP, is GRAS.

References

Aachary AA, Prapulla SG. Value addition to corncob: Production and characterization of xylooligosaccharides from alkali pretreated lignin-saccharide complex using *Aspergillus oryzae* MTCC 5154. *Bioresource Tech.* 2009;100:991-995.

Ai Z, Jiang Z, Li L, Deng W, Kusakabe I, Li H. Immobilization of *Streptomyces olivaceoviridis* E-86 xylanase on Eudragit S-100 for xylo-oligosaccharide production. *Process Biochem.* 2005;40:2707-2714.

Alles MS, Hartemink R, Meyboom S, Harryvan JL, Van Laere KMJ, Nagengast FM, Hautvast JGAJ. Effect of transgalactooligosaccharides on the composition of the human intestinal microflora and on putative risk markers for colon cancer. *Am J Clin Nutr.* 1999; 69:980-991.

Alles MS, Hautvast JGAJ, Nagengast FM, Hartemink R, Van Laere, KMJ, Jansen JBMJ. Fate of fructo-oligosaccharides in the human intestine. *Br J Nutr.* 1996;76:211-221.

Anthony JC, Merriman TN, Heimbach JT. 90-day oral (gavage) study in rats with galactooligosaccharides syrup. *Food Chem Toxicol.* 2006;44:819-826.

Bouhnik Y, Flourie B, D'Agay-Abensour L, Pochart P, Gramet G, Durand M, Rambaud JC. Administration of transgalacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. *J Nutr.* 1997;127:444-448.

Bouhnik Y, Raskine L, Simoneau G, Vicaud E, Neut C, Flourie B, Brouns F, Bornet FR. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: A double-blind, randomized, placebo-controlled, parallel-group, dose response relation study. *Am J Clin Nutr.* 2004;80:1658-1664.

Campbell JM, Fahey GC Jr, Wolf BW. 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.

CDC. 2010. Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES). Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS); Hyattsville, Maryland. Available from: http://www.cdc.gov/nchs/data/nhanes/nha0n3e_s04/nhanes_analytic_guidelines_dec_2010.

Cho SS, Clark C. Wheat Bran: Physiological effects. *In* . Handbook of Dietary Fiber, Marcel Dekker, Inc. New York, NY. 2001. pp 453-472.

Cho SS, Rickard S, Clark C. Psyllium: Food Applications, Efficacy and Safety. *In Handbook of Dietary Fiber*, Marcel Dekker, Inc. New York, NY. 2001. pp 473-496.

Cho SS, Clark C, Uribe-Saucedo S. Gastrointestinal and other physiological effects of wheat bran. *Cereal Foods World*. 2004; 49:140-144.

Chung M, Chien C, Huang P, Tung T. Effects of prolonged feeding of D-xylose on rats. *J Formosan Med Assoc*. 1973;72:467-471.

Collins T, Gerday C, Feller G. Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiol Rev*. 2005;29:3-23.

Courtin CM, Swennen K, Verjans P, Delcour JA. Heat and pH stability of prebiotic arabinoxylooligosaccharides, xylooligosaccharides and fructooligosaccharides. *Food Chem*. 2009;112:831-837.

Crittenden R, Karppinen S, Ojanen S, Tenkanen M, Fagerstrom R, Matto J, Saarela M, Mattila-Sandholm T, Poutanen K. In vitro fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. *J Sci Food Agric*. 2002;82:781-789.

Deguchi Y, Matsumoto K, Watanuki M, Ito A. Effects of β 1-4 galactooligosaccharides administration on defecation of healthy volunteers with constipation tendency. *Eiyogaku Zasshi*. 1997;55:13-22.

Dohnalek MIH, Ostrom KM, Hilty MD. Use of indigestible oligosaccharides to prevent gastrointestinal infections and reduce duration of diarrhea in humans. USA Patent, US 5827526. 1998a.

Dohnalek MIH, Ostrom KM, Hilty MD. Use of Indigestible Oligosaccharides to Reduce the Incidence of Otitis Media in Humans. USA Patent, US 5849324. 1998b.

Ebringerova' A, Heinze T. Xylan and xylan derivatives - biopolymers with valuable properties, 1. Naturally occurring xylans structures, isolation procedures and properties. *Macromol Rapid Commun*. 2000;21:542-556.

Food and Agriculture Organization of the United Nations (FAO). Technical Meeting Report on Prebiotics. September 15-16, 2007.

Fleming SE, Marthinsen D, Kuhnlein H. Colonic function and fermentation in men consuming high fiber diets. *J Nutr*. 1983a;113:2535-2544.

Fleming SE, Rodriguez MA. Influence of dietary fiber on fecal excretion of volatile fatty acids by human adults. *J Nutr*. 1983b;113:1613-1625.

Fleming SE, Lee B. Growth performance and intestinal transit time of rats fed purified and natural dietary fibers. *J Nutr*. 1983c;113:592-601.

- Fujikawa S, Okazaki M, Matsumoto N. Effect of xylooligosaccharide on growth of intestinal bacteria and putrefaction products'. J. Jpn. Soc. Nutr. Food Sci. 1991;44:37-40.
- Garcia RB, Ganter J, Carvalho RR. Solution properties of D-xylans from corn cobs. Eur Polym J. 2000;36:783-787.
- Garrote G, Dominguez H, Parajo JC. Kinetic modelling of corncob autohydrolysis. Proc. Biochem. 2001;36:571-578.
- Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota. Introducing the concept of prebiotics. J Nutr. 1995;125:1401-1412.
- Gobinath D, Madhu AN, Prashant G, Srinivasan K, Prapulla SG. Beneficial effect of xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats. Br J Nutr. 2010;104:40-47.
- Graham H, Apajalahti J, Peuranen S. Xylo-oligosaccharides alter metabolism of gut microbes and blood xylose levels in chicks. In Dietary Fibre; bioactive carbohydrates for food and feed. Van de Kamp et al. (Ed). Wageningen Academic Publishers, The Netherlands. 2004, pp 329-333.
- Grootaert C, Delcour JA, Courtin CM, Broekaert WF, 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.
- Harbak L, Thygesen HV. Safety evaluation of a xylanase expressed in *Bacillus subtilis*. Food Chem Toxicol. 2002;40:1-8.
- Hardinge MG, Swarner JB, Grooks H. Carbohydrates in foods. J Am Diet Assoc. 1965;46:197-204.
- Holma R, Juvonen P, Asmawi MZ, Vapaatalo H, Korpela R. Galacto-oligosaccharides stimulate the growth of bifidobacteria but fail to attenuate inflammation in experimental colitis in rats. Scand J Gastroenterol. 2002;37:1042-1047.
- Holzapfel WH, Schillinger U. Introduction to pre- and probiotics. Food Research Intl. 2002; 35:109-116.
- Howard MD, Gordon DT, Garleb KA, Kerley MS. Dietary fructooligosaccharide, xylooligosaccharide and gum arabic have variable effects on cecal and colonic microbiota and epithelial cell proliferation in mice and rats. J Nutr. 1995;125:2604-2609.

Hsu CK, Liao JW, Chung YC, Hsieh CP, Chan YC. Xylooligosaccharides and fructooligosaccharides affect the intestinal microbiota and precancerous colonic lesion development in rats. *J Nutr.* 2004;134:1523-1528.

Imazawa T, Nishikawa A, Furukawa F, Ikeda T, Nakamura H, Miyauchi M, Hirose M. A 13-week subchronic toxicity study of D-xylose in F344 rats. *Bull Natl Inst Health Sci.* 1999;117:115-118 (in Japanese).

Imaizumi K, Nakatsu Y, Sato M, Sedarnawati 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.

Iino T, Nishijima Y, Sawada S, Sasaki H, Harada H, Suwa Y, Kiso Y. Improvement of constipation by a small amount of xylooligosaccharides ingestion in adult women. *J Jpn Assoc Dietary Fiber Res.* 1997;1:19-24.

Institute of Medicine (IOM). Dietary Reference Intakes for energy, carbohydrates, fiber, fat, fatty acids, cholesterol, protein, and amino acids. National Academy Press, Washington, DC. 2002.

Ito M, Deguchi Y, Miyamori A, Matsumoto K, Kikuchi H, Matsumoto K, Kobayashi Y, Yajima T, Kan T. Effects of administration of galactooligosaccharides on the human fecal microflora, stool weight and abdominal sensation. *Microb Ecol Health Dis.* 1990;3:285-292.

Jeong KJ, Park IY, Kim MS, Kim SC. High-level expression of an endoxylanase gene from *Bacillus* sp. in *Bacillus Subtilis* DB104 for the production of xylobiose from xylan. *Appl Microbiol Biotechnol.* 1998;50:113-118.

JETRO (Japan External Trade Organization), Specifications and Standards for Foods, Food Additives, etc. Under the Food Sanitation Law. 2004. Available from: <http://www.jetro.go.jp/se/e/standards_regulation/foodadd2004apr-e.pdf>.

Jiang KS. Effects of dietary cellulose and xylan on absorption and tissue contents of zinc and copper in rats. *J Nutr.* 1986;116:999-1006.

Jiang ZQ, Deng W, Li XT, Ai ZL, Li LT, Kusakabe I. Characterization of a novel, ultra-large xylanolytic complex (xylanosome) from *Streptomyces olivaceoviridis* E-86. *Enz Microbial Technol.* 2005;36:923-929.

Jiang Z, Deng W, Yan Q, Zhai Q, Li L, Kusakabe I. Subunit composition of a large xylanolytic complex (xylanosome) from *Streptomyces olivaceoviridis* E-86. *J Biotechnol.* 2006;126:304-312.

Joo GJ, Rhee IK, Kim SO, Rhee SJ. Effect of dietary xylooligosaccharide on indigestion

and retarding effect of bile acid movement across a dialysis membrane. Han'guk Sikp'um Yongyang Kwahak Hoechi. 1998;27:705-711.

Kabel MA, Kortenoeven L, Schols HA, Voragen AG. In vitro fermentability of differently substituted xylo-oligosaccharides. J Agric Food Chem. 2002;50:6205-6010.

Kobayashi T, Uchida K, Kaneko K, Mizutani T, Onoue M. Ninety-day repeated oral dose toxicity study of GOS in rats. Yakuruto Kenkyujo Kenkyu Hokokushu 2003;23:25-42.

Kontula P, von Wright A, Mattila-Sandholm T. Oat bran beta-gluco- and xylo-oligosaccharides as fermentative substrates for lactic acid bacteria. Int J Food Microbiol. 1998;45:163-169.

Kunimasa K, Shigeaki. Xylooligosaccharides. Jpn Technol Rev. 1991;3:131-143.

Kuroiwa Y, Nishikawa A, Imazawa T, Kitamura Y, Kanki K, Umemura T, Hirose M. Lack of carcinogenicity of D-xylose given in the diet to F344 rats for two years. Food Chem Toxicol. 2005;43:1399-1404.

Marlett JA, Fischer MH. The active fraction of psyllium seed husk. Proc Nutr Soc. 2003;62:207-209.

Marteau P, Flourié B, Cherbut C, Corrèze JL, Pellier P, Seylaz J, Rambaud JC. Digestibility and bulking effect of ispaghula husks in healthy humans. Gut. 1994;35:1747-1752.

Marthinsen D, Fleming SE. Excretion of breath and flatus gases by humans consuming high-fiber diets. J Nutr. 1982;112:1133-1143.

Matsumoto K, Kobayashi Y, Ueyama S, Watanabe T, Tanaka R, Kan T, Kuroda A, Sumihara Y. Galactooligosaccharides. Jpn Technol Rev. 1993;3:90-106, 222-225.

MHLW (Ministry of Health, Labor and Welfare of Japan). List of existing food additives, Notification No. 120 of the Ministry of Health and Welfare. 1996a.

MHLW (Ministry of Health, Labor and Welfare of Japan). Guidelines for Designation of Food Additives, and for Revision of Standard for Use of Food Additives, Article No. 29 of the Life and Sanitation Bureau. 1996b.

Moura P, Cabanas S, Lourenço P, Gírio F, Maria C, Loureiro-Dias M, Esteves P. In vitro fermentation of selected xylo-oligosaccharides by piglet intestinal microbiota Food Sci Technol. 2008;41:1952-1961.

Moure A, Gullón P, Domínguez H, Parajó JC. Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. Process Biochem. 2006;41:1913-1923.

Ninawe S, Kuhad RC. Use of xylan-rich cost effective agro-residues in the production of xylanase by *Streptomyces cyaneus* SN32. J Appl Microbiol. 2005;99:1141-1148.

Oh HG, Park YJ, Lee UT, Lee JW, Lee CS, Rhew BK, Yang CK, Yoon SW, Kang BH. Bacterial reverse mutation assay of xylooligosaccharide. J Food Hyg Safety. 1999;14:259-264.

Okazaki M, Koda H, Izumi R, Fujikawa S, Matsumoto N. In vitro digestibility and in vivo utilization of xylobiose. Nippon Eiyo Shokuryo Gakkaishi. 1991;44:41-44.

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

Oku T, Sadako N. Digestion, absorption, fermentation, and metabolism of functional sugar substitutes and their available energy. Pure Appl Chem. 2002;74:1253-1261.

Oliveira EE, Silva AE, Júnior TN, Gomes MC, Aguiar LM, Marcelino HR, Araújo IB, Bayer MP, Ricardo NM, Oliveira AG, Egito ES. Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization. Bioresour Technol. 2010;101:5402-5406.

Parajo JC, Garrote G, Cruz JM, Domínguez H. Production of xylooligosaccharides by autohydrolysis of lignocellulosic materials. Trends Food Sci Technol. 2004;15:115-120.

Park JH, Yoo JY, Shin OH, Shin HK, Lee SJ, Park KH. Growth effect of branched oligosaccharides on principal intestinal bacteria. Kor J Appl Microbiol Biotechnol. 1992; 20:237-242.

Park, YJ, Lee UT, Lee JW, Lee CS, Rhew BK, Yang CK, Yoon SW, Kang BH. Subacute toxicity of xylooligosaccharide in rats. J Food Hyg Safety. 2000;15:151-166.

Park YJ, Oh HG, Lee UT, Lee JW, Lee CS, Rhew BK, Yang CK, Yoon SW, Kang BH. Acute oral toxicity of xylooligosaccharide in rats. J Food Hyg Safety. 1999;14:255-258.

Pazur JH, Budovich T, Shuey EW, Georgi CE. The hydrolysis of xylan and xylooligosaccharides by ruminal enzymes. Arch Biochem Biophys. 1957;70:419-425.

Rycroft CE, Jones MR, Gibson GR, Rastall RA. A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. J Appl Microbiol. 2001;91:878-887.

Ryu BG, Lee JW, Lee CS, Hyeon SI, Park YJ, An JB, Yang CG. Effects of xylooligosaccharides on the growth of intestinal microflora. Korean J Microbiol Biotechnol. 2002; 30:380-386.

Sakata T. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: A possible explanation for trophic effects of fermentable fiber, gut microbes, and luminal trophic factor. *Br J Nutr.* 1987; 58:95-103.

Sakata T, von Engelhardt W. Stimulatory effect of short-chain fatty acids on the epithelial cell proliferation in rat large intestine. *Comp Biochem Physiol.* 1983;74A:459-462.

Scheppach W, Luehrs H, Menzel T. Beneficial health effects of low digestible carbohydrate consumption. *Br J Nutr.* 2001;85:S23-S30.

Schutte JB, de Jong J, Polziehn R, Verstegen MW. Nutritional implications of D-xylose in pigs. *Br J Nutr.* 1991a; 66:83-93.

Schutte JB, van Leeuwen P, Lichtendonk WJ. Ileal digestibility and urinary excretion of D-xylose and L-arabinose in ileostomized adult roosters. *Poult Sci.* 1991b;70:884-891.

Schutte JB, de Jong J, van Weerden EJ, van Baak MJ. Nutritional value of D-xylose and L-arabinose for broiler chicks. *Br Poult Sci.* 1992;33:89-100.

Shadid R, Haarman M, Knol J, Theis W, Beermann C, Rjosk-Dendorfer D, Schendel DJ, Koletzko BV, Krauss-Etschmann S. Effects of galactooligosaccharide and long-chain fructooligosaccharide supplementation during pregnancy on maternal and neonatal microbiota and immunity - A randomized, double-blind, placebo-controlled study. *Am J Clin Nutr.* 2007; 86:1426-1437.

Sheu WH, Lee IT, Chen W, Chan YC. Effects of xylooligosaccharides in type 2 diabetes mellitus. *J Nutr Sci Vitaminol (Tokyo).* 2008;54:396-401.

Southgate DAT. The definition, analysis and properties of dietary fiber. In: *Dietary Fiber: Current Developments of Importance to Health* (Heaton, K. W., ed.), pp 9-19, J. Libbey & Co., London. 1979.

Suzuki I, Nojima S, Tanimura A. (Eds.) *The commentary of the Japan's specifications and standards for food additives*, 7th ed. Hirokawa Publishing Co., Tokyo, 1999; D-347 (in Japanese).

Tateyama I, Hashii K, Johno I, Iino T, Hirai K, Suwa Y, Kiso Y. Effect of xylooligosaccharide intake on severe constipation in pregnant women. *J Nutr Sci Vitaminol (Tokyo).* 2005;51:445-448.

Teuri U, Korpela R. Galacto-oligosaccharides relieve constipation in elderly people. *Ann Nutr Metab.* 1998;42:319-327.

Teuri U, Korpela R, Saxelin M, Montonen L, Salminen S. Increased fecal frequency and gastrointestinal symptoms following ingestion of galacto-oligosaccharide containing yogurt. J Nutr Sci Vitaminol (Tokyo). 1998;44:465-471.

Umetsu G. Knowledge of home chemicals (20). J Practical Pharm “Yakkyoku”. 1981;32:67-72 (in Japanese).

U S. Department of Agriculture. USDA Nutrient Database for Standard Reference, Release 19. 2010. Available at:
<http://www.nal.usda.gov/fnic/foodcomp/Data/SR19/sr19.html>

U.S. Department of Agriculture. 2005 Dietary Guidelines Advisory Committee Report. U.S. Government Printing Office, Washington, DC. 2004.

U.S. Department of Agriculture. 2010 Dietary Guidelines Advisory Committee Report. U.S. Government Printing Office, Washington, DC. 2010.

U.S. FDA. U.S. Code of Federal Regulations (CFR). Title 21-Food and Drugs (Food and Drug Administration). U.S. Government Printing Office (GPO), Washington, DC. 2008. Available from: <http://www.access.gpo.gov/cfr/cfrassemble.cgi?title=21>[S2e1e Table for CFR sections].

Van Laere KMJ, Hartemink R, Bosveld M, Schols HA, Voragen AGJ. Fermentation of plant cell wall derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria. J Agric Food Chem. 2000; 48:1644-1652.

van Loo J, Cummings J, Delzenne N, Englyst H, Franck A, Hopkins M, Kok N, Macfarlane G, Newton D, Quigley M, Roberfroid M, van Vliet T, van den Heuvel E. Functional food properties of non-digestible oligosaccharides: A consensus report from the ENDO project (DGXII AIRII-CT94-1095). Br J Nutr. 1999;81:121-132.

Vázquez MJ, Alonso JL, Domínguez H, Parajó JC. Xylooligosaccharides: Manufacture and applications. Trends Food Sci Technol. 2000;11:387-393.

Vulevic J, Drakoularakou A, Yaqoob P, Tzortzis G, Gibson GR. Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. Am J Clin Nutr. 2008;88:1438-1446.

Wang L, Zhao B, Liu B, Yang C, Yu B, Li Q, Ma C, Xu P, Ma Y. Efficient production of L-lactic acid from cassava powder by *Lactobacillus rhamnosus*. Bioresource Technol. 2010;101:7895-7901.

WHO. Toxicological versus physiological responses. In: Principles for the Safety Assessment of Food Additives and Contaminants in Food. World Health Organization

(WHO), International Programme on Chemical Safety (IPCS); Geneva. Environmental Health Criteria, 1987;70:82.

Xu B, Wang Y, Li J, Lin Q. Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (*Carassius auratus gibelio*). Fish Physiol Biochem. 2009;35:351-357.

Yasutake N, Oyama W, Gonda M, Ikeda M, Onoue M. Safety of GOS: Bacterial reverse mutation, micronucleus, and chromosomal aberration tests. Yakuruto Kenkyujo Kenkyu Hokokushu. 2003;23:13-24.

Yazawa K, Imai K, Tamura Z. Oligosaccharides and polysaccharides specifically utilizable by bifidobacteria. Chem Pharm Bull. 1978;26:3306-3311.

Yen GC, Tsai LC, Lii JD. Antimutagenic effect of Maillard browning products obtained from amino acids and sugars. Food Chem Toxicol. 1992;30:127-132.

Yoshino K, Higashi N, Koga K. Inhibitory effects of acidic xylooligosaccharide on stress-induced gastric inflammation in mice. Shokuhin Eiseigaku Zasshi. 2006;47:284-287.

APPENDIX

山东龙力生物科技有限公司

No. 20100126**Shandong Longlive Bio-
Technology Co., Ltd.**

检 验 证 书

CERTIFICATE OF ANALYSIS

Add: High-Technology

Development

Zone of Yucheng, Shandong,

China

Tel: 0086-532-85769015

Fax: 0086-532-85762209

品名及型号： Product & model:	XYLO-OLIGOSACCHARIDE XOS95P (95% POWDER of XOS)	批号： Lot NO. :	20100126
生产日期： Produce date:	Jan 26, 2010	有效期： Expiry date :	24 months

Result of Inspection:

Test item 测试项目	Specification 标 准	Test Result 检测结果
Appearance 感官	White or yellowish powder, sweet, no peculiar smell 白色或略泛微黄色粉末, 味甜, 无异味	Yellowish powder, sweet, no peculiar smell 略泛微黄色粉末, 味甜, 无异味
pH 酸碱度	3.0-6.0	3.85
Ash 灰分	Not more than 0.3% ≤0.3%	0.22
Protein 蛋白质	Negative	Negative
Lipid 脂肪	Negative	Negative
Moisture 水分	Not more than 5.0% ≤5.0%	2.14%
XOS ₂₋₇ contents 低聚木糖含量	Not less than 95 g/100 g ≥95 g/100 g	96.71 g/100 g
XOS ₂₋₄ contents 低聚木糖含量	Not less than 65 g/100g ≥65 g/100 g	78.28 g/100 g

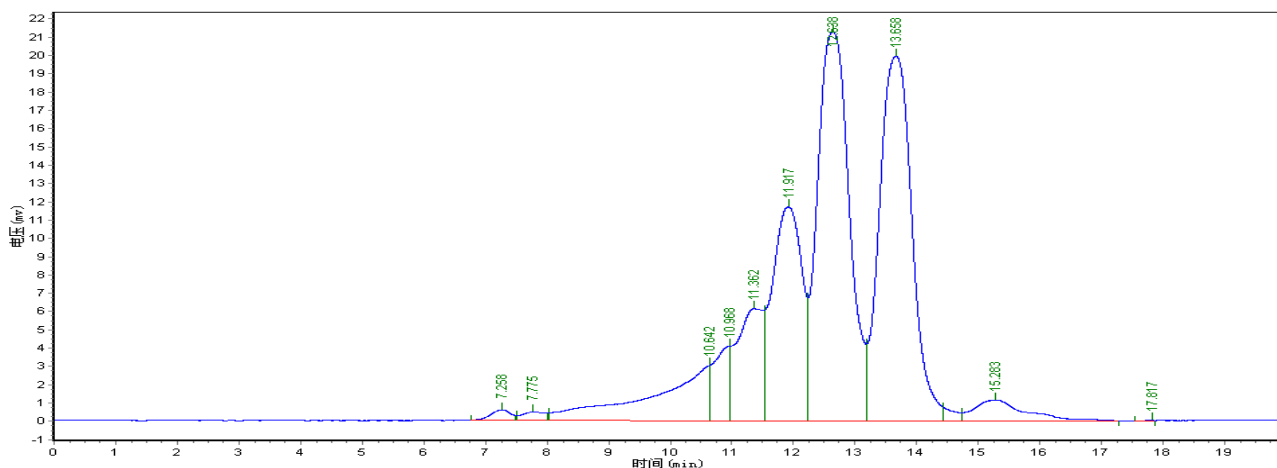
Lead 铅	Not more than 0.5 mg/kg ≤0.5 mg/kg	< 0.5 mg/kg
Arsenic 砷	Not more than 0.3 mg/kg ≤0.3 mg/kg	< 0.3 mg/kg
Copper 铜	Not more than 5 mg/kg ≤5 mg/kg	< 5 mg/kg
Coliform Bacteria 大肠菌群	Not more than 30 MPN/100 g ≤30 MPN/100 g	< 30 MPN/100 g
Total plate count 菌落总数	Not more than 1000cfu/g ≤1000 cfu/g	20 cfu/g
Mold 霉菌	Not more than 25 cfu/g ≤25cfu/g	5 cfu/g
Yeast 酵母菌	Not more than 25 cfu/g ≤25 cfu/g	5 cfu/g
Pathogen 致病菌	Negative 不得检出	Negative 未检出

Components of 95P XOS

Experimental Methods: HPLC

Calculation Methods: Peak Area External Standard Calibration

95P 20100126



Peak	Reserved time	Peak height	Peak area	Contents (g/100 g)
X7	10.642	3019.579	194382.3	8.1
X6	10.968	4065.387	70561.24	2.94
X5	11.362	6121.361	177393.5	7.39
X4	11.917	11666.73	395890.3	16.49
X3	12.638	21250.52	761325.3	31.71
X2	13.658	19916.04	730009.1	30.08
Totally				96.71
Glucose	13.658	740.056	9626.108	0.4
Xylose and Arabinose	15.283	1142.755	65850.21	2.92

Opinion of the analyst:

Representative samples were inspected and found in conformity with the required specifications

Annotations:

1. If there are any doubts about the results, please inform us for re-test in a month. The sample for re-test shall be the original one.
2. This report should not be used for advertisement and propaganda.

Analyst

Corrector:

Approver:

山东龙力生物科技有限公司
**Shandong Longlive Bio-
 Technology Co., Ltd.**
 Adds: High-Technology
 Development
 Zone of Yucheng, Shandong,
 China
 Tel: 0086-532-85769015
 Fax: 0086-532-85762209

No. 20100412

检 验 证 书
CERTIFICATE OF ANALYSIS

品名及型号： Product & model:	XYLO-OLIGOSACCHARIDE XOS95P (95% POWDER of XOS)	批号： Lot NO.：	20100412
生产日期： Produce date:	Apr 12, 2010	有效期： Expiry date：	24 months

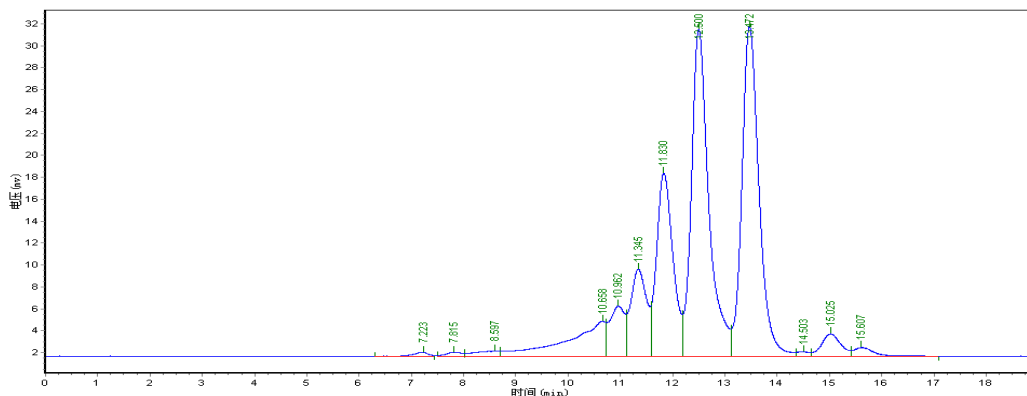
Result of Inspection:

Test Item 测试项目	Specification 标 准	Test result 检测结果
Appearance 感官	White or yellowish powder, sweet, no peculiar smell 白色或略泛微黄色粉末, 味甜, 无异 味	Yellowish powder, sweet, no peculiar smell 略泛微黄色粉末, 味甜, 无异味
pH 酸碱度	3.0-6.0	4.08
Ash 灰分	Not more than 0.3% ≤0.3%	0.16%
Moisture 水分	Not more than 5.0% ≤5.0%	2.25%
Protein 蛋白质	Negative	Negative
Lipid 脂肪	Negative	Negative
XOS ₂₋₇ contents 低聚木糖含量	Not less than 95 g/100 g ≥95 g/100 g	95.22 g/100 g
XOS ₂₋₄ contents 低聚木糖含量	Not less than 65 g/100 g ≥65 g/100 g	73.78 g/100 g
Lead 铅	Not more than 0.5 mg/kg ≤0.5 mg/kg	< 0.5 mg/kg

Arsenic 砷	Not more than 0.3 mg/kg ≤0.3 mg/kg	< 0.3 mg/kg
Copper 铜	Not more than 5 mg/kg ≤5 mg/kg	< 5 mg/kg
Coliform Bacteria 大肠菌群	Not more than 30 MPN/100 g ≤30 MPN/100 g	< 30 MPN/100 g
Total plate count 菌落总数	Not more than 1000 cfu/g ≤1000 cfu/g	5 cfu/g
Mold 霉菌	Not more than 25 cfu/g ≤25 cfu/g	5 cfu/g
Yeast 酵母菌	Not more than 25cfu/g ≤25cfu/g	5 cfu/g
Pathogen 致病菌	Negative 不得检出	Negative 未检出

Components of 95P XOSExperimental Methods: HPLCCalculation Methods: Peak Area External Standard Calibration

95P 20100412



Peak	Reserved time	Peak height	Peak area	Contents (g/100 g)
X7	10.658	3159.42	208550.3	8.7
X6	10.962	4563.52	128823	5.37
X5	11.345	7922.64	176628.6	7.37
X4	11.83	16683.8	403890.4	16.85
X3	12.5	29781.02	720244.8	30.04
X2	13.472	30040.33	651690.6	26.89
Totally				95.22
Glucose	14.503	382.667	6043.588	0.25
Xylose	15.025	2033.837	11260.01	0.5
Arabinose	15.607	791.026	7164.664	0.32

Opinion of the analyst:

Representative samples were inspected and found in conformity with the required specifications.

Annotations:

1. If there is any doubt about the results, please inform us for re-test in a month. The sample for re-test shall be the original one.
2. This report should not be used for advertisement and propaganda.

Analyst

Corrector:

Approver:

山东龙力生物科技有限公司
Shandong Longlive Bio-Technology Co., Ltd.
 Add: High-Technology
 Development
 Zone of Yucheng, Shandong,
 China
 Tel: 0086-532-85769015
 Fax: 0086-532-85762209

No. 20100413

检 验 证 书
CERTIFICATE OF ANALYSIS

品名及型号： Product & model:	XYLO-OLIGOSACCHARIDE XOS95P(95% POWDER of XOS)	批号： Lot NO.：	20100413
生产日期： Produce date:	Apr 13, 2010	有效期： Expiry date：	24 months

Result of Inspection:

Test Item 测试项目	Specification 标 准	Test Result 检测结果
Appearance 感官	White or yellowish powder, sweet, no peculiar smell 白色或略泛微黄色粉末, 味甜, 无异 味	Yellowish powder, sweet, no peculiar smell 略泛微黄色粉末, 味甜, 无异味
pH 酸碱度	3.0-6.0	3.92
Ash 灰分	Not more than 0.3% ≤0.3%	0.14%
Moisture 水分	Not more than 5.0% ≤5.0%	2.64%
Protein 蛋白质	Negative	Negative
Lipid 脂肪	Negative	Negative
XOS ₂₋₇ contents 低聚木糖含量	Not less than 95 g/100 g ≥95g/100 g	95.26 g/100 g
XOS ₂₋₄ contents 低聚木糖含量	Not less than 65 g/100 g ≥65 g/100 g	74.57 g/100 g
Lead 铅	Not more than 0.5 mg/kg ≤0.5 mg/kg	< 0.5 mg/kg

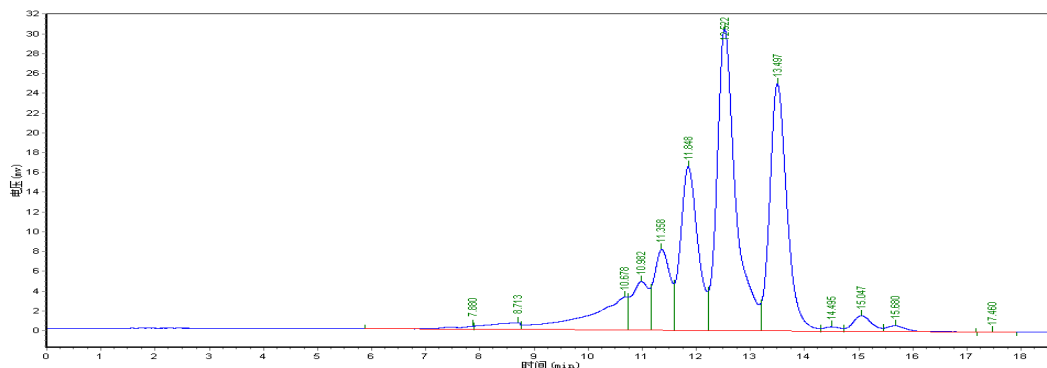
Arsenic 砷	Not more than 0.3 mg/kg ≤0.3 mg/kg	< 0.3 mg/kg
Copper 铜	Not more than 5 mg/kg ≤5 mg/kg	< 5 mg/kg
Coliform bacteria 大肠菌群	Not more than 30 MPN/100 g ≤30 MPN/100 g	< 30 MPN/100 g
Total plate count 菌落总数	Not more than 1000 cfu/g ≤1000 cfu/g	35 cfu/g
Mold 霉菌	Not more than 25 cfu/g ≤25 cfu/g	20 cfu/g
Yeast 酵母菌	Not more than 25 cfu/g ≤25 cfu/g	5 cfu/g
Pathogen 致病菌	Negative 不得检出	Negative 未检出

Components of 95P XOS

Experimental Methods: HPLC

Calculation Methods: Peak Area External Standard Calibration

95P 20100413



Peak	Reserved time	Peak height	Peak area	Contents (g/100 g)
X7	10.678	3358.089	225208.6	8.87
X6	10.982	4920.664	107075.9	4.22
X5	11.358	8178.531	192948.3	7.6
X4	11.848	16542.96	407727.1	16.06
X3	12.522	30497.17	753425.6	29.68
X2	13.497	24974.86	739590.3	28.83
Totally				95.26
Glucose	14.495	460.284	6390.634	0.25
Xylose	15.047	1604.654	26927.66	1.13
Arabinose	15.68	592.946	9357.842	0.39

Opinion of the Analyst:

Representative samples were inspected and found in conformity with the required specifications.

Annotations:

1. If there are any doubts about the results, please inform us for re-test in a month. The sample for re-test shall be the original one.
2. This report should not be used for advertisement and propaganda.

Analyst

Corrector:

Approver:

APPENDIX: DETAILED RESULTS OF TOXICITY STUDIES

Acute toxicity test in the mouse (SD Longlive, 2010):

Twenty mice weighing 18-22 g were selected, half male and half female. Stomach perfusion was conducted twice daily with a dosage of 20 g/kg BW. Observed 14 d and recorded the toxic actions and death of mice. Grade II Kunming mice (Qualified Certificate: MANO: 01-3001) and Grade II Wistar rats (Qualified Certificate: MANO:01-3008). Both were provided by the Laboratory Animal Breeding Plant, Laboratory Animal Research Institute, Chinese Academy of Medical Sciences. After the testing material was given by gastric perfusion, there was no obvious toxicity symptoms in both male and female mice and no death of animals in 14 d (Table A1). So, the acute toxicity LD₅₀ of testing material in both male and female mice was greater than 20 g/kg BW.

Table A1. Results of acute toxicity studies in mice

Animal species	Sex	Route	LD 50 (g/kg BW)
Mice	Male	Oral	>20
Mice	Female	Oral	>20

Subacute Toxicity of XOS in Rats (Park et al., 2000)

As shown in Table A2, no treatment-related changes in BWs were noted during the treatment period.

Table A2. Body weights (g) in male and female rats treated orally with XOS^a

Gender	Male				Female			
Dose, mg/kg	0	333	1000	3000	0	333	1000	3000
0 d	132.7 ± 9.2	133.2 ± 9.2	135.4 ± 10.1	134.5 ± 8.8	110.7 ± 6.4	112.1 ± 8.1	111.4 ± 7.0	111.6 ± 7.0
28	353.2 ± 29.2	355.3 ± 21.9	350.4 ± 17.9	351.0 ± 22.0	217.1 ± 18.7	211.7 ± 18.4	211.9 ± 17.4	215.6 ± 17.3
56	456.8 ± 44.2	453.2 ± 29.3	460.1 ± 30.8	451.0 ± 29.3	262.0 ± 19.3	252.7 ± 26.5	253.1 ± 19.8	257.6 ± 22.4
89	511.9 ± 49.7	502.6 ± 36.8	508.3 ± 46.1	510.1 ± 35.3	287.5 ± 22.8	277.1 ± 26.6	276.6 ± 17.3	279.6 ± 21.0
118	531.3 ± 56.4	NM	NM	550.7 ± 52.3	292.4 ± 9.6	NM	NM	294.7 ± 16.9

From Park et al., 2000; NM=not measured.

Table A3. Food consumption by male and female rats treated orally with XOS (daily food intakes in the XOS-treated groups of both sexes were not significantly different from the control groups except transient changes noted on day 1 in males.)

Gender	Male				Female			
Dose, mg/kg	0	333	1000	3000	0	333	1000	3000
1 d	26.0 ± 1.0	25.1 ± 0.8	24.6 ± 1.2**	24.7 ± 1.0**	19.8 ± 2.3	19.6 ± 1.5	21.6 ± 1.9	20.0 ± 1.6
29	34.1 ± 3.3	33.1 ± 2.8	31.5 ± 1.1	32.9 ± 2.0	22.6 ± 2.6	21.0 ± 2.4	22.4 ± 1.6	21.5 ± 2.0
57	32.1 ± 3.3	32.0 ± 1.9	31.9 ± 1.5	31.0 ± 2.2	22.3 ± 1.7	21.3 ± 1.5	22.7 ± 0.8	22.2 ± 1.9
90	31.9 ± 2.6	32.5 ± 2.6	31.9 ± 2.8	30.7 ± 3.1	21.6 ± 2.3	20.5 ± 1.5	20.5 ± 5.5	18.6 ± 3.2
119	30.8 ± 2.4	NM	NM	31.9 ± 1.9	20.9 ± 1.4	NM	NM	20.7 ± 1.7

From Park et al., 2000; ** Significantly different from control (p<0.01); NM=not measured.

Hematological values

As shown in Table A4, no treatment-related changes in hematological values were noted during the treatment period. In males at 4 wk recovery, significant decreases ($P < 0.05$ or 0.01) in WBC, RBC, HCT, MCHC, and lymphocyte values were observed in the 3,000 mg/kg group. However, all the hematological values were within the physiologically normal ranges.

Table A4a. Hematological values in male rats treated orally with XOS at 13 wk

Item	Dose, mg/kg			
	0	333	1,000	3,000
WBC, 10 ³ /μl	13.73 ± 2.8	11.56 ± 4.2	13.31 ± 4.4	12.31 ± 2.6
RBC, 10 ⁶ /μl	8.22 ± 0.3	8.33 ± 0.3	8.25 ± 0.5	8.22 ± 0.3
HGB, 10 ³ /μl	15.6 ± 0.4	15.8 ± 0.3	15.5 ± 0.7	15.6 ± 0.6
HCT, %	45.2 ± 1.5	45.7 ± 1.4	45.2 ± 2.0	45.3 ± 1.8
MCV, fl	55.0 ± 1.5	54.9 ± 1.1	54.9 ± 1.9	55.0 ± 1.3
MCH, pg	18.9 ± 0.7	18.9 ± 0.4	18.8 ± 0.7	19.0 ± 0.4
MCHC, g/μl	34.4 ± 0.5	34.5 ± 0.4	34.3 ± 0.4	34.5 ± 0.4
PLT, 10 ³ /μl	1034 ± 84.0	959 ± 55.4	1004 ± 100.1	1014 ± 55.2
Neutrophil, 10 ³ /μl	1.73 ± 0.8	1.46 ± 0.6	2.35 ± 1.9	1.44 ± 0.6
Eosinophil, 10 ³ /μl	0.11 ± 0.11	0.13 ± 0.1	0.1 ± 0.1	0.09 ± 0.1
Basophil, 10 ³ /μl	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Lymphocyte, 10 ³ /μl	11.85 ± 2.9	9.94 ± 4.0	10.84 ± 4.2	10.74 ± 2.6
Monocyte, 10 ³ /μl	0.05 ± 0.1	0.03 ± 0.1	0.03 ± 0.1	0.04 ± 0.1

From Park et al. (2000); *Significantly different from control (p<0.05); **Significantly different from control (p<0.01); HGB=hemoglobin; MCV= mean corpuscular volume; PLT=platelets.

Table A4b. Hematological values in female rats treated orally with XOS at 13 wk

Item	Dose, mg/kg			
	0	333	1,000	3,000
WBC, 10 ³ /μl	7.71 ± 2.4	7.76 ± 3.0	6.52 ± 1.7	6.5 ± 1.3
RBC, 10 ⁶ /μl	7.38 ± 0.6	7.61 ± 0.4	7.81 ± 0.3	7.59 ± 0.3
HGB, 10 ³ /μl	14.6 ± 1.1	14.6 ± 0.6	15.1 ± 0.5	15.1 ± 0.5
HCT, %	42.5 ± 2.9	42.7 ± 2.0	44.3 ± 1.8	43.6 ± 1.5
MCV, fl	57.6 ± 1.8	56.1 ± 1.0	56.8 ± 1.4	57.5 ± 1.5
MCH, pg	19.8 ± 0.5	19.2 ± 0.4*	19.4 ± 0.5	19.8 ± 0.5
MCHC, g/μl	34.3 ± 0.5	34.2 ± 0.3	34.2 ± 0.4	34.5 ± 0.2
PLT, 10 ³ /μl	9641 ± 84.4	9461 ± 15.3	942 ± 71.3	930 ± 79.7
Neutrophil, 10 ³ /μl	0.98 ± 0.4	1.15 ± 0.6	0.66 ± 0.3	0.75 ± 0.2
Eosinophil, 10 ³ /μl	0.04 ± 0.1	0.06 ± 0.1	0.05 ± 0.1	0.04 ± 0.1
Basophil, 10 ³ /μl	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Lymphocyte, 10 ³ /μl	6.67 ± 2.2	6.55 ± 2.5	5.81 ± 1.8	5.69 ± 1.3
Monocyte, 10 ³ /μl	0.01 ± 0.0	0.01 ± 0.0	0.0 ± 0.0	0.01 ± 0.0

From Park et al. (2000); *Significantly different from control (p<0.05); **Significantly different from control (p<0.01); HGB=hemoglobin; MCV= mean corpuscular volume; PLT=platelets.

Table A4c. Hematological values in male and female rats treated orally with XOS at 4 wk of recovery

	Males		Females	
Dose, mg/kg; Item	0	3000	0	3000
WBC, 10 ³ /μl	12.27 ± 2.7	9.22 ± 2.4*	7.6 ± 1.4	8.11 ± 2.3
RBC, 10 ⁶ /μl	8.45 ± 0.4	8.01 ± 0.3*	7.74 ± 0.3	7.84 ± 0.3
HGB, 10 ³ /μl	15.4 ± 0.7	14.9 ± 0.7	15.1 ± 0.6	15.4 ± 0.5
HCT, %	45.5 ± 2.0	43.6 ± 1.7*	44.0 ± 1.6	44.8 ± 1.2
MCV, fl	53.9 ± 1.2	54.5 ± 1.4	56.9 ± 1.5	57.2 ± 0.6
MCH, pg	18.2 ± 0.5	18.6 ± 0.6	19.5 ± 0.6	19.6 ± 0.4
MCHC, g/μl	33.8 ± 0.2	34.2 ± 0.4**	34.2 ± 0.3	34.4 ± 0.4
PLT, 10 ³ /μl	984 ± 65.6	1028 ± 87.5	893 ± 71.4	913 ± 68.6
Neutrophil, 10 ³ /μl	1.64 ± 1.1	1.19 ± 0.4	0.77 ± 0.5	0.75 ± 0.3
Eosinophil, 10 ³ /μl	0.14 ± 0.1	0.1 ± 0.1	0.09 ± 0.1	0.10 ± 0.1
Basophil, 10 ³ /μl	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Lymphocyte, 10 ³ /μl	10.49 ± 2.7	7.92 ± 2.6*	6.7 ± 1.4	7.27 ± 2.2
Monocyte, 10 ³ /μl	0.0 0.0	0.01 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

From Park et al. (2000); *Significantly different from control (p<0.05); **Significantly different from control (p<0.01); HGB=hemoglobin; MCV= mean corpuscular volume; PLT=platelets.

Serum biochemistry

Serum analysis showed no treatment-related changes in serum metabolite concentrations such as AST, ALT, ALP, BUN, creatine (CREA), glucose, total cholesterol (T-C), total bilirubin (T-BIL), TP, ALB, A/G ratio, CPK, TG, CA, IP, Na, K, and Cl during the treatment period (Table A5). The only exception was PL-E concentration noted in the 1,000 mg/kg group in males at 13 wk, but it was not considered as a treatment related change.

Table A5. Serum metabolite concentrations in male and female rats treated orally with XOS^a: At 16 wk

Item	Dose, mg/kg							
	Male				Female			
	0	333	1,000	3,000	0	333	1,000	3,000
AST, IU/L	120.7 ± 28.3	110.7 ± 24.4	120.1 ± 38.0	113.0 ± 22.6	125.5 ± 17.3	117.2 ± 29.1	100.6 ± 21.7	100.0 ± 17.7
ALT, IU/L	42.0 ± 6.5	47.2 ± 6.7	45.0 ± 7.5	48.0 ± 7.5	39.8 ± 9.6	41.6 ± 17.1	29.8 ± 2.8	32.6 ± 7.0
ALP, IU/L	173.5 ± 35.1	195.8 ± 13.6	169.8 ± 29.1	185.8 ± 41.1	82.6 ± 18.1	96.1 ± 19.7	87.4 ± 20.4	107.2 ± 26.5
BUN, mg/dl	15.1 ± 0.9	16.2 ± 1.7	15.4 ± 2.5	15.4 ± 1.7	17.8 ± 3.0	18.2 ± 3.7	16.2 ± 2.9	17.2 ± 2.1
CREA, mg/dl	0.51 ± 0.1	0.55 ± 0.1	0.50 ± 0.1	0.48 ± 0.1	0.53 ± 0.1	0.50 ± 0.1	0.5 ± 0.1	0.54 ± 0.1
GLU, mg/dl	122.2 ± 12.8	129.6 ± 13.8	124.8 ± 18.4	141.1 ± 24.8	117.6 ± 12.9	110.5 ± 14.6	113.4 ± 20.5	118.0 ± 6.7
T-C, mg/dl	94.3 ± 23.5	94.3 ± 16.3	78.1 ± 14.1	88.0 ± 16.7	89.2 ± 10.5	78.7 ± 18.4	71.0 ± 16.0	81.1 ± 11.1
T-BIL, mg/dl	0.10 ± 0.0	0.09 ± 0.0	0.09 ± 0.0	0.09 ± 0.0	0.11 ± 0.0	0.1 ± 0.4	0.11 ± 0.0	0.12 ± 0.0
TP, g/dl	6.27 ± 0.3	6.43 ± 0.3	6.12 ± 0.6	6.39 ± 0.3	6.29 ± 0.5	6.10 ± 0.4	5.84 ± 0.7	6.12 ± 0.3
ALB, g/dl	4.34 ± 0.2	4.42 ± 0.2	4.24 ± 0.3	4.44 ± 0.1	4.65 ± 0.3	4.54 ± 0.3	4.44 ± 0.5	4.56 ± 0.1
A/G ratio	2.26 ± 0.2	2.22 ± 0.2	2.33 ± 0.4	2.32 ± 0.3	2.96 ± 0.6	2.97 ± 0.5	3.27 ± 0.5	2.95 ± 0.3
CPK, IU/L	334.8 ± 170.8	240.4 ± 134.4	301.1 ± 197.8	245.7 ± 92.6	340.4 ± 106.0	262.0 ± 110.6	237.9 ± 103.4	222.7 ± 88.6
TG, mg/dl	115.8 ± 48.1	100.4 ± 19.6	82.6 ± 25.0	100.0 ± 34.5	54.9 ± 20.8	47.2 ± 27.8	44.5 ± 27.7	52.0 ± 14.9
CA, mg/dl	10.18 ± 0.5	10.33 ± 0.4	10.21 ± 1.1	10.54 ± 0.5	10.33 ± 0.8	10.11 ± 0.7	9.72 ± 1.5	10.38 ± 0.7
IP, mg/dl	7.14 ± 0.8	7.08 ± 0.8	7.14 ± 1.1	7.36 ± 1.0	6.42 ± 1.3	6.5 ± 1.1	6.31 ± 1.4	6.42 ± 1.6
PL-E, mg/dl	148.6 ± 28.1	145.8 ± 19.5	123.8 ± 13.2*	139.4 ± 16.2	174.7 ± 13.1	156.4 ± 32.1	145.0 ± 27.5	163.2 ± 16.4
Na, mmol/l	144.4 ± 0.8	144.4 ± 1.2	143.8 ± 2.8	143.1 ± 2.1	148.1 ± 10.5	144.2 ± 2.2	147.6 ± 7.5	143.6 ± 4.8
K, mmol/l	4.95 ± 0.3	4.79 ± 0.3	5.49 ± 1.21	5.49 ± 1.2	5.03 ± 0.7	4.67 ± 0.3	5.23 ± 1.1	4.51 ± 0.5
Cl, mmol/l	107.6 ± 0.8	108.0 ± 1.2	108.1 ± 2.4	107.0 ± 2.3	112.3 ± 9.8	109.8 ± 3.29	112.2 ± 6.2	108.7 ± 4.7

From Park et al., 2000.

Table A6. Serum metabolite concentrations in male and female rats treated orally with XOS: At 4 wk of recovery

Item	Dose, mg/kg			
	Male		Female	
	0	3,000	0	3,000
AST, IU/L	116.9 ± 19.5	129.3 ± 30.5	122.9 ± 37.3	126.2 ± 21.8
ALT, IU/L	47.9 ± 7.7	48.5 ± 6.6	35.5 ± 5.3	39.8 ± 12.3
ALP, IU/L	170.2 ± 35.3	159.1 ± 17.5	74.4 ± 16.0	80.7 ± 18.1
BUN, mg/dl	16.2 ± 1.5	17.6 ± 2.0	21.0 ± 3.5	20.2 ± 2.5
CREA, mg/dl	0.60 ± 0.1	0.56 ± 0.1	0.68 ± 0.0	0.66 ± 0.1
GLU, mg/dl	127.4 ± 10.8	134.6 ± 11.5	150.2 ± 26.9	138.4±19.3
T-CHO, mg/dl	88.2 ± 13.2	95.5 ± 13.9	98.2 ± 15.0	89.3±19.1
T-BIL, mg/dl	0.10 ± 0.0	0.1 ± 0.0	0.12 ± 0.0	0.12 ± 0.0
TP, g/dl	6.37 ± 0.3	6.34 ± 0.4	6.81 ± 0.3	6.63±0.5
ALB, g/dl	4.25 ± 0.1	4.19 ± 0.2	4.80 ± 0.2	4.76±0.3
A/G ratio	2.03 ± 0.2	1.96 ± 0.2	2.40 ± 0.2	2.56±0.2
CPK, IU/L	267.4 ± 138.2	340.8 ± 172.1	309.5 ± 176.5	313.1±133.7
TG, mg/dl	96.53 ± 0.4	125.1 ± 49.3	53.6 ± 24.6	52.0 ±21.3
CA, mg/dl	10.60 ± 0.3	10.63 ± 0.4	11.24 ± 0.5	11.37 ± 0.8
IP, mg/dl	6.78 ± 0.7	6.60 ± 0.6	6.97 ± 0.9	7.31 ± 1.1
PL-E, mg/dl	133.3 ±15.5	143.2 ± 21.1	180.8 ± 23.3	167.3 ± 25.2
Na, mmol/l	143.6 ± 2.6	144.0 ± 1.4	143.8 ± 1.3	144.1 ± 2.0
K, mmol/l	5.44 ± 1.6	5.42 ± 1.1	4.84 ± 0.9	5.27 ± 1.0
Cl, mmol/l	107.2 ± 1.6	107.5 ± 1.9	106.6 ± 1.5	106.9 ± 1.1

From Park et al., 2000. *Significantly different from control (p<0.05).

Gross findings in male and female rats treated orally with XOS^a

No abnormalities were observed in brain, hypophysis, adrenal gland, liver, spleen, kidney, heart, testis, ovary, prostate/uterus, lung, thymus, thyroid gland, salivary gland, urinary bladder, seminal vesicle, epididymis, preputial gland, pancreas, skin, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, artery, cervical spinal cord, lumbar spinal cord, tongue, trachea, esophagus, sciatic nerve, muscle, femur, sternum, eyes, harderian gland, mesenteric lymph node, submandibular lymph node, or abdominal cavity (Table A7).

Table A7a. Absolute and relative organ weights in male rats treated orally with XOS at 13 wk^a

Dose, mg/kg	0	333	1,000	3,000
Brain, g	2.076 ± 0.066	2.068 ± 0.141	2.014 ± 0.062	2.010 ± 0.066
Rel.wt., % ^b	.427 ± 0.045	0.435 ± 0.034	0.424 ± 0.034	0.425 ± 0.026
Hypophysis, g	0.013 ± 0.003	0.014 ± 0.003	0.013 ± 0.002	0.013 ± 0.002
Rel.wt., %	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001
Adrenal gland-left, g	0.033 ± 0.007	0.030 ± 0.004	0.030 ± 0.004	0.029 ± 0.007
Rel.wt., %	0.007 ± 0.002	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.001
Adrenal gland-right, g	0.030 ± 0.003	0.029 ± 0.003	0.029 ± 0.005	0.032 ± 0.007
Rel.wt., %	0.006 ± 0.000	0.006 ± 0.001	0.006 ± 0.001	0.007 ± 0.001
Adrenal gland-total, g	0.063 ± 0.008	0.059 ± 0.006	0.059 ± 0.007	0.061 ± 0.007
Rel.wt., %	0.013 ± 0.002	0.013 ± 0.001	0.012 ± 0.002	0.013 ± 0.001
Liver, g	13.158 ± 1.492	12.322 ± 1.207	12.429 ± 1.786	12.135 ± 0.814
Rel.wt., %	2.678 ± 0.110	2.584 ± 0.154	2.590 ± 0.176	2.562 ± 0.112
Spleen, g	0.758 ± 0.073	0.709 ± 0.070	0.750 ± 0.129	0.674 ± 0.126
Rel.wt., %	0.155 ± 0.010	0.150 ± 0.018	0.158 ± 0.027	0.142 ± 0.024
Kidney-left, g	1.485 ± 0.190	1.422 ± 0.150	1.447 ± 0.167	1.466 ± 0.159
Rel.wt., %	0.303 ± 0.033	0.298 ± 0.018	0.302 ± 0.018	0.309 ± 0.028
Kidney-right, g	1.498 ± 0.153	1.449 ± 0.153	1.451 ± 0.166	1.426 ± 0.127
Rel.wt., %	0.306 ± 0.029	0.304 ± 0.018	0.303 ± 0.015	0.301 ± 0.020
Kidney-total, g	2.984 ± 0.338	2.871 ± 0.297	2.897 ± 0.329	2.892 ± 0.282
Rel.wt., %	0.609 ± 0.061	0.602 ± 0.034	0.605 ± 0.032	0.610 ± 0.047
Heart, g	1.487 ± 0.120	1.371 ± 0.115	1.403 ± 0.149	1.426 ± 0.116
Rel.wt., %	0.304 ± 0.029	0.288 ± 0.023	0.294 ± 0.027	0.301 ± 0.014
Testis/ovary-left, g	1.715 ± 0.307	1.777 ± 0.112	1.814 ± 0.150	1.773 ± 0.169
Rel.wt., %	0.351 ± 0.070	0.374 ± 0.021	0.382 ± 0.045	0.375 ± 0.038
Testis/ovary-right, g	1.713 ± 0.354	1.765 ± 0.124	1.814 ± 0.119	1.724 ± 0.179
Rel.wt., %	0.350 ± 0.077	0.371 ± 0.027	0.382 ± 0.039	0.365 ± 0.041
Testis/ovary-total, g	3.427 ± 0.650	3.542 ± 0.232	3.628 ± 0.267	3.497 ± 0.345
Rel.wt., %	0.702 ± 0.145	0.745 ± 0.048	0.764 ± 0.084	0.740 ± 0.079
Prostate/uterus, g	0.650 ± 0.136	0.740 ± 0.131	0.720 ± 0.109	0.705 ± 0.147
Rel.wt., %	0.135 ± 0.038	0.156 ± 0.030	0.151 ± 0.019	0.148 ± 0.027
Lung, g	1.626 ± 0.132	1.588 ± 0.139	1.597 ± 0.130	1.644 ± 0.191
Rel.wt., %	0.332 ± 0.013	0.334 ± 0.025	0.335 ± 0.018	0.347 ± 0.033
Thymus, g	0.446 ± 0.144	0.311 ± 0.089*	0.303 ± 0.069**	0.331 ± 0.095*
Rel.wt., %	0.091 ± 0.027	0.065 ± 0.018*	0.064 ± 0.015*	0.070 ± 0.018
Thyroid gland-left, g	0.011 ± 0.003	0.013 ± 0.003	0.010 ± 0.002	0.010 ± 0.003
Rel.wt., %	0.002 ± 0.001	0.003 ± 0.001	0.002 ± 0.001	0.002 ± 0.001
Thyroid gland-right, g	0.011 ± 0.003	0.012 ± 0.003	0.012 ± 0.003	0.012 ± 0.003
Rel.wt., %	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001
Salivary gland, g	0.762 ± 0.111	0.758 ± 0.058	0.734 ± 0.082	0.746 ± 0.062
Rel.wt., %	0.155 ± 0.017	0.160 ± 0.013	0.154 ± 0.015	0.158 ± 0.010

From Park et al., 2000; *Significantly different from control (p<0.05); **Significantly different from control (p<0.01).

Table A7b. Absolute and relative organ weights in female rats treated orally with XOS^a at 13 wk^a

Dose, mg/kg	0	333	1,000	3,000
Brain, g	1.807 ± 0.253	1.882 ± 0.081	1.823 ± 0.166	1.899 ± 0.111
Rel.wt., % ^b	0.667 ± 0.110	0.729 ± 0.059	0.702 ± 0.078	0.732 ± 0.044
Hypophysis, g	0.014 ± 0.004	0.013 ± 0.002	0.014 ± 0.003	0.014 ± 0.002
Rel.wt., %	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
Adrenal gland-left, g	0.044 ± 0.008	0.038 ± 0.005	0.040 ± 0.005	0.036 ± 0.006*
Rel.wt., %	0.016 ± 0.002	0.015 ± 0.002	0.015 ± 0.002	0.014 ± 0.003
Adrenal gland-right, g	0.038 ± 0.004	0.033 ± 0.006*	0.038 ± 0.004	0.034 ± 0.004
Rel.wt., %	0.014 ± 0.001	0.013 ± 0.003	0.015 ± 0.002	0.013 ± 0.002
Adrenal gland-total, g	0.083 ± 0.012	0.071 ± 0.010*	0.077 ± 0.008	0.070 ± 0.009*
Rel.wt., %	0.030 ± 0.003	0.027 ± 0.005	0.030 ± 0.004	0.027 ± 0.004
Liver, g	7.017 ± 0.857	6.241 ± 0.644*	6.457 ± 0.426	6.164 ± 0.397**
Rel.wt., %	2.565 ± 0.148	2.404 ± 0.099	2.482 ± 0.164	2.379 ± 0.1093*
Spleen, g	0.488 ± 0.055	0.458 ± 0.067	0.420 ± 0.047	0.423 ± 0.071
Rel.wt., %	0.179 ± 0.018	0.178 ± 0.032	0.161 ± 0.015	0.163 ± 0.022
Kidney-left, g	0.879 ± 0.153	0.824 ± 0.063	0.826 ± 0.095	0.811 ± 0.056
Rel.wt., %	0.320 ± 0.033	0.318 ± 0.016	0.318 ± 0.038	0.312 ± 0.013
Kidney-right, g	0.920 ± 0.206	0.829 ± 0.060	0.857 ± 0.048	0.833 ± 0.085
Rel.wt., %	0.335 ± 0.057	0.320 ± 0.015	0.330 ± 0.020	0.321 ± 0.025
Kidney-total, g	1.799 ± 0.355	1.653 ± 0.116	1.683 ± 0.132	1.644 ± 0.135
Rel.wt., %	0.655 ± 0.090	0.638 ± 0.027	0.647 ± 0.054	0.633 ± 0.035
Heart, g	0.962 ± 0.106	0.882 ± 0.065	0.931 ± 0.075	0.948 ± 0.091
Rel.wt., %	0.353 ± 0.029	0.342 ± 0.035	0.358 ± 0.033	0.365 ± 0.027
Testis/ovary-left, g	0.046 ± 0.013	0.045 ± 0.010	0.047 ± 0.008	0.047 ± 0.007
Rel.wt., %	0.017 ± 0.004	0.017 ± 0.003	0.018 ± 0.003	0.018 ± 0.003
Testis/ovary-right, g	0.048 ± 0.010	0.048 ± 0.009	0.053 ± 0.010	0.048 ± 0.007
Rel.wt., %	0.018 ± 0.003	0.019 ± 0.004	0.020 ± 0.005	0.018 ± 0.003
Testis/ovary-total, g	0.094 ± 0.021	0.094 ± 0.015	0.100 ± 0.014	0.095 ± 0.011
Rel.wt., %	0.035 ± 0.007	0.036 ± 0.006	0.038 ± 0.007	0.037 ± 0.005
Prostate/uterus, g	0.549 ± 0.183	0.558 ± 0.213	0.466 ± 0.084	0.540 ± 0.179
Rel.wt., %	0.201 ± 0.065	0.213 ± 0.066	0.180 ± 0.041	0.207 ± 0.062
Lung, g	1.249 ± 0.138	1.118 ± 0.100	1.171 ± 0.104	1.201 ± 0.135
Rel.wt., %	0.458 ± 0.046	0.434 ± 0.049	0.450 ± 0.041	0.461 ± 0.033
Thymus, g	0.285 ± 0.062	0.267 ± 0.044	0.234 ± 0.028	0.252 ± 0.043
Rel.wt., %	0.104 ± 0.018	0.103 ± 0.015	0.090 ± 0.009	0.097 ± 0.015
Thyroid gland-left, g	0.009 ± 0.004	0.007 ± 0.003	0.008 ± 0.002	0.010 ± 0.002
Rel.wt., %	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.004 ± 0.001
Thyroid gland-right, g	0.009 ± 0.003	0.009 ± 0.002	0.007 ± 0.002	0.009 ± 0.002
Rel.wt., %	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001

From Park et al., 2000; *Significantly different from control (p<0.05); **Significantly different from control (p<0.01).

Table A7c. Absolute and relative organ weights in male and female rats treated orally with XOS^a at 4 wk of recovery^a

Dose, mg/kg	0	3,000	0	3,000
Brain, g	2.115 ± 0.067	2.090 ± 0.058	1.908 ± 0.070	1.915 ± 0.070
Rel.wt., % ^b	0.428 ± 0.047	0.406 ± 0.040	0.698 ± 0.032	0.698 ± 0.043
Hypophysis, g	0.013 ± 0.002	0.014 ± 0.002	0.014 ± 0.003	0.014 ± 0.003
Rel.wt., %	0.003 ± 0.001	0.003 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
Adrenal gland-left, g	0.033 ± 0.007	0.030 ± 0.003	0.033 ± 0.005	0.039 ± 0.007*
Rel.wt., %	0.007 ± 0.001	0.006 ± 0.001	0.012 ± 0.002	0.015 ± 0.003
Adrenal gland-right, g	0.028 ± 0.007	0.026 ± 0.003	0.035 ± 0.004	0.038 ± 0.006
Rel.wt., %	0.006 ± 0.001	0.005 ± 0.001	0.013 ± 0.001	0.014 ± 0.002
Adrenal gland-total, g	0.061 ± 0.012	0.057 ± 0.006	0.068 ± 0.006	0.077 ± 0.012
Rel.wt., %	0.012 ± 0.002	0.011 ± 0.002	0.025 ± 0.003	0.028 ± 0.005
Liver, g	13.128 ± 1.902	13.699 ± 1.742	6.781 ± 0.228	6.934 ± 0.642
Rel.wt., %	2.644 ± 0.127	2.634 ± 0.133	2.478 ± 0.075	2.520 ± 0.170
Spleen, g	0.769 ± 0.116	0.739 ± 0.105	0.446 ± 0.048	0.452 ± 0.041
Rel.wt., %	0.155 ± 0.017	0.143 ± 0.018	0.163 ± 0.016	0.165 ± 0.019
Kidney-left, g	1.575 ± 0.170	1.501 ± 0.132	0.825 ± 0.034	0.884 ± 0.092
Rel.wt., %	0.316 ± 0.019	0.290 ± 0.024*	0.302 ± 0.018	0.322 ± 0.032
Kidney-right, g	1.572 ± 0.183	1.525 ± 0.146	0.846 ± 0.046	0.929 ± 0.095*
Rel.wt., %	0.316 ± 0.023	0.295 ± 0.029	0.309 ± 0.018	0.339 ± 0.038*
Kidney-total, g	3.146 ± 0.349	3.026 ± 0.265	1.672 ± 0.072	1.813 ± 0.179*
Rel.wt., %	0.632 ± 0.041	0.5854 ± 0.050*	0.612 ± 0.034	0.660 ± 0.068
Heart, g	1.526 ± 0.184	1.500 ± 0.161	0.961 ± 0.085	0.927 ± 0.068
Rel.wt., %	0.306 ± 0.020	0.290 ± 0.025	0.352 ± 0.035	0.338 ± 0.31
Testis/ovary-left, g	1.834 ± 0.170	1.814 ± 0.123	0.041 ± 0.006	0.047 ± 0.009
Rel.wt., %	0.371 ± 0.048	0.351 ± 0.027	0.015 ± 0.002	0.017 ± 0.004
Testis/ovary-right, g	1.808 ± 0.135	1.817 ± 0.108	0.041 ± 0.007	0.048 ± 0.008
Rel.wt., %	0.366 ± 0.047	0.352 ± 0.023	0.015 ± 0.002	0.018 ± 0.004*
Testis/ovary-total, g	3.642 ± 0.301	3.630 ± 0.228	0.082 ± 0.011	0.095 ± 0.016*
Rel.wt., %	0.737 ± 0.094	0.702 ± 0.050	0.030 ± 0.003	0.035 ± 0.007
Prostate/uterus, g	0.5959 ± 0.150	0.602 ± 0.184	0.549 ± 0.121	0.506 ± 0.125
Rel.wt., %	0.119 ± 0.028	0.116 ± 0.035	0.201 ± 0.048	0.185 ± 0.048
Lung, g	1.663 ± 0.146	1.590 ± 0.150	1.174 ± 0.090	1.238 ± 0.120
Rel.wt., %	0.336 ± 0.033	0.308 ± 0.038	0.429 ± 0.031	0.450 ± 0.039
Thymus, g	0.294 ± 0.086	0.275 ± 0.093	0.252 ± 0.049	0.200 ± 0.044*
Rel.wt., %	0.060 ± 0.019	0.053 ± 0.018	0.092 ± 0.016	0.073 ± 0.015*
Thyroid gland-left, g	0.013 ± 0.004	0.010 ± 0.002*	0.008 ± 0.002	0.008 ± 0.002
Rel.wt., %	0.003 ± 0.001	0.002 ± 0.001*	0.003 ± 0.001	0.003 ± 0.001
Thyroid gland-right, g	0.013 ± 0.004	0.011 ± 0.002	0.009 ± 0.003	0.007 ± 0.001
Rel.wt., %	0.003 ± 0.001	0.002 ± 0.001	0.003 ± 0.001	0.003 ± 0.001
Salivary gland, g	0.748 ± 0.097	0.732 ± 0.084	0.477 ± 0.044	0.447 ± 0.057
Rel.wt., %	0.151 ± 0.018	0.142 ± 0.020	0.174 ± 0.018	0.163 ± 0.017

From Park et al., 2000.; ^aValues are expressed as means±S.D.; ^bRelative organ weights were expressed as the percentage of organ weights to BWs; *Significantly different from control (p<0.05); **Significantly different from control (p<0.01).

A 30 day feeding test in rats (SD Longlive, 2010)

Methods: 80 weaning rats weighing 50-60 g were randomly divided into 4 groups (10 male and 10 female mice/group). The dosages of testing material in three test groups were 1.00, 2.00, and 4.00 g/kg BW in each group, respectively. The results of BW and food availability are shown in Tables A8 and A9. No animal refused to eat in any group during the experiment. All showed normal growth activity.

Body weight, food intake, and food availability

There were no significant differences in BW, food intake, and food availability between any test group and control group.

Table A8. Effects of 30 d feeding on BW (g) in rats

Sex	Dose, g/kg BW	Original BW, g	1 st wk	2 nd wk	3 rd wk	4th wk
Male	0.00	58.00±2.31	113.50±7.84	140.50±6.85	176.50±5.30	210.00±10.80
	1.00	59.10±1.60	111.50±5.30	169.00±5.68	190.50±6.85	211.50±8.51
	2.00	59.80±1.20	121.50±5.30	170.00±3.33	197.00±5.37	214.00±3.94
	4.00	58.60±2.27	111.00±4.37	161.50±8.18	200.00±5.77	217.00±6.32
Female	0.00	57.90±2.47	119.50±4.97	135.50±5.90	161.00±3.94	173.50±5.80
	1.00	58.50±1.78	122.00±4.22	143.00±5.87	160.50±4.38	175.00±5.77
	2.00	58.90±1.66	119.50±3.69	140.50±4.38	159.50±3.69	171.50±7.09
	4.00	59.20±1.59	118.50±3.37	138.50±3.37	157.50±4.25	176.50±5.80

From SD Longlive, 2010.

Blood routine and biochemical indices

Table A10 showed that hemoglobin, red blood cell count, total number of white blood cells and their classification, aminotransferase, blood urea nitrogen (BUN), creatine, cholesterol, nitroglycerine, blood sugar, total protein, and albumin all were in the normal range and there were no significant differences between any test group and control group.

Table A10. Hematological results of a 30 d feeding test in rats

Sex	Dose, g/kg BW	Hemoglobin, g/L	RBC count, $\times 10^{12}/L$	WBC count, $\times 10^9/L$	Neutrophil, %	Lymphocyte, %	Others, %
Male	0.00	147.60 \pm 10.04	7.08 \pm 0.49	10.68 \pm 0.50	15.88	82.22	1.56
	1.00	143.20 \pm 2.68	7.11 \pm 0.48	10.56 \pm 0.17	16.23	82.20	1.58
	2.00	141.20 \pm 5.12	7.05 \pm 0.26	10.90 \pm 0.85	16.13	82.43	1.40
	4.00	143.20 \pm 8.87	7.32 \pm 0.54	10.50 \pm 0.27	14.58	83.74	1.88
Female	0.00	140.40 \pm 9.09	6.76 \pm 2.03	10.42 \pm 0.23	17.08	81.32	1.60
	1.00	140.40 \pm 6.69	6.94 \pm 0.14	10.66 \pm 0.10	16.82	81.30	1.88
	2.00	142.80 \pm 4.21	7.21 \pm 0.12	10.54 \pm 0.31	14.63	83.53	1.83
	4.00	148.20 \pm 7.01	7.63 \pm 0.44	10.86 \pm 0.75	15.15	83.30	1.55

Sex	Dose, g/kg BW	GPT, U/L	GOT, U/L	BUN, m mol/L	Creatinine, μ mol/L	Cholesterol, m mol/L
Male	0.00	54.43 \pm 9.46	108.44 \pm 12.28	8.57 \pm 1.67	34.38 \pm 1.20	1.72 \pm 0.09
	1.00	51.72 \pm 8.10	121.69 \pm 25.91	9.15 \pm 1.05	35.09 \pm 2.82	1.82 \pm 0.14
	2.00	41.64 \pm 3.80	105.61 \pm 10.77	8.48 \pm 1.06	40.64 \pm 1.47	1.87 \pm 0.27
	4.00	59.10 \pm 5.06	112.16 \pm 14.32	9.26 \pm 0.92	36.31 \pm 3.67	2.14 \pm 0.35
Female	0.00	56.33 \pm 6.94	136.25 \pm 22.82	9.46 \pm 0.80	36.34 \pm 1.81	1.93 \pm 0.22
	1.00	39.18 \pm 5.47	123.30 \pm 38.78	10.16 \pm 1.89	38.74 \pm 1.35	1.96 \pm 0.20
	2.00	50.13 \pm 4.61	141.61 \pm 21.35	10.42 \pm 0.95	35.84 \pm 3.60	1.84 \pm 0.18
	4.00	39.44 \pm 7.45	127.02 \pm 33.60	9.15 \pm 2.17	39.36 \pm 7.02	1.75 \pm 0.16

Sex	Dose, g/kg BW	Triglyceride, mmol/L	Blood sugar, mmol/L	Total protein, g/L	Albumin, g/L
Male	0.00	0.30 \pm 0.17	7.72 \pm 0.71	65.36 \pm 2.21	39.15 \pm 2.24
	1.00	0.29 \pm 0.17	7.39 \pm 1.10	76.63 \pm 2.88	49.20 \pm 1.17
	2.00	0.36 \pm 0.16	6.91 \pm 0.21	76.50 \pm 4.06	50.35 \pm 2.94
	4.00	0.34 \pm 0.38	7.55 \pm 0.80	80.33 \pm 2.84	52.03 \pm 1.83
Female	0.00	0.22 \pm 0.03	7.41 \pm 0.41	69.45 \pm 2.66	44.66 \pm 0.90
	1.00	0.26 \pm 0.18	7.51 \pm 1.13	78.52 \pm 3.97	51.74 \pm 3.34
	2.00	0.31 \pm 0.12	6.82 \pm 1.27	75.64 \pm 2.33	49.72 \pm 1.64
	4.00	0.26 \pm 0.20	7.06 \pm 0.33	79.32 \pm 1.90	49.86 \pm 3.00

From SD Longlive, 2010.

General and histological examination

There was no abnormality upon general inspection, no abnormality of urine bladder stone and liver duct stone, and no abnormality of organ coefficient (Table A11). There was no significant microscopic pathological change in liver, spleen, kidney, gastric, duodenum, testis, and ovary in any test group compared to the control group.

Table A11. Results of organ ratio of a 30 d feeding test in rats

Sex	Dose, g/kg BW	Liver/body, %	Spleen/body, %	Kidney/body, %
-----	---------------	---------------	----------------	----------------

Male	0.00	4.31±0.32	0.31±0.01	0.97±0.02
	1.00	4.69±0.13	0.32±0.01	0.98±0.01
	2.00	4.80±0.21	0.31±0.03	1.01±0.02
	4.00	4.79±0.16	0.29±0.03	0.99±0.01
Female	0.00	4.29±0.14	0.32±0.02	0.92±0.05
	1.00	4.79±0.20	0.31±0.02	1.01±0.02
	2.00	4.55±0.25	0.35±0.03	0.97±0.01
	4.00	4.34±0.09	0.32±0.02	0.99±0.02

From SD Longlive, 2010.

Ames Test (SD Longlive, 2010)

Certified *Salmonella typhimurium* (histidine-defect) TA97, TA98, TA100, and TA102 were used as lab test strains (Table A13). S-9 mixture (polychlorinated biphenyl induced rat liver homogenate) was used as in vitro activating system. Based on the results of toxicity test, 5 dosage levels (250, 500, 1,000, 2,500, and 5,000 µg/plate) were prepared. In addition, spontaneous revertant group, solvent control group, and positive control group were set up. According to plate penetration method, the tests were conducted with S-9 mixture added and not added. Three plates were used for each group. If the number of revertant colonies was more than twice the number of spontaneous revertant colonies and certain dosage-reaction relationships could be observed, a positive judgment could be concluded. The full set of tests was conducted twice under the same conditions. Table A13 show that the number of revertant colonies did not exceed two times that of spontaneous revertant colonies in all dosage groups in two experiments. There was no dose-response relationship either, indicating that the sample had no inherited toxicity on those four strains of *Salmonella typhimurium* with or without S-9.

Table A13a. Results of Ames test (First replication)

Dose, ug/plate	TA97		TA98		TA100		TA102	
	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
5000	132±7.8	138±10.5	30±2.8	33±1.4	135±14.1	137±11.3	135±14.1	137±11.3
2500	144±8.4	147±9.8	33±4.2	36±1.4	141±11.3	145±11.3	141±11.3	145±11.3
1000	151±11.3	157±8.4	35±7.1	38±4.2	147±9.8	148±15.5	147±9.8	148±15.5
500	146±5.6	157±12.7	39±5.6	41±4.2	153±12.7	158±13.4	153±12.7	158±13.4
250	156±12.7	161±9.8	40±4.2	39±5.6	159±9.8	164±14.1	159±9.8	164±14.1
Spontaneous revertant	137±8.4	144±7.1	32±4.2	35±2.8	148±9.8	156±11.3	148±9.8	156±11.3
Solvent control	141±10.5	147±12.7	31±5.6	35±5.6	140±14.1	148±12.7	140±14.1	148±12.7

Table A13b. Results of Ames test (Second replication)

Dose, ug/plate	TA97	TA98	TA100	TA102
-------------------	------	------	-------	-------

	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
5000	136±8.4	140±8.4	31±2.8	32±5.6	139±14.1	143±10.5	251±19.7	260±16.9
2500	145±9.8	144±8.4	32±7.1	33±2.8	146±7.10	150±19.7	263±21.2	272±14.1
1000	150±7.1	149±7.1	34±2.8	36±4.2	151±10.5	154±8.4	265±17.6	276±15.5
500	156±7.1	155±12.7	38±5.6	39±2.8	157±12.0	162±13.4	277±19.0	282±18.4
250	161±8.4	165±8.4	36±1.4	40±4.2	162±14.8	166±15.5	282±20.4	288±21.2
Spontaneous revertant	142±7.1	151±5.6	36±2.8	40±4.2	153±12.7	160±13.4	263±19.7	275±25.4
Solvent control	145±8.4	150±9.8	35±4.2	38±7.1	146±14.1	153±10.5	275±18.3	266±24.0

From SD Longlive, 2010; Note: (1) Atebrin, 250 µg/dish, (2) Rudomycin, 50 µg/dish, (3) Sodium azide, 100 µg/dish, (4) Mitomycin, 0.5 µg/dish, (5) 2-AF, 50 µg/dish

Polychromatophilic normocyte micronucleus test in mice (SD Longlive, 2010)

Fifty mice (25-30 g in BW) were randomly divided into 5 groups (5 males and 5 females per group). The dosages of testing material were 2.5, 5.0, and 10.0 g/kg BW. Distilled water was given to the negative control group and cyclophosphamide (CTX, 40 mg/kg BW) to the positive control group by gastric perfusion two times with an interval of 24 h. Animals were sacrificed 6 h after the second administration of testing material.

Polychromatophilic normocyte for each animal were examined under the microscope, the number of micronuclei cell was recorded, and the rate of micronuclei cell was calculated. Table A14 shows that there was no significant difference in micronucleus rate between any testing group and control group, indicating that the sample did not cause micronucleus change of polychromatophilic normocyte of bone marrow in mice.

Table A14. Results of polychromatophilic normocyte micronucleus test of bone marrow (BM) in mice

Sex	Dose, g/kg BW	Number of animals	Number of testing cells	Number of micronucleus cells	Rate of micronucleus cells (permil)
Male	2.5	5	1,000	10	2.0
	5.0	5	1,000	10	2.0
	10.0	5	1,000	10	2.0
	Negative control	5	1,000	10	2.0
	Positive control	5	1,000	146	29.2**
Female	2.5	5	1,000	10	2.0
	5.0	5	1,000	10	2.0
	10.0	5	1,000	10	2.0
	Negative control	5	1,000	10	2.0
	Positive control	5	1,000	155	31.0**

From SD Longlive, 2010; **P<0.01(Poisson distribution test, compared with negative control group).

Sperm abnormality test in mice (SD Longlive, 2010)

Fifty mice (25-30 g in BW) were randomly divided into 5 groups (5 males and 5 females per group). The dosages of testing material were 2.5, 5.0, and 10.0 g/kg BW. Distilled water was given to the negative control group and cyclophosphamide (CTX, 40 mg/kg BW) to the positive control group by gastric perfusion continuously for 5 d. Animals were sacrificed 35 d after the first gastric perfusion. The percentage of abnormal sperms were calculated. Table A15 shows that there were no significant differences in sperm abnormality rate between any test group and control group, indicating that the sample did not cause sperm abnormality in mice.

Table A15. Results of sperm abnormality test in mice

Dose, g/kg BW	Number of animal	Abnormality rate of sperm, %
2.5	5	2.10
5.0	5	2.50
10.0	5	2.28
Negative control	5	2.22
Positive control	5	5.84**

From SD Longlive, 2010; **P<0.01 (X^2 test, compared with negative control group).

Testis chromosome aberration test in mice (SD Longlive, 2010)

Fifty mice (25-30 g in BW) were randomly divided into 5 groups (5 males and 5 females per group). The dosages of testing material were 2.5, 5.0, and 10.0 g/kg BW. Distilled water was given to the negative control group and cyclophosphamide (CTX, 40 mg/kg BW) to the positive control group by gastric perfusion continuously for 5 d. Animals were sacrificed 13 d after the first gastric perfusion. The type and number of testis chromosome aberrations were recorded. Table A16 shows that there were no significant differences in chromosome aberration rate between any test group and control group, indicating that the sample did not cause chromosome aberration of testis in mice.

Table A16. Results of testis chromosome aberration test in mice

Dose, g/kg BW	Number of test cells	No. of chromosome aberrations
2.5	100	11
5.0	100	11
10.0	100	17
Negative control	100	13
Positive control	100	33

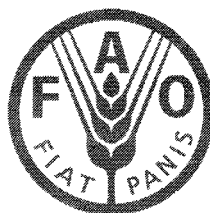
From SD Longlive, 2010; **P<0.01 (X^2 test, compared with negative control group).

FAO Technical Meeting Report

Food Quality and Standards Service

Food and Agriculture Organization of the United Nations

FAO Technical Meeting on PREBIOTICS



FAO TECHNICAL MEETING ON PREBIOTICS

Food Quality and Standards Service (AGNS)
Food and Agriculture Organization of the United Nations (FAO)
September 15-16, 2007

This report was prepared for the Food Quality and Standards Service (AGNS), Food and Agriculture Organization of the United Nations (FAO) based on the technical meeting convened by AGNS/FAO (FAO secretariat: Maya Pineiro, Senior Officer, AGNS) with the international experts namely: Nils-Georg Asp, Oscar Brunser, Sandra Macfarlane (Chair), Lorenzo Morelli, Gregor Reid and Kieran Tuohy (Rapporteur).

The views expressed in this publication are those of the author(s) and do not necessarily reflect the views of the Food and Agriculture Organization of the United Nations (FAO). The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of FAO concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

For further information, please contact:

Food Quality and Standards Service
Nutrition and Consumer Protection Division
Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla
00153, Rome, Italy
Fax: (+39) 06 57054593
E-Mail: food-quality@fao.org
Web site: www.fao.org/ag/agn/agns/index_en.stm

Table of Contents

1. Objective of the Meeting	4
2. Current prebiotic standing and state of the art	4
3. Defining the term prebiotic	5
3.1. Definition	6
3.2. Qualifications	6
4. How to evaluate and substantiate that a product is a prebiotic	6
4.1. Product specification/characteristics of the prebiotic.....	6
4.2. Functionality.....	6
4.3. Qualifications	7
4.4. Safety.....	7
5. Management issues	7
6. Monitoring.....	8
7. Future research areas	8
Figure 1:	9
8. References	10
9. List of Participants	11

1. Objective of the Meeting

This **Technical Meeting** of experts was convened to begin discussions on guidelines, recommended criteria and methodology for conducting a systematic approach for the evaluation of prebiotics, leading to their safe and efficacious use in food. The purpose was to discuss the prebiotic concept and its application to human health. An aim was to determine if prebiotics is an area of food research which would benefit from an Expert Consultation drawn from independently recognised leading experts convened under the auspices of the FAO.

Prebiotics have become a recognised functional food commodity. The Technical Meeting concluded that advances in prebiotic research provide sufficient substance for the FAO to consider a full Expert Consultation.

2. Current prebiotic standing and state of the art

Currently, there are no industry-wide accepted guidelines governing the usage of the term prebiotic on food products. The market for prebiotics in food is growing rapidly. A 2007 report on the world prebiotic market states that there are over 400 prebiotic food products and more than 20 companies producing oligosaccharides and fibres used as prebiotics [1]. A Frost & Sullivan review reported that the European prebiotics market is currently worth €87 million, and will reach €179.7 million by 2010. This is a dramatic growth spurt, in part explained by the increase in diversity of food products to which prebiotics have been added.

The basis for the expanded use of prebiotics is several-fold, not the least of which is a belief that modern day humans do not ingest sufficient quantities of lactic acid bacteria or their growth stimulants including non-digestible carbohydrates. In addition, there is a growing recognition that events taking place in the intestine and influenced by microbes, have major consequences for human health. Thus, not only are prebiotics being examined for anti-pathogenic effects (such as inhibiting adhesion of pathogenic organisms to the gut mucosa), but they are also being developed to decrease faecal transit time, lower cholesterol and the glycaemic response, improve bone health, lower daily energy (fat) intake, relieve symptoms of inflammatory bowel disease, and attempt to lower colon cancer rates [2]. The latter effects are also promoted for dietary fibres, and this raises the question of if and how prebiotics are differentiated from, or the same as, dietary fibres.

A prebiotic was originally defined in 1995 as a “non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” [3]. A more recent definition stated that “A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health” [4].

The principal concept associated with both of these definitions is that the prebiotic has a selective effect on the microbiota that results in an improvement in health of the host. The definitions arose from observations that particular dietary fibres bring about a specific modulation of the gut microbiota, particularly increased numbers of bifidobacteria and/or lactobacilli, and that ingestion of these compounds was associated with improved host health. However, as our ability to determine the microbial ecology of the gastrointestinal microbiota

increases, along with our understanding of how this complex and diverse collection of bacteria functions, we now recognise that a beneficial modulation of the microbiota encompasses far more than bifidogenesis.

Common prebiotics in use include inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), soya-oligosaccharides, xylo-oligosaccharides, pyrodextrins, isomalto-oligosaccharides and lactulose. The majority of studies have so far focused on inulin, FOS and GOS [5, 6]. These saccharides have now a long history of safe use and are generally regarded as safe, although there is some concern over increased gas production with some compounds, particularly when ingested in higher amounts or during the first few days of intake.

There is also a range of new prebiotic compounds emerging, and these include: pecticoligosaccharides, lactosucrose, the sugar alcohols, gluco-oligosaccharides, levans, resistant starch, xylosaccharides and soy-oligosaccharides. These compounds have been studied to varying degrees *in vitro*, in animal feeding studies, but rarely in human feeding studies. Novel compounds new to the human diet fall under the European regulatory category of “novel foods” and will require legislated levels of safety and toxicological assessment before they can be included in food products. However, little legislation exists governing the use of the word “prebiotic” itself on functional food products and there is a growing collection of commercially available products which bear the prebiotic label but for which supportive scientific literature is sparse or lacking all-together.

The call for a scientific evaluation of the functional and health properties of prebiotics is thus timely. The FAO Technical Meeting on Prebiotics addressed guidelines, recommended criteria and methodologies for conducting a systematic approach for the evaluation of prebiotics leading to their safe and efficacious use in food.

3. Defining the term prebiotic

The existing definitions of a prebiotic, as stated above, while differentiating this class of non-digestible food ingredient within the dietary fibres and broadly serving the more common and well studied prebiotic oligosaccharides, is restrictive in its applicability for target sites outside the gastrointestinal tract. It is also restricted by necessitating a single mechanism of action (e.g. anti-adhesive activities) in addition to the selective changes in the composition and/or activity in the gastrointestinal microbiota. These definitions too, were drawn up early in the current wave of interest surrounding the impact of the gut microbiota on human health and disease, specifically, before metagenomic demonstration of the high species richness, novelty (with up to 70% of the gut microbiota commonly categorised as “new to science” upon direct 16S rRNA gene fragment sequencing) and degree of metabolic cross-feeding or co-dependence within the gut microbiota.

The stipulation of selective fermentation or selective increase in growth and/or activity encompassed within these current definitions, has become synonymous with the preferential increased abundance of bifidobacteria and/or lactobacilli. However, this is now inadequate to describe a beneficial modulation of a microbiota dominated by members of the *Clostridium* *coccoides*, *C. leptum* groups and the *Bacteroides*, regarded as key species together with the bifidobacteria in saccharolytic fermentation within the colon. These considerations and their implications warrant a reconsideration of the prebiotic definition itself.

The Technical Meeting proposes a broader definition to encompass new prebiotics, and to more accurately reflect current understanding of the microbial ecology of the human microbiota. This revised definition follows.

3.1. Definition

A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota.

3.2. Qualifications

1. Component – not an organism or drug; a substance that can be characterized chemically; in most cases this will be a food grade component.
2. Health benefit – measurable and not due to absorption of the component into the bloodstream or due to the component acting alone; and over-riding any adverse effects
3. Modulation – show that the sole presence of the component and the formulation in which it is being delivered changes the composition or activities of the microbiota in the target host. Mechanisms might include fermentation, receptor blockage or others.

A prebiotic can be a fibre but a fibre need not be a prebiotic.

4. How to evaluate and substantiate that a product is a prebiotic

4.1. Product specification/characteristics of the prebiotic

The component to which the claim of being prebiotic is attributed, must be characterized for any given product. This includes:

- Source, origin
- Purity
- Chemical composition and structure
- Vehicle, concentration and amount in which it is to be delivered to the host

4.2. Functionality

At a minimum, there needs to be evidence of a correlation between the measurable physiological outcomes and modulation of the microbiota at a specific site (primarily the gastrointestinal tract, but potentially also other sites such as vagina and skin). Need to correlate a specific function at a specific site with the physiological effect and its associated timeframe.

- Within a study, the target variable should change in a statistically significant way and the change should be biologically meaningful for the target group consistent with the claim to be supported.

- Substantiation of a claim should be based on studies with the final product type, tested in the target host.
- A suitably sized randomized control trial (compared to placebo or a standard control substance) is required, preferably with a second independent study.
- Examples of physiological outcomes due to administration of prebiotics could be: satiety (measured towards carbohydrates, fats, total energy intake); endocrine mechanisms regulating food intake and energy usage in the body; effects on absorption of nutrients (e.g. calcium, magnesium, trace elements, protein); reduced incidence or duration of infection; blood lipid and classic endocrine parameters; bowel movement and regularity; markers for cancer risk; changes in innate and acquired immunity that are evidence of a health benefit.

4.3. Qualifications

- Bifidogenic effects are not sufficient without demonstrated physiological health benefits.
- It is recognized that at this time, determining events that take place within compartments of the intestine are often difficult. Until such times as specific site sampling or more sophisticated methods can reliably link microbiota modulation with health benefits, faecal analysis will be deemed suitable, with limitations.

4.4. Safety

As with any food component, safety parameters are established by all national regulations. It is recommended that the following issues need to be covered in any safety assessment of a prebiotic final product formulation:

- If , according to local legislation, the product has a history of safe use in the target host, such as GRAS or its equivalents, then it is suggested that further animal and human toxicological studies may not be necessary.
- Safe consumption levels with minimal symptoms and side effects should be established.
- The product must not contain contaminants and impurities.
- Based upon current knowledge, the prebiotic should not alter the microbiota in such a way as to have long term detrimental effects on the host.

5. Management issues

- **Production** – the onus is on the manufacturer to ensure substances considered prebiotics should have purity and consistency in composition between product lots.
- **Formulation and storage** – It is recommended that the limit of stability in different product types, effects of processing and production technologies on prebiotic composition, and the desired biological activity in the target host be evaluated.
- **Regulatory** – Prebiotics are components designed for specific health effects through modulation of the host microbial population. The onus is on the producer to provide the regulatory agency where sales are to be made with an appropriate level of documentation supporting the health claims. It is possible that these may refer to

disease prevention, treatment and risk reduction claims. A number of documents available in the public domain, such as PASSCLAIM, EFSA guidelines and others [7,8], provide criteria for evaluating the quality of data suitable for making health claims on food and food components.

- The status of prebiotics is not established on an international basis. The term prebiotic must be used only when a health benefit related to modulation of the target site microbiota has been demonstrated in the target host.
- The issues of product specific testing were considered. The consensus was that the onus should be on the producer to show that a new formulation e.g. yoghurt, is equivalent to the one (e.g. dried powder) proven in target host studies to confer the prebiotic effect.

6. Monitoring

The Technical Meeting recommends that prebiotic producers, medical professionals and public health officers consider some form of system to monitor the health outcomes of long-term prebiotic administration. This is suggested as a means to gain insight into potential side effects as well as assess long-term benefits. A necessary prerequisite for surveillance is a proper trace-back system.

7. Future research areas

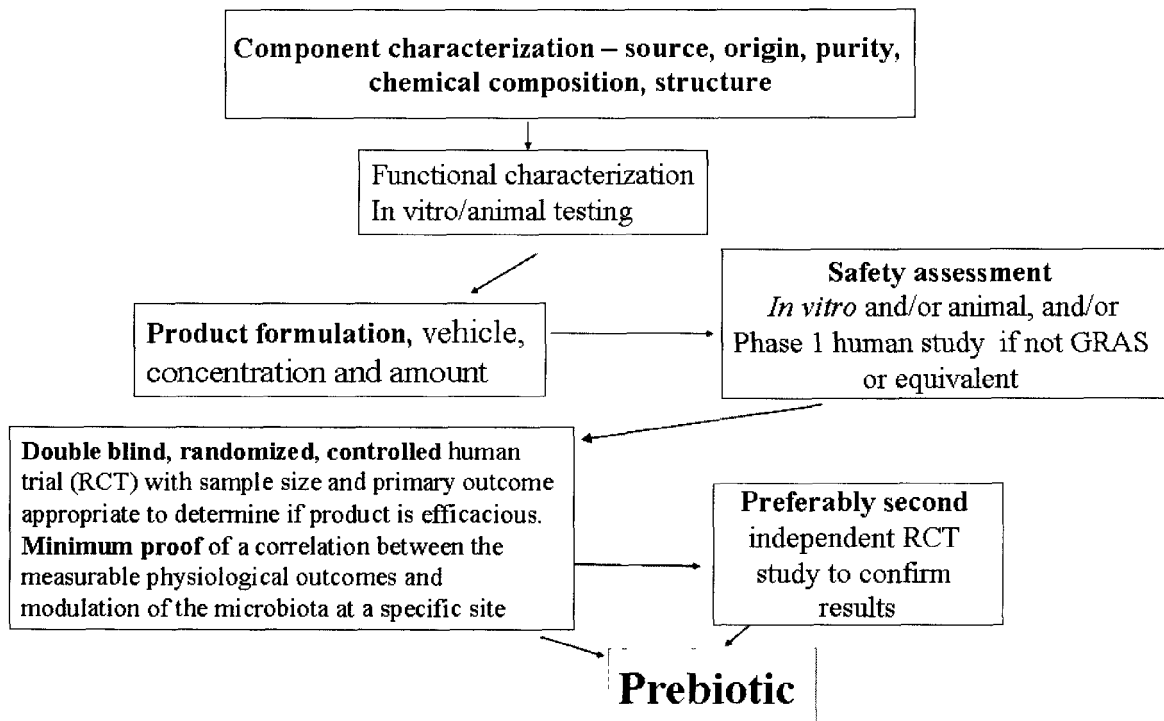
- It is recognised that there are numerous potential new applications being considered for prebiotic use e.g. prevention and or management of type 2 diabetes mellitus; drug bioavailability; effects on autoimmune diseases and allergy; modulation of pathogenic biofilms. There is a need for more randomised, placebo controlled clinical trials with adequate statistical power. We encourage publication in peer-reviewed journals of all clinical trials, whether the outcome is positive, negative or adverse.
- It is recognised that prebiotics may be used in conjunction with probiotics; this is considered a synbiotic. Depending on the nature of the two components, the net effect may not be synergistic. We recommend that the term synbiotic only be used if the net health effect is synergistic. It is also recommended that the issue of synbiotics be addressed by a separate Technical Meeting.

Figure 1:

Guidelines for the evaluation and substantiation of prebiotics



A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota.



8. References

1. <http://www.ubic-consulting.com/template/fs/The-World-Prebiotic-Ingredient-Market.pdf>
2. Conway, P. Prebiotics and human health: the state-of-the-art and future perspectives. *Scand. J. Nutrition/Naringsforskning* 2001; 45:13-21.
3. Gibson, GR, Roberfroid, M. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 1995; 125:1401-1412.
4. Gibson, GR, Probert, HM, Van Loo, J, Rastall, RA, Roberfroid, M. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr. Res. Rev.* 2004; 17:259-275.
5. Macfarlane, S, Marfarlane, GT, Cummings, JH. Prebiotics: key issues. *Aliment. Pharmacol. Ther.* 2006; 24:701-714.
6. Macfarlane, GT, Steed, H, Macfarlane, S. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *J. Appl. Microbiol.* 2008; 104: 305-344.
7. Aggett PJ, Antoine J-M, Asp N-G, Bellisle F, Contor L, Cummings JH, Howlett J, Müller DJG, Persin C, Pijls LTJ, Rechkemmer G, Tuitelaars S, Verhagen H. PASSCLAIM Process for the Assessment of Scientific Support for Claims on Foods. Consensus on Criteria. *E J Nutr* 2005; 44 (Suppl 1):i/1-I/30.
8. Anonymous. EFSA Scientific and technical guidance for the preparation and presentation of the application for authorization of a health claim. http://www.efsa.europa.eu/EFSA/Scientific_Opinion/nda_op_ej530_guidance_%20health_claim_en.pdf.pdf (Accessed 5 Oct 2007)

9. List of Participants

International Experts

Nils-Georg Asp
Professor Applied Nutrition
Lund University and
Director, SNF Swedish Nutrition
Foundation, Sweden
E-mail: asp@snf.ideon.se

Oscar Brunser
Ultrastructure Laboratory
Institute of Nutrition and Food Technology
(INTA) and University of Chile
Santiago, Chile
E-mail: obrunser@inta.cl

Sandra Macfarlane (Chair)
Division of Pathology and Neuroscience
Dundee University, United Kingdom
E-mail: s.macfarlane@dundee.ac.uk

Lorenzo Morelli
Istituto di Microbiologia UCSC, Italy
E-mail: Lorenzo.morelli@unicatt.it

Gregor Reid
University of Western Ontario and
Canadian R&D Centre for Probiotics
F2-116, Lawson Health Research Institute,
268 Grosvenor Street, London, Ontario
N6A 4V2, Canada
E-mail: gregor@uwo.ca

Kieran Tuohy (Rapporteur)
Food Biosciences
University of Reading
United Kingdom
E-mail: k.m.tuohy@reading.ac.uk

FAO

Maya Piñeiro
Senior Officer
Food Quality and Standards Service, Food
and Agriculture Organization of the United
Nations (FAO), Rome, Italy
E-mail: Maya.Pineiro@fao.org

SUBMISSION END