ORIGINAL SUBMISSION

# GRAS Notification for Shangdong Longlive Biotechnology, Ltd.'s Xylooligosaccharides

Prepared for: U.S. Food and Drug Administration
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740-3835

Prepared by: NutraSource, Inc.
6309 Morning Dew Court
Clarksville, MD 21029
Tel: 410-531-3336
Susanscho1@yahoo.com

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# I. GRAS EXEMPTION CLAIM

## I.A. Claim of exemption from the requirement for premarket approval requirements pursuant to Proposed 21 CFR 5 170.36(~)(1)

Shangdong Longlive (the notifier) has determined that its xylooligosaccharides (XOS), water soluble non-digestible oligosaccharides (NDO) derived from corncob xylans, are Generally Recognized As Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act.* This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food. Therefore, the use of XOS is exempt from the requirement of premarket approval.

Signed

Susan Cho Date:
Agent for
Shandong Longlive Bio-technology Co., Ltd.
Room 806, R&D Fortune Center, No.182-6 Haier Road, Laoshan District, Qingdao, China

## I.B. Notifier's Name and Address

Shandong Longlive Bio-technology Co., Ltd.
Attention: Alice Fu
Address: Room 806, R&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

## I.C. Name of GRAS substance

Xylooligosaccharides (XOS 95P, 70P, 35P, and 20P) manufactured by Shangdong Longlive Bio-Technology Co., Ltd.

## I.D. Product description

Xylooligosaccharides (beta-1,4-(D-pentose); n=2~6) are sugar oligomers made up of xylose units. They consist of a main backbone of xylose linked by β 1→4 bonds. Shangdong Longlive's XOS are mostly composed of non-digestible xylose-based disaccharide (xylobiose, 35%) and 60% total fiber (non-digestible oligomers; n=3-6) with xylose backbones. Total fiber is defined as the sum of non-digestible carbohydrates (with degree of polymerization [DP] of 3 and higher) and lignin.

Xylooligosaccharides are hydrolysis products of xylan (a type of dietary fiber), a common hemicellulose of cereal grains (corncob, corn bran, rice bran, wheat bran, and psyllium), where it occurs in many different compositions and structures. Hemicelluloses and cellulose are considered major dietary fiber components. The Shangdong Longlive's manufacturing process involves a mild pretreatment of corncobs, followed by enzymatic hydrolysis by endoxylanase,

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(b) (6)

1/21/2013

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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, fructooligosaccharides (FOS), galactooligosaccharides (GOS), soya-oligosaccharides, and isomalto-oligosaccharides (IMO) as prebiotics. These are defined as a food component that confers a health benefit on the host associated with modulation of the microbiota.

**I.E. Applicable conditions for use of the notified substance**

Xylooligosaccharides can be used as ingredients in foods and beverages, such as milk and milk products, flavored and soy milk, milk desserts, evaporated or condensed milk, powdered milk, cheese, ready- to-drink milk-based meal replacements and protein, yogurt, puddings, custards, and other milk desserts, gelatin desserts or salads, ready to eat cereals, cereal bars, granola bars, protein bars, and power bars, breakfast bars, processed fruits, juice drinks and punch, chewing gums, sports drinks, isotonic beverages, beers and ales, and medical foods at an average level of 1.3 g per serving (ranged from 0.2 to 2.4 g per serving). Shangdong Longlive's XOS are not intended for use in infant formulas, or in meat, egg, or catfish products, which are regulated by the USDA.

**I.F. Basis for GRAS determination**

In accordance with 21CFR 170.30, the intended use of XOS has been determined to be GRAS based on scientific procedures. A comprehensive search of the scientific literature also was conducted for this review. There is sufficient qualitative and quantitative scientific evidence, including human and animal data, to determine safety-in-use for XOS. The safety determination of XOS is based on the totality of available evidence, including human clinical trials. A variety of animal, as well as *in vitro*, studies further corroborate its safety.

To calculate an estimated daily intake (EDI; estimated exposure) for XOS, we considered the intended use levels of the ingredient per serving as well as the number of servings to be consumed daily. The analysis of the 2009-2010 National Health and Nutrition Examination Survey (NHANES) dataset revealed that approximately 89% of Americans are the users of one or more foods under the intended use. The mean and 90$^{th}$ percentile intakes, including XOS by all users (aged 2-99 years) from all GRAS-proposed use categories, are 3.9 and 7.5 servings per day, respectively. Xylooligosaccharides will be added to foods in the categories listed in the previous section at an average level of 1.3 g per serving. Assuming all the foods will be used under the intended use, the mean and 90$^{th}$ percentile intakes of all users aged 2-99 years are estimated to be 5.1 g per day and 9.8 g per day, respectively. Assuming a 60 kg body weight (BW) per person, these levels may correspond to a mean of 85 mg/kg BW/day and the 90th percentile of 163 mg/kg BW/day. These exposure estimates are very likely to be overestimated since it is not possible that all the foods can contain XOS. Medical foods are not included in the exposure estimates.

These overestimated levels are far below the no-observed-adverse-effect-level (NOAEL) values found from subchronic rat studies (up to 11,510-14,950 mg XOS/kg BW/day). Human intervention studies reported that doses up to 12 g per day were well tolerated by humans. Also, in the Finnish population, whose grain fiber consumption levels are much higher than that of Americans, daily xylan intake is estimated to be 13.8 g at the 99$^{th}$ percentile. Grain fiber intake

and resulting xylan intake were inversely associated with the risk of type 2 diabetes; there were no adverse effects. The data indicate that high intake of xylans (as high as 13.8 g per day) is not only safe, but also offers health benefits. Xylans and XOS are metabolized in a similar way to produce short chain fatty acids (SCFA), $H_2$, and $CO_2$ in the colon. Therefore, the metabolism of XOS does not raise safety concerns, and no systemic toxicity is expected following ingestion of XOS.

The Institute of Medicine (IOM) recommends increased intakes of total fiber: Recommended intakes of total fiber are 25 g per day for adult women and 38 g per day for adult men. Unfortunately, Americans consume only one-half of the recommended intakes, an average of 15.9 g per day. Since XOS function as a prebiotic fiber, its intended use may be to help Americans improve their fiber intake.

The information/data provided by Shangdong 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 in the proposed use as a food ingredient that is prepared according to appropriate specifications and used according to good manufacturing process (GMP).

Therefore, the proposed use of XOS is safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm). It is also *Generally Recognized as Safe* (GRAS) by consensus among experts. In addition, it is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion.

**I.G. Availability of information**

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

## II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

**II.A. Common or trade name:** Xylooligosaccharides manufactured by Shangdong Longlive Bio-technology, Ltd.

**II.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 in the Title 21 of the Code of Federal Regulations.

### II.C. Background

Xylooligosaccharides (beta-1,4-(D-pentose); n=2~6) are sugar oligomers made up of xylose units. They consist of a main backbone of xylose linked by β 1→4 bonds. Shangdong Longlive's XOS are mostly composed of non-digestible xylose-based disaccharide (xylobiose, 35%) and 60% total fiber (non-digestible oligomers; n=3-6) with xylose backbones. Total fiber is defined as the sum of non-digestible carbohydrates with a DP of 3 or above and lignin (IOM, 2002).

Xylooligosaccharides can be produced on an industrial scale by enzymatic hydrolysis of xylan or arabinoxylan. Xylan and arabinoxylan are the major components of plant hemicelluloses that are composed of xylose and arabinose backbones with side chains of galactose, glucose, and (or) mannose (Broekaert et al., 2011). Xylooligosaccharides have a sweet taste and are used as an alternative sweetener.

Xylooligosaccharides have been used as a food ingredient predominantly in Asia, particularly in Japan. Suntory Limited (Japan) was the first commercial-scale producer using enzymatic hydrolysis of xylan derived from corncob. The annual production volume of XOS was estimated at 650 tons in 2004 (Taniguchi, 2004). More recently, XOS have been manufactured by the Chinese producer Shangdong Longlive. The company's XOS are made from corncob by enzymatic hydrolysis (Moure et al., 2006). Corncob contains approximately 35% xylan (Aachary and Prapulla, 2009; Moure et al., 2006) and is an important byproduct of the corn industry. Corncobs are used to produce XOS, xylose, and xylitol (Aachary and Prapulla, 2009).

Like other NDOs, including FOS and GOS, XOS escape digestion in the upper gastrointestinal tract and are fermented in the lower gastrointestinal tract to SCFA (Campbell et al., 1997; Fleming and Rodriguez, 1983; Holma et al., 2002), where they exert prebiotic activities by promoting the growth of bifidobacteria (Table 1; Bouhnik et al., 2004; Chung et al., 2007; Fu et al., 2012; Grootaert et al., 2007; Kabel et al., 2002; Makelainen et al., 2009; van Laerke et al., 2000). The 2007 FAO Technical Meeting Report on Prebiotics classified XOS, FOS, GOS, soya-oligosaccharides, and IMO as prebiotics. These are defined as a food component that confers a health benefit on the host associated with modulation of the microbiota (FAO, 2007). The metabolism of XOS produces SCFA, $H_2$, and $CO_2$ in the colon. This does not raise safety concerns and, thus, no systemic toxicity is expected after ingestion of XOS. In addition, the long history of human exposure to xylose-based fibers (as hemicelluoses from grain sources; Broekaert et al., 2011) indicates that they do not pose a safety concern.

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

| Item | Chemical classification | Digestibility in upper GI tract | Fermentability in the lower GI tract | GRAS status, GRN No. |
|---|---|---|---|---|
| **Xylose-based carbohydrates** | | | | |
| Xylose | monosaccharide | Mostly absorbed | Negligible | |
| Xylan | polysaccharide | Non-digestible | Yes | |
| XOS | oligosaccharide | Non-digestible | Yes | 343 |
| AXOS | oligosaccharide | Non-digestible | Yes | |
| **Other non-digestible oligosaccharides** similar in nature to XOS | | | | |
| FOS | oligosaccharide | Non-digestible | Yes | 44 |
| GOS | oligosaccharide | Non-digestible | Yes | 236, 285, 286 & 334 |
| Soya oligosaccharides | oligosaccharide | Non-digestible | Yes | |
| IMO | oligosaccharide | Non-digestible | Yes | 246 |

AXOS=arabinoxylooligosaccharides

Table 2 presents the neutral sugar composition of various fibers. A major component of hemicelluloses, which are part of grain fibers, is xylose. It ranges from 14 to 35% of total fiber. Data show that humans have been exposed to xylose-based carbohydrates for a long time with no adverse effects.

Table 2. Neutral sugar composition of various grain fibers

| | Wheat bran | Rye bread | Oat bran |
|---|---|---|---|
| Total fiber, % | 40-84 | 10.3 | 18.4 |
| Neutral sugar composition, % total fiber residue | | | |
| Arabinose | 17.5 | 16.5 | 9.2 |
| Xylose | 34.8 | 27.2 | 14.1 |
| Mannose | 0.6 | 4.8 | 2.7 |
| Galactose | 1.5 | 3.9 | 2.2 |
| Glucose | 21.1 | 33.0 | 53.2 |
| Uronic acid | 5.2 | 5.8 | 5.4 |
| Other components, % of total fiber | | | |
| Lignin | 18.4 | 8.7 | 10.3 |

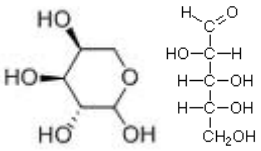
Data from Theander et al., 1995 (data supporting AOAC 994.13); To correct for $H_2O$ molecule loss during the polymerization process, neutral sugar residue was calculated from the following formula: neutral sugar residue (as polymer) = neutral sugar as free sugar (as measured by GLC after acid hydrolysis) x 0.9.

**II.D. Molecular formula:** $C5^{n}H^{8n+2}O^{4n+1}$, where n=2-6.

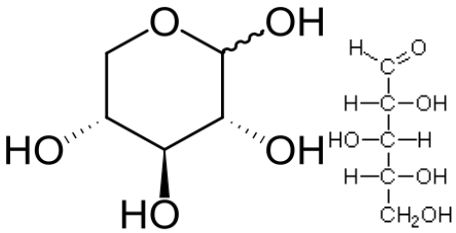
## II.E. Chemical structure of XOS

Figure 1. Chemical structure of various monosaccharides

Pentoses

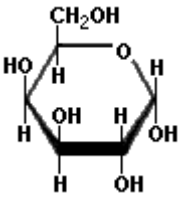


Arabinose

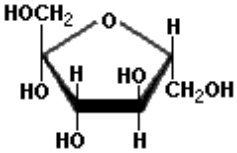


D-Xylose

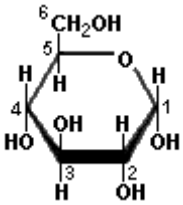
Hexoses



Galactose

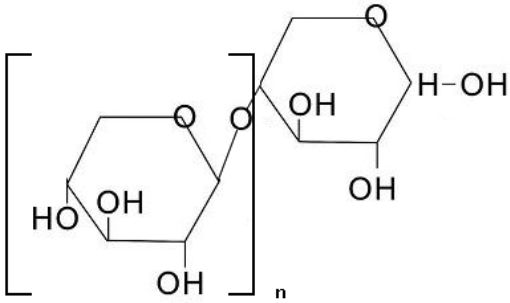


Fructose

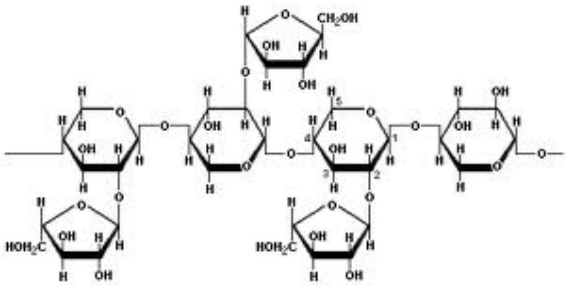


Glucose

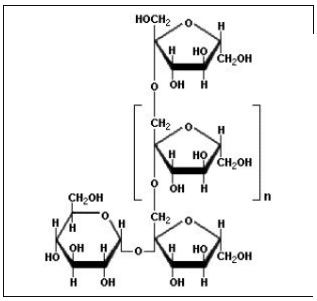
Figure 2. Chemical structure of various oligosaccharides (XOS, AXOS, FOS, and GOS; n=2-9)



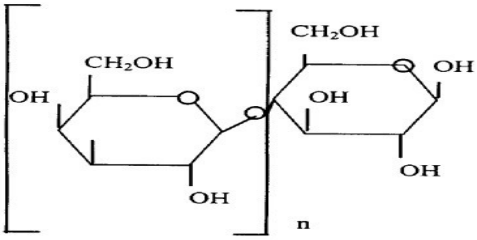
XOS



AXOS



FOS



GOS

**II.F. Acid and thermal stability**

Xylooligosaccharides are stable after heating to 100℃ under acid conditions (pH=2.5-8) that cover the pH values of the vast majority of food systems (Courtin et al., 2009; Vazquez et al., 2000). In food processing, XOS show advantages over inulin in terms of resistance to both acidity and heat, allowing their utilization in low-pH juices (Vazquez et al., 2000).

**II.G. Manufacturing process**

Xylooligosaccharides are produced from xylan-rich corncob by chemical methods, autohydrolysis, direct enzymatic hydrolysis of a susceptible substrate, or a combination of chemical and enzymatic treatments (Moure et al., 2006; Vazquez et al., 2000). However, enzymatic production of XOS is preferred in the food industry. Corncobs are 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).

The Shangdong Longlive's manufacturing process involves a mild pretreatment of corncobs, followed by enzymatic hydrolysis by endoxylanase. All the chemicals used in the production of XOS are of food grade.

Process description

1. Size mixing: mix corncob powder with water in a ratio of 1:6-10 and heat to 75 ℃ or above.
2. Cooking/pretreatment: add glacial acetic acid (0.2-1.5% by weight of corncob) and hold the mixture at 155-180℃ 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℃, pH 5.0-6.0 (adjusted by 1 mol/L hydrochloric acid or 1 mol/L sodium hydroxide) for 4-10 hours 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℃ for 25-40 minutes, and then filter by plate and frame filter (equipment for filtration) to remove the pigment and other impurities.
6. Ion exchange at ≤45℃:
   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 a nanofilter membrane at 15-30℃.
8. Concentration: concentrate the liquid to 40-75% at 60-80℃.
9. Adjust the saccharide content: prepare several different concentrations of liquids by adding maltodextrin as an excipient.
10. Spray drying: spray dry with inlet temperatures of 130-160°C and outlet temperatures 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 days 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 were used for verification).

## II.H. Product Description and Specifications

As shown in Table 3, about 95% of Shangdong Longlive's XOS is carbohydrate; the remaining 5% is water. The carbohydrate typically consists of approximately 95% XOS and about 5% digestible sugars, such as xylose, glucose, and arabinose. The DP of the XOS are concentrated in the range 2 to 6. Maltodextrin is used as an excipient to adjust the XOS concentrations of the three XOS preparations—70P, 35P, and 20P. Tables 3 and 4 list specifications of XOS.

Table 3. Specifications of Shangdong Longlive's XOS preparations

| Item | XOS 95P | XOS 70P | XOS 35P | XOS 20P | Analytical method |
|---|---|---|---|---|---|
| | Powder | Powder | Powder | Powder | |
| Dry substance, % | ≥95 | ≥95 | ≥95 | ≥95 | |
| Moisture, % | ≤6.0 | ≤6.0 | ≤6.0 | ≤6.0 | AOAC 934.01 |
| Ash, % | ≤0.3 | ≤0.3 | ≤0.3 | ≤0.3 | AOAC 900.02 |
| XOS content, % dry wt. basis | ≥95% | ≥ 70% | ≥ 35% | ≥ 20% | MHLW*, 2005 |
| XOS content, % as is basis | ≥90.2% | ≥66.5% | ≥33.25% | ≥19.0% | MHLW*, 2005 |
| Arsenic, ppm by wt. | ≤0.3 | ≤0.3 | ≤0.3 | ≤0.3 | |
| Lead, ppm by wt. | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 | |

*MHLW (Ministry of Health, Labor and Welfare of Japan), 2005=modified AOAC method 2001.03 (measured xylose oligomers instead of glucose oligomers)

Table 4. Microbiologial specifications

| Microorganism | Limit | Analytical method |
|---|---|---|
| Standard plate count, cfu/g | ≤1000 | FDA-BAM* Chap. 3 |
| Coliform | Negative | FDA-BAM* Chap. 4 |
| Yeast, cfu/g | ≤25 | FDA-BAM* Chap. 18 |
| Mold, cfu/g | ≤25 | FDA-BAM* Chap. 18 |
| Pathogenic bacteria (salmonella, E. coli, shigella, and staphylococci) | Negative | FDA-BAM* Chap. 4 and 5 |

*BAM=Bacteriological Analytical Manual, January, 2001.

## II.I. Suitability tests for XOS in dairy products

### II.I.1. Stability

As shown in Table 5, XOS were stable in various dairy products. The XOS content was analyzed by HPLC (MHLW, 2005) at the beginning and end of the storage period.

Table 5. Stability of XOS in dairy products

| XOS concentration tested | Item | The length of stable period, the maximum length tested | Recovery at the end of the study |
|---|---|---|---|
| 0.5-4% | Liquid milk | 4 weeks | 88.3% |
| 2% | Yogurt | 16 days | 95.5% |
| 2% | Powdered milk | 5 months | 97.6% |

**II.I.2. Sensory properties**

Mumtaz et al. (2008) studied the effects of XOS enrichment on physicochemical and sensory analysis of yogurt. The addition of XOS up to 3.5% to yogurt did not influence taste or overall acceptability.

Also, the tests done in the laboratory of Shangdong Longlive showed that XOS addition did not change sensory and physical properties of liquid milk (up to 1%), yogurt (up to 3.5%), or powdered milk (up to 4%).

**II.J. Intended technical effects**

The intended technical effect of XOS is as a source of dietary fiber.

**II.K. Current regulatory status**

Wheat bran extract composed primarily of XOS and AXOS (GRN 343) is listed as a GRAS ingredient. 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 the 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, rice bran, and wheat bran, are considered GRAS ingredients by the U.S. FDA (21 CFR, Part 184.1890, 182.8890, and 182.8892) when used in accordance with GMP. The FDA (2008) approved a health claim for psyllium fiber and reduced risk of heart disease. Xylooligosaccharides are considered dietary fiber, an essential nutrient that is low in the American diet. The IOM (2002, 2006) has recommended increased consumption of total fiber for Americans of all ages.

Xylooligosaccharides are commercially used as an ingredient for ‘Food for Specified Health Use’ (FOSHU) foods in Japan since 1991 (Vazquez et al., 2000). The 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, 286, and 334) and IMO (GRN 246), are listed as GRAS substances.

## III. INTENDED USES AND EXPOSURE ESTIMATES

### III.A. Food sources of XOS

Xylooligosaccharides are hydrolysis products of xylan, a common hemicellulose of cereal grains (corncob, rice bran, wheat bran, and psyllium), where it occurs in many different compositions and structures (Garcia et al., 2000). Hemicelluloses and cellulose are considered major dietary fiber components that are naturally present in many plant-based foods. Xylose, a complete hydrolysis product of XOS, is naturally present in small quantities in foods. Due to abundance of xylans in cereal grains, human bodies have been exposed to xylose-based nondigestible carbohydrates for a long time.

Since 1997, 32 product launches (including XOS) have been recorded by Mintel's global new products database (Mintel, 2008). Japan accounts for more than half of the new product launches since 1997.

### III.B. Intended use

For the purpose of this GRAS notice, Shangdong Longlive's XOS, manufactured in accordance with GMP as specified in 21 CFR 110, are intended to be used as ingredients at the level of 0.2-2.4 g per serving in the following food categories: milk and milk products, flavored and soy milk, milk desserts, condensed milk, powdered milk, cheese, ready-to-drink milk-based meal replacements and protein, yogurt, puddings, custards, and other milk desserts, gelatin desserts or salads, ready- to-eat cereals, cereal bars, granola bars, protein bars, power bars, and breakfast bars, processed fruits, juice drinks and punch, chewing gums, sports drinks, isotonic beverages, beers and ales, and medical foods (Table 6). Medical foods are defined as food that is formulated to be consumed or administered enterally under the supervision of a physician, and for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation. Medical foods are not included in the exposure estimates. Intended uses exclude infant formulas and foods that fall under USDA jurisdiction, such as catfish, poultry, pork, beef, and egg products. National Health and Nutrition Examination Survey food codes are listed for each food group (Table 6). Table 7 presents the amounts of various XOS ingredients to deliver 0.2, 1.3, and 2.4 g of XOS.

Table 6. List of foods under the intended use

| NHANES code | Food group |
|---|---|
| 113 | Milk, fluid, and imitations, including soy milk |
| 115 | Flavored milk and milk drinks, fluid |
| 131 | Milk desserts, frozen |
| 111 | Milk, fluid (regular, filled, buttermilk, and dry reconstituted) |
| 112 | Milk, fluid, evaporated and condensed |
| 118 | Milk, dry, and powdered mixtures with dry milk, not reconstituted |
| 14 | Cheese (cheese, cream cheese, cottage cheese, imitation cheese, cheese mixtures, cheese soups) |
| 116 | Ready-to-drink milk-based meal replacements and protein |
| 114 | Yogurt |
| 132 | Puddings, custards, and other milk desserts |
| 915 | Gelatin desserts or salads |
| 571-574,535 | Ready-to-eat cereals, cereal bars, granola bars, protein bars, and power bars |
| 5354 | Breakfast bars |
| 61,64 | Processed fruits, juice drinks, and punch |
| 918 | Chewing gum |
| 9256 | Sports drink |
| 9257 | Isotonic beverages (fluid replacement drinks) |
| 931 | Beers and ales |

Table 7. Intended use levels (g per serving) of various XOS ingredients.

| Item | XOS 95 | XOS 70 | XOS 35 | XOS 20 |
|---|---|---|---|---|
| | Powder | Powder | Powder | Powder |
| Dry substance, % | ≥95 | ≥95 | ≥95 | ≥95 |
| Moisture, % | ≤6.0 | ≤6.0 | ≤6.0 | ≤6.0 |
| XOS content, dry wt. basis | ≥ 95% | ≥ 70% | ≥ 35% | ≥ 20% |
| XOS content, As is basis | ≥90.2% | ≥66.5% | ≥33.25% | ≥19.0% |
| The amount (g) to deliver 0.2 g XOS per serving | 0.22 | 0.30 | 0.61 | 1.05 |
| The amount (g) to deliver 1.3 g XOS per serving | 1.44 | 1.95 | 3.91 | 8.10 |
| The amount (g) to deliver 2.4 g XOS per serving | 2.66 | 3.61 | 7.22 | 12.63 |

### III.C. Current consumer intake levels

Since the XOS levels in each food are not listed in the USDA food composition tables and the NHANES databases, the current exposure levels from food sources were not estimated. Polymers of XOS, xylans, and arabinoxylans are abundant in nature. However, XOS are not naturally present in significant quantities in foods.

However, humans have consumed grain fibers, which consist of at least 25% xylan (fibers with xylose backbones; Rasper, 1979). Our analysis of the National Institute of Health-American Association of Retired Persons (NIH-AARP's) longitudinal study dataset for American elderly individuals showed that mean and 90$^{th}$ percentile daily intakes of grain fibers were 5.27 g and 10.22 g, respectively, and corresponding daily intakes of xylans are estimated to be at least 1.3 g and 2.6 g, respectively.

### III.D. Estimated daily intake estimate from the intended use

To calculate an estimated exposure for XOS, we used levels of the ingredient per serving as well as the number of servings to be consumed daily. The analysis of the 2009-2010 NHANES data set revealed that approximately 89% of Americans are the users of one or more foods under the intended use. The mean and 90$^{th}$ percentile intakes, including XOS by all users (aged 2-99 years) from all GRAS proposed use categories, are 3.9 and 7.5 servings per day, respectively.

Table 8. Estimated food intakes, total daily servings of foods under the intended use

| Users of one or more foods under the intended use | | | | | | |
|---|---|---|---|---|---|---|
| Age | Gender | % users | Mean* | SE* | 90$^{th}$ Pctl* | SE* |
| 2-5 yr | All | 97.5 | 3.85 | 0.103 | 6.63 | 0.316 |
| 6-12 yr | All | 95.3 | 3.52 | 0.133 | 6.37 | 0.249 |
| 13-18 yr | All | 86.3 | 3.92 | 0.113 | 7.48 | 0.205 |
| 13-18 yr | Male | 88.9 | 4.61 | 0.158 | 8.93 | 0.399 |
| 13-18 yr | Female | 83.8 | 3.21 | 0.175 | 6.32 | 0.400 |
| 19-50 yr | All | 86.8 | 4.21 | 0.077 | 8.66 | 0.300 |
| 19-50 yr | Male | 87.0 | 5.26 | 0.159 | 10.81 | 0.507 |
| 19-50 yr | Female | 86.6 | 3.14 | 0.103 | 6.21 | 0.204 |
| 51-99 yr | All | 88.9 | 3.58 | 0.084 | 7.09 | 0.147 |
| 51-99 yr | Male | 89.6 | 4.26 | 0.137 | 8.62 | 0.477 |
| 51-99 yr | Female | 88.3 | 2.97 | 0.061 | 5.88 | 0.142 |
| 2-99 yr | All | 88.9 | 3.89 | 0.058 | 7.50 | 0.103 |

*Total daily servings of foods

Xylooligosaccharides will be added to foods in the categories listed in the previous section at an average level of 1.3 g per serving. Assuming all foods will be used under the intended use, the mean and the 90$^{th}$ percentile intakes of all users aged 2-99 years are estimated to be 5.1 g per day and 9.8 g per day, respectively (Table 8). Assuming a 60 kg BW per person, these levels may correspond to mean of 85 mg/kg BW/day, and the 90th percentile of 163 mg/kg BW/day. These exposure estimates are very likely to be overestimated since it is not possible that all the foods can contain XOS. Medical foods are not included in the exposure estimates.

These overestimated levels are far below the NOAEL values found in 2 subchronic rat studies (up to 11,510-14,950 mg XOS/kg BW/day). Human clinical trials report benefits with no adverse effects of XOS. Doses of up to 12 g per day were well-tolerated in humans. Also, the IOM recommends increased intakes of total fiber, which are defined as the sum of non-digestible carbohydrates (with DP of 3 and higher) and lignin. Recommended intakes are 25 g per day for adult women and 38 g per day for adult men (IOM, 2002, 2006). Unfortunately, Americans consume only one half of the recommended intakes, or an average of 15.9 g per day (King et al., 2012). Since XOS functions as a prebiotic fiber, its use as intended may help Americans increase their total fiber intake.

## IV. BASIS FOR GRAS DETERMINATION

### IV.A. Metabolic fate of XOS

The metabolic fate of the carbohydrate moiety of XOS fully resembles that of other prebiotic fibers and non-digestible carbohydrates, such as inulins, FOS, and GOS. These remain largely undigested in the upper gastrointestinal tract and are fermented in the colon to gases and SCFA (acetate, propionate, and butyrate), which lower the pH of the colon (Fleming and Rodriguez , 1983; Macfarlane and Macfarlane, 2011). These SCFA 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 reduced risk of colon cancer (Scheppach et al., 2001).

Gibson et al. (2004) established the following 3 criteria to meet the definition of prebiotics: 1) resistance to hydrolysis by human alimentary enzymes, and to gastrointestinal absorption; 2) fermentation by intestinal microbiota; and 3) selective stimulation of the growth and/or activity of intestinal bacteria that contribute to health and well-being. Xylooligosaccharides meet all of these criteria.

<u>Gibson's criterion 1: Resistance to gastric acidity, hydrolysis by mammalian alimentary enzymes</u>
The beta-1,4-bond linking the xylose moiety that form the xylan backbone of XOS resists enzymatic digestion in the upper gastrointestinal tract (Okazaki et al., 1990). Xylooligosaccharides are stable under pH and temperature conditions that simulate the human gastric environment (Courtin et al., 2009).

<u>Gibson's criterion 2: fermentation by intestinal microbiota</u>
A few human interventions show that XOS are fermented to SCFAs in the colon, and that they lower colonic pH. Chung et al. (2007) reported that supplementation of 3.8 g XOS/day for 3 weeks decreased fecal pH (XOS group, 6.50 vs. control, 7.39, $p<0.05$), indicating increased colonic fermentation leading to SCFA production.

<u>Gibson's Criterion 3: selective stimulation of growth and/or activity of beneficial intestinal bacteria</u>
Administration of XOS to human subjects promotes the growth of bifidobacteria (Chung et al., 2007; Fu et al., 2012; Makelainen et al., 2009). *In vitro* studies support the findings from human

studies: different *Bifidobacterium* species, when grown in pure cultures, efficiently utilize XOS (Van Laere et al.,2000; Crittenden et al., 2002).

In summary: as a prebiotic carbohydrate, XOS promotes the growth of beneficial intestinal bacteria, such as bifidobacteria. This does not raise safety concerns. In addition, any healthy diet rich in plant-derived fiber comprised of sources of fermentable fiber leads to generation, absorption, and excretion of the same metabolites ($H_2$, $CO_2$, SCFA) as those produced upon digestion of XOS. Therefore, the metabolism of XOS does not raise safety concerns and no systemic toxicity is expected following ingestion of XOS.

**IV.B. Preclinical animal safety studies of XOS**

Table 9 summarizes the toxicity studies of XOS in mice, rats, and chicks. Published studies indicate that XOS are of low toxicity to animals. The $LD_{50}$ of XOS has been reported to be over 10 g/kg BW in the rat (Park et al., 1999) and over 32 g/kg BW in mice (Gao et al., 2012). A compound which has a $LD_{50}$ value of over 5 g/kg BW in rats is classified as 'practically nontoxic', and the compound with a $LD_{50}$ value of over 15 g/kg BW as 'relatively harmless' (Altug, 2003). Xylooligosacharides, like other oligosaccharides and carbohydrates, belong to the group with the lowest toxicity rating.

In subchronic toxicity studies of rats (13 weeks), the NOAELs were found to be 11,510 mg/kg BW/day for males and 14,950 mg/kg BW/day for females; these were the highest doses tested (Gao et al., 2012).

Other animal efficacy studies measuring various endpoints reported no adverse effects of XOS (Table 10; Gobinath et al., 2010; Howard et al., 1995; Hsu et al., 2004). Dosages tested were up to 7-10% in the diet and the lengths of the studies ranged from 5 days to 6 months. In addition, various studies showed no mutagenic, teratogenic, or genotoxicity effects of XOS (Oh et al., 1999; Fu et al., 2012).

A related compound, AXOS, did not show adverse effects in animal toxicity studies. In addition, xylose, xylan, and fibers that contain a xylose backbone, have been reported to be safe (Fleming and Lee, 1983; Jiang et al., 1986; Kuroiwa et al., 2005; Marlett and Fischer, 2003).

Table 9. Oral toxicity studies of XOS in animals*

| Animals | Length of the study; route of administration | Results | Reference |
|---|---|---|---|
| Single dose studies | | | |
| 40 Rats | Single dose by gavage, observed for 14 days | $LD_{50}$≥10 g/kg BW | Park et al., 1999; Fu et al., 2012 |
| 40 Mice | Single oral dose by gavage, observed for 14 days | NOAEL ≥32 g/kg BW/day | Gao et al., 2012 |
| Repeated dose studies | | | |
| 216 Broiler chicks | 21 days by diet | 4% in the diet in chicks; the highest dose administered | Graham et al., 2004 |
| 120 SD rats | 13 weeks by gavage | NOAEL - 3,000 mg/kg BW/day; the highest dose administered | Fu et al., 2012*; Park et al., 2000* |
| 80 Wistar rats | 13 weeks by diet | NOAEL - 11,510 mg/kg BW/day for males and 14,950 mg/kg BW/day for females; the highest doses tested | Gao et al., 2012* |

*Studies are based on observations/analysis of food consumption, body weight, serum biochemistry, hematology, organ weights, and histopathology; SD=Sprague Dawley®

**IV.B.1. Details of a single dose acute toxicity test in rats** (Table 9; Fu et al., 2012; Park et al., 1999)

Fu et al. (2012) reviewed an acute toxicity study conducted by Park et al. in 1999. In the study of Park et al. (1999), the $LD_{50}$ of XOS (from corncob) was above 10 g/kg BW in 30 Sprague Dawley® rats (5 week old; 15 males and 15 females; males weighed 126-141 g; females weighed 98.6-112.7 g). In this study, a single dose of 0, 5, or 10 g XOS was administered by gavage. Body weights and clinical signs were observed for 14 days. Soft stool and diarrhea appeared right after the overdose, but these clinical signs disappeared the next day. No deaths or abnormalities in body weights and clinical signs were observed. The authors concluded that the $LD_{50}$ of XOS was above 10 g/kg BW in rats.

**IV.B.2. Details of a single dose acute toxicity test in mice** (Table 9; Gao et al., 2012)

Young adult Kunming mice (18-22 g) were assigned to either control or treatment groups (10 males and 10 females per group). After an overnight fast, animals received a single oral dose of 32 g/kg of XOS (source; Shangdong Longlive, 95% XOS powder from corncob) by gavage (day 1), and an equal number of animals served as vehicle controls. Animals were checked for clinical signs and mortality twice a day, BW was measured on days 1, 7, 10, and 14, and gross tissue changes were observed at necropsy on day 15. No overt signs of toxicity, adverse effects, or abnormal behavior were observed during the course of the study. However, watery stool was found in XOS-treated animals. Moreover, asthenia, hypoactivity, and piloerection were noticed in some animals (7/20), but these abnormalities returned to normal the next day. On day 15, no

treatment-related changes were observed in any organs of either sex at the end of the test. The authors concluded that the $LD_{50}$ of XOS was above 32 g/kg BW in mice.

**IV.B.3. Details of a 21-day toxicity study in chicks** (Table 9; Graham et al., 2004)
A total of 216 day-old chicks were fed diets containing 0, 0.04, 0.4, and 4% XOS (with 3 pen-replicates of 18 chicks per diet). At 14 days of age, 2 replicates per diet were orally challenged with *Eimeria maxima* oocytes, a small intestinal parasite. Xylooligosaccharides 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 SCFA, indicating fermentation of XOS in the colon. Xylooligosaccharides 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.

**IV.B.4. Details of 13-week toxicity studies in rats (**Table 9; Fu et al., 2012; Park et al., 2000; Gao et al., 2012).
Fu et al. (2012) reviewed a 13 week toxicity study conducted by Park et al. in 2000. In this report, groups of 60 male and 60 female Sprague Dawley® rats (4 week old; females weighing 98.6-112.7 g; males weighing 126-141 g) were administered with 0, 333, 1,000 or 3,000 mg/kg by gavage for 13 weeks. Xylooligosaccharides were prepared from corncob using hydrolysis by xylanase. Hematological values, serum biochemistry, organ weights, and histopathological findings were investigated at the end of weeks 13 and 17 (i.e., at the end of a 4-week recovery period). No deaths or toxic effects were observed during the test periods. No treatment-related abnormalities were observed. According to the results, the NOAEL of XOS was estimated to be > 3,000 mg/kg, the highest dose tested.

In a subchronic toxicity study of Gao et al. (2012), weaning Wistar rats (10 males and 10 females per group) were fed diets containing 0% (control), 0.9%, 2.9%, 8.8%, or 10% XOS (source; Shangdong Longlive, 95% XOS powder from corncob) for 13 weeks (Fu et al., 2012; Gao et al., 2012). Measurement outcomes included clinical observations, BW, food consumption, food conversion efficiency, hematology, blood biochemistry, gross necropsy, organ weight, and histopathology. No treatment-related changes were noted in behavior or appearance of the rats and no mortalities occurred. No treatment-related adverse effects were found in food consumption, food conversion efficiency, hematology, clinical biochemistry, or organ weights in either sex. Hematological and serum chemistry values, such as hemoglobin, red blood cell count, white blood cells, aminotransferase, blood urea nitrogen (BUN), creatinine, cholesterol, blood sugar, total protein, and albumin were within normal ranges and there were no significant differences among treatment and control groups. Also, there were no significant microscopic pathological changes in the liver, spleen, kidney, stomach, duodenum, testes, or ovaries in any treatment group compared with the control group. The authors concluded that the NOAELs of XOS were 11,510 mg/kg BW/day for males and 14,950 mg/kg BW/day for females, the highest dose levels tested.

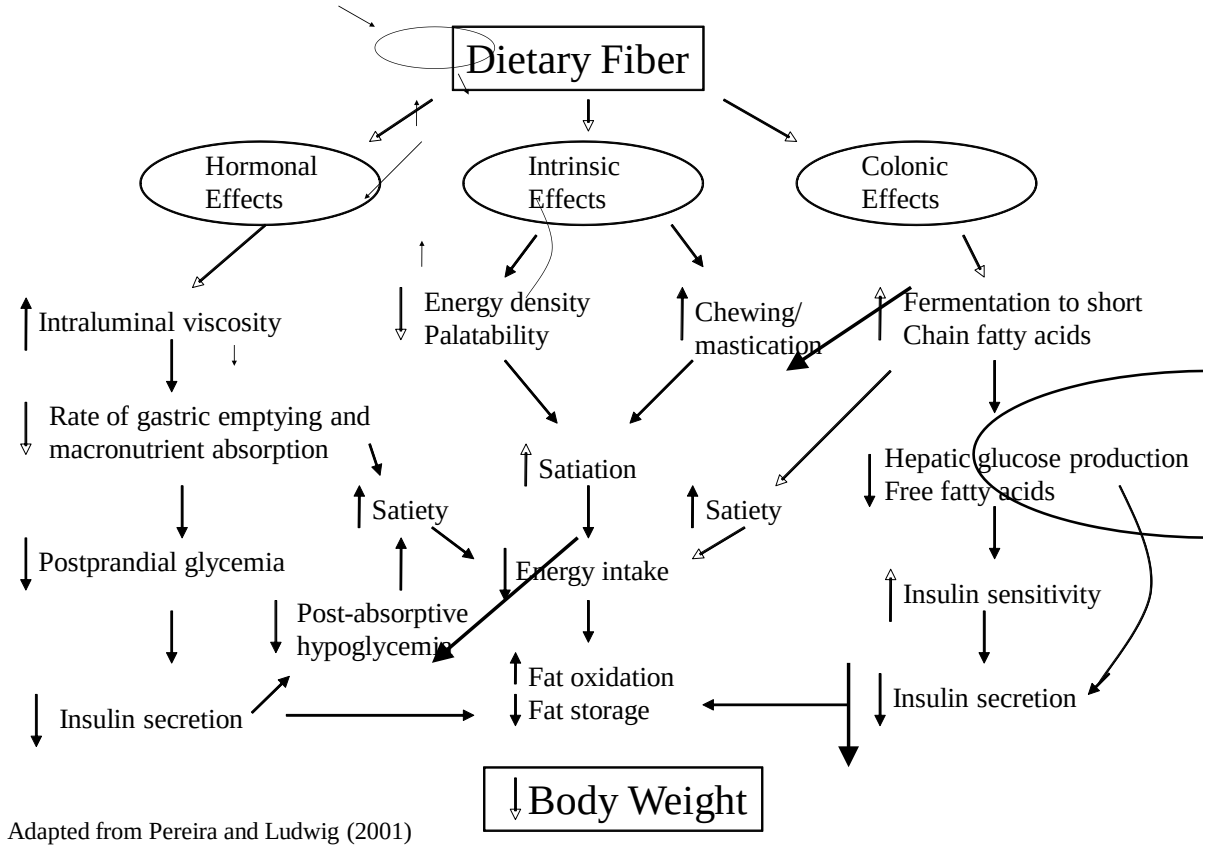
In this study, the only changes noted were health benefits of XOS, such as slight decreases (mostly without statistical significance) in feed intakes, body weights, and serum triglycerides. These are not considered to be of toxicological concern. It is well known that consumption of fiber-rich foods is associated with decreased food intakes and body weight loss. Howarth et al.

(2001) summarized 10 human randomized clinical trials (RCT) on the effects of dietary fiber on energy intake and BW. The majority of studies with controlled energy intake reported an increase in post-meal satiety and a decrease in subsequent hunger with increased dietary fiber. Ad libitum energy intake has an average effect of increasing dietary fiber across all the studies. This indicated that an additional 14 g of fiber per day resulted in a 10% decrease in energy intake and a weight loss of over 1.9 kg through approximately 3.8 months of intervention. Several mechanisms of actions have been proposed for the action of dietary fiber/non-digestible carbohydrates in weight control (Figure 3; Pereira and Ludwig, 2001): 1) Fiber displaces available calories and nutrients from the diet; dietary fiber or non-digestible carbohydrates have lower calories (0-2 kcal/g) than digestible carbohydrates (4 kcal/g), 2) fiber increases chewing, which limits intake by promoting the secretion of saliva and gastric juice, resulting in an expansion of the stomach and increased satiety; 3) fiber delays gastric emptying, 4) fiber improves insulin sensitivity, leading to weight control, and 5) fiber has hormonal effects that lead to increased satiety.

These characteristics of fiber have become the basis of a few authoritative statements on the beneficial effects of fiber on calorie intake and body weight control. The term “authoritative statement” comes from the Food and Drug Administration Modernization Act (FDAMA), legislation that was enacted in November 1997 (FDA, 1998). FDAMA permits claims based on current, published authoritative statements from "a scientific body of the United States with official responsibility for public health protection or research directly related to human nutrition . . . or the National Academy of Sciences (NAS) or any of its subdivisions." The FDA states that other federal agencies (the CDC, the NIH, and the Surgeon General within the Department of Health and Human Services [DHHS]; the Food and Nutrition Service, the Food Safety and Inspection Service, and the Agricultural Research Service within the U.S. Department of Agriculture [USDA]) also may qualify as appropriate sources for such authoritative statements (FDA, 1998).

1) The NAS (2001) stated that the satiety benefits of dietary fiber are as follows: “Diets high in fiber are associated with slower stomach emptying, which induces a short-term increase in satiety (Roberfroid, 1993). This may modulate caloric intake and the rate of nutrient absorption. In addition, the reduced caloric density of diets rich in fiber has been seen as an asset in weight maintenance.”
2) “Foods low in fat and high in naturally occurring fiber appear to induce satiety in humans at lower levels of caloric intake than do high fat, low fiber foods.” (Surgeon General’s Report, U.S. DHHS, 1988, p. 292).
3) “There are two kinds of fiber: insoluble fiber that exerts its effects primarily in the digestive system, and soluble fiber, which affects substances in the blood stream. Diets high in both kinds of fiber tend to be bulky, and since fiber itself does not contribute calories, foods high in fiber tend to contain fewer calories in the same volume of food. These characteristics of high fiber diets may help assuage hunger, and, thus, contribute to weight loss.” (NAS, Eat for Life, 1992)
4) “To reduce calories in response to the epidemic obesity crisis in the U.S., non-nutrient-dense carbohydrate sources, should be reduced. Because fiber has known health benefits, it is advisable to select foods high in dietary fiber, -- whenever possible.” (Dietary Guidelines for Americans, USDA and US DHHS, 2010)

Figure 3. Effects of dietary fiber on body weight




Adapted from Pereira and Ludwig (2001)

**IV.C. Animal efficacy studies showing no adverse effects of XOS**

As shown in Table 10, other animal efficacy studies reported no adverse effects of XOS in mice, rats, and piglets. Dosages tested were up to 7-10% in the diet and the lengths of the studies ranged from 5 days to 6 months (Campbell et al., 1997; Gobinath et al., 2010; Howard et al., 1995; Hsu et al., 2004; Imaizumi et al.,1991; Murai et al., 1994; Santos et al., 2006). Animal efficacy studies support findings from the two 13-week toxicity studies that reported the NOAEL values of 3,000 -14,950 mg/kg BW/day, the highest doses tested.

Table 10. Animal efficacy studies showing no adverse effects of XOS

| Source of XOS | Species | Dose in diet | Dura-tion | Results | Ref |
|---|---|---|---|---|---|
| Prebiotic effects | | | | | |
| Corncob | Mouse, healthy | 0.95% | 6 mo | Increased bifidobacteria, lactobacilli, bacteroides, and total anaerobes in the colon; Increased bifidobacteria in the ileum; Reduced enterobacteriaceae and sulphate-reducing bacteria in the colon | Santos et al., 2006 |
| Birch wood | Rat treated with STZ* | 7.5% | 35 d | Increased cecal SCFA (acetate from 70 to 190 umol/cecum) | Imaizumi et al.,1991 |
| Corncob | Rat | 5.4% | 14 d | Increased cecal and fecal bifidobacteria; Increased cecal and fecal total anaerobic bacteria; Increased cecal SCFA | Campbell et al., 1997 |
| Corncob | Rat, wild type treated with DMH* | 5.4% | 35 d | Increased cecal bifidobacteria Reduced cecal *E. coli* | Hsu et al., 2004 |
| Corncob, Suntory | Rat | 7.0% | 5 d | Tend to increase fecal SCFA from 3.9 to 7.0 umol/g feces (p=NS). No significant reduction of fecal pH; Fecal microflora –not measured | Murai et al., 1994 |
| Not specified | Mouse, weaning | 3% in water | 15 d | No increase in cecal bifidobacteria | Howard et al., 1995 |
| Corncob | Piglet | 2% | 4 wk | No increase in cecal bifidobacteria | Moura et al., 2007 |
| Other endpoints | | | | | |
| Corncob | Rat, STZ*-induced diabetic Wistar | 10% | 6 wk | Activity of antioxidant enzymes, fecal microflora | Gobinath et al., 2010 |

*DMH=1.2-dimethylhydrazine; STZ=streptozotocin (diabetes inducer); d=days; wk=weeks

Anti-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 Sprague Dawley® rats treated with 1,2-dimethylhydrazine (DMH, 15 mg/kg BW/week for 2 weeks). 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). Xylooligosaccharides 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). No adverse effects of XOS were reported.

### IV.D. Mutagenicity and genotoxicity tests of XOS

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

Table11. Summary of mutagenicity and genotoxicity studies showing no adverse effects of XOS

| Test | Dosage of XOS | Reference |
|---|---|---|
| Ames test: *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537 with or without S-9 | 5,000 ug/plate | Oh et al., 1999 |
| Ames test: *Salmonella typhimurium* TA97, TA98, TA100, and TA102 with or without S-9 | 5,000 ug/plate | Fu et al., 2012 |
| Polychromatophilic normocyte micronucleus test of bone marrow (BM) in mice, 5 d | 10.0 g/kg BW | Fu et al., 2012 |
| Sperm abnormality test in mice, tested 35 days after XOS treatment | 10.0 g/kg BW | Fu et al., 2012 |
| Testis chromosome aberration test in mice, sacrificed animals 13 days after the first time of gastric perfusion | 10.0 g/kg BW | Fu et al., 2012 |

### IV.E. Information pertaining to the safety of bacterial enzymes

Xylanase from *Streptomyces olivaceoviridis* is of food grade, and has been widely used in the manufacture of XOS in the food industry (Ai et al., 2005; Jiang et al., 2005, 2006). Shangdong Longlive's unpublished data support the fact that xylanase from *Streptomyces olivaceoviridis* is safe in rats.

In addition, a xylanase preparation from another microorganism (expressed in a self-cloned strain of *Bacillus subtilis*) has been proven safe for food processing. This has been demonstrated in acute and subchronic oral toxicity studies in rats, mutagenicity, and chromosomal aberrations assays (Harbak and Thygesen, 2002). Xylanase derived from *Fusarium venenatum*, which carries a gene encoding xylanase from *Thermomyces lanuginosus*, has a GRAS notice status (GRN 54).

### IV.F. Toxicity studies of related compounds

#### IV.F. 1. Arabinoxylooligosaccharides (AXOS)

Table 12 indicates that AXOS showed consistent prebiotic effects in rats, mice, and chickens (Courtin et al., 2008; Eeckhaut et al., 2008; Van Craeyveld et al. 2008; Yamada et al.1993) and that AXOS improved iron bioavailability (Kobayashi et al., 2011). As described in GRN 343, dosages tested were 0.25-4.3% AXOS in the diet, and the lengths of the studies were 14-37 days. No adverse effects of AXOS were reported in any of these studies (Broekaert et al., 2011).

Table 12. Animal studies showing no adverse effects of AXOS.

| Source of AXOS | Species | Dose | Duration | Results | Ref |
|---|---|---|---|---|---|
| Wheat bran | Mice, healthy | 2.15-4.3% in diet | 21 d | Increased cecal SCFA (acetate) Increased bifldobacteria and reduced cecal *E. coli*, staphylococcus, and streptococcus | Yamada et al.,1993 |
| Wheat bran | Rat, healthy | 2.15-4.3% in diet | 21 d | Increased cecal SCFA (acetate) Increased bifidobacteria and reduced cecal *E. coli* | Yamada et al.,1993 |
| Wheat bran | Rat, healthy | 3.6-4.0% in diet | 14 d | Increased cecal bifidobacteria; Increased colonic SCFA (acetate); Increased cecal branched SCFA (marker of protein fermentation) | Van Craeyveld et al., 2008 |
| Wheat bran | Chicken, healthy | 0.25 % in diet | 21 d | Increased cecal Bifidobacteria | Courtin et al., 2008 |
| Wheat bran | Chicken, healthy | 0.2-0.4% in diet | 37 d | Reduced *Salmonella enteritidis* colonization of cecum after oral infection. Reduced translocation to the spleen | Eeckhaut et al., 2008 |
| Hardwood pulp | Rat | 2% in iron-deficient diet | 1-4 wk | Improved iron bioavailability | Kobayashi et al., 2011 |

d=days; wk=weeks

**IV.F. 2. D-Xylose**

D-xylose (a monosaccharide), a hydrolysis product of XOS and xylan, is found in the juice of fruits, such as apples and peaches (Vázquez et al., 2000). 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. In Japan, D-xylose is widely used as a natural food additive in the fishery products and baking industries (MHLW, 1996a, 1996b).

<u>Two year chronic toxicity/carcinogenicity study of xylose</u>

Kuroiwa et al. (2005) conducted a 2-year 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%. No treatment related abnormalities were observed. Thus, it was concluded that D-xylose was not carcinogenic to F344 rats. The NOAEL, determined from a chronic toxicity study, was found to be over 5% in the diet, the highest dose tested.

**IV.F. 3. Xylan, a xylose based polysaccharide**

Several beneficial effects associated with xylans have been reported by many authors. No adverse effects were reported from any animal studies on xylan (Fleming and Lee, 1983; Jiang et al., 1986; Oliveira et al., 2009).

Fleming and Lee (1983) compared the effects of select purified fibers to those derived from cereals or legume seeds in a 9 week 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. No adverse effects of xylan were noted.

Jiang et al. (1986) determined the effects of xylan on the apparent absorption and tissue concentration of zinc and copper in a 26 week study with male weanling Sprague Dawley rats. The control group was fed a fiber-free diet and six other groups were fed a diet containing 3, 6, or 12% xylan. After 26 weeks of consuming the test diets, there were no significant differences between groups in regard to weight gain, feed intake, or feed efficiency. Xylan did not exert a significant influence on apparent absorption and tissue concentrations of the minerals.

## IV.G. HUMAN CLINICAL STUDIES

### IV.G. 1. Safety of XOS in humans

Table 13 shows that human clinical studies have reported benefits 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 12 g/person/day for periods of up to 8 weeks (Chung et al., 2007; Fu et al., 2012; Kobayashi et al., 1991; Oku et al., 2002; Sheu et al., 2008; Tateyama et al., 2005; Xiao et al., 2012). Kobayashi et al. (1991; reviewed in Fu et al., 2012) reported that daily doses of up to 10 g XOS were well tolerated in humans. In this study, administration of 2-5 g of XOS decreased both fecal hardness and the frequency of diarrhea in 10 men. However, the daily dose of 10 g XOS increased the frequency of diarrhea (10 g XOS, 18% vs. control, 8%) on the first day, although diarrhea was controlled on day 5 (6.7%).

Xiao et al. (2012) showed similar results. In this study, 60 healthy volunteers were divided into 5 groups: maltodextrin control, no treatment control, and 3 XOS groups (XOS source: Shangdong Longive's 95P). All 3 XOS groups had a daily dose of 3 g XOS for the first 2 weeks with an increased dosage to 5 g at 3 weeks. At the 4th week, the 3 XOS groups received a daily XOS dose of 8, 10, or 12 g per day. The diarrhea ratios (approximately 8%) were comparable between the control and high dose XOS groups (8-12 g per day). A small dose of XOS (1-3 g per day) decreased the diarrhea ratio to 2.40%. The authors concluded that daily doses of up to 12 g per day were well tolerated. In a review article, Oku et al. (2002) reported the maximum permissible dose that does not cause gastrointestinal symptoms as 0.12 g/kg BW in men. This figure is based on Suntory company's internal data on XOS from corn cob. Overall, the data suggest that daily doses of up to 12 g XOS were well tolerated (Fu et al., 2012; Xiao et al., 2012).

Xylooligosaccharides, like other fibers, are fermented by intestinal microflora to produce SCFAs that improve colonic health (Broekaert et al., 2011; Fu et al., 2012). Fermentation in the large intestine can result in a minor side effect, such as the formation of gases (including hydrogen, carbon dioxide, and methane), that is often associated with flatulence and intestinal discomfort. Since the human body is able to adapt to higher intakes of dietary fiber, intestinal discomfort can be a transient symptom (IOM, 2002).

Table 13. Human clinical studies showing no adverse effects of XOS

| Source of XOS | Dose, g/day* | Duration | Subject | Results | Ref |
|---|---|---|---|---|---|
| Studies on gastrointestinal tolerance | | | | | |
| Corncob | 0.12 g/kg BW | single dose | Men | Maximum permissible dose not causing GI symptoms in men=0.12 g/kg BW or 7.2 g/day | Oku et al., 2002 |
| Corncob | 2, 5, or 10 | single dose | 10 men | Daily dose of 2-10 g XOS were well tolerated | Kobayashi et al., 1991; Fu et al., 2012 |
| Corncob | 4.2 | 4 wk | 30 constipated pregnant women | Improved stool consistency | Tateyama et al., 2005 |
| Corncob | Wk 1, 3 g; wk 3 & 4, 5g; wk 4-8,10, or 12 g | 4 wk | 60 men and women | Daily doses of 10-12 g XOS were well tolerated | Xiao et al., 2012 |
| Studies on other effects | | | | | |
| Corncob | 4 | 8 wk | Type 2 diabetics | Improved blood sugar and lipid profiles | Sheu et al., 2008 |

Wk=week; d=day; *g/day unless noted otherwise

### IV.G. 2. Safety of AXOS in humans

Human clinical studies have not shown any adverse effects of AXOS when up to 15 g per day was administered for 22 days (Table 14; Broekaert et al., 2011; Cloetens et al., 2009, 2010; Grasten et al., 2003; Grootaert et al., 2007).

Table 14. Human clinical studies showing no adverse effects of AXOS

| Source | Dose, g/day | Duration | Subject | Results | Reference |
|---|---|---|---|---|---|
| Prebiotic effects | | | | | |
| Wheat bran | 2.2-5.0 g in water | 2 wk | 11 healthy subjects; mean age 24 year | Increased fecal bifidobacteria; Decreased ammonium biomarker secretion via urine and feces. | Cloetens et al., 2010 |
| Wheat bran | 15 g | 19-22 d | 14 healthy subjects | Increased fecal SCFA (acetate, propionate, and lactate) | Grasten et al., 2003 |

Wk=week; d=day

### IV.G. 3. Safety of xylan and xylose-containing fibers in humans

**Intervention studies**

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/day) 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. No adverse effects of these fibers, including xylan, were reported in humans.

In addition, other fiber ingredients based on xylose backbones, such as wheat bran, rice bran, and psyllium (Marlett and Fischer, 2003; Theander et al., 1995), are known for their fecal bulking effects and improvement of gastrointestinal regularity (Cho et al., 2001; Marteau et al., 1994) These benefits are accomplished without having negative effects on mineral bioavailability (Marlett and Fischer, 2003).

**Epidemiological studies**

Throughout human history, people have consumed grain fibers that consist of approximately 25% xylans (fibers with xylose backbones; Rasper, 1979). In some countries—where intake levels of total fiber, cereal fiber, and xylans are much higher than those of Americans—no adverse effects from cereal fiber or xylans have been reported. For example, the Finnish population has much higher fiber intakes than do Americans; their mean daily intake levels of total and grain fibers are estimated to be 26 g and 18 g, respectively (Montonen et al., 2003), and the 87.5$^{th}$ and 99$^{th}$ percentile intakes of grain fibers are 29 and 51 g, respectively. Finnish's major source of grain fiber intake is rye bread; its fiber consists of 27% xylans. Daily xylan intakes are estimated to be 7.8 and 13.8 g at the 87.5$^{th}$ and 99$^{th}$ percentiles, respectively. In this Finnish population, grain fiber intake was inversely associated with reduced risk of type 2 diabetes. No adverse effects were observed. The data indicated that high intakes of grain fiber and xylans (as high as 13.8 g per day) are not only safe but also offer health benefits. In other studies with various populations, including Americans, consumption of grain fiber and resulting xylans have been associated with positive health benefits (risk reductions of cardiovascular disease, type 2 diabetes, and obesity) without any adverse effects (Flight and Clifton, 2006; Koh-Banerjee et al., 2004; Schulze et al., 2007). Xylans from grain fibers are subjected to colonic fermentation to produce SCFA, $CO_2$, and $H_2$, the same compounds produced from the metabolism of XOS. Thus, no systematic risks related to XOS consumption are expected.

## IV.H. Allergy

No case reports of allergy to XOS were identified in the literature.

## IV.I. Potential adverse effects of XOS

<u>Gastrointestinal distress</u>

The only side effect that may be associated with XOS consumption (when eaten in excessively large quantities) is gastrointestinal discomfort, which is a transient symptom. The IOM (2002) states that "while occasional adverse gastrointestinal symptoms are observed when consuming one of the above isolated or synthetic fibers, serious chronic adverse effects have not been observed. Furthermore, due to bulking effects of fibers, excess consumption is likely to be self-limiting. Therefore, an UL (Tolerable Upper Intake Level) was not set for these individual fibers." The UL is defined as a safe upper level for consumption over a lifetime or the highest level of a daily nutrient's intake that will be most likely to present no risk of adverse health effects in almost all individuals in the general population. In addition, the American Dietetic

Association's position paper (Slavin, 2008) found no serious health hazard related to fiber intakes in the American population.

Consistent with the IOM, the FDA had not established the maximum daily dosage levels of dietary fiber when it issued the final rule of health claims related to fiber-containing grain products or psyllium (FDA, 1993a, 1993b, 2008).

Mineral balance

Prebiotic fibers (such as XOS, AXOS, GOS, and FOS) are well-known for improving bioavailability of minerals, including calcium and iron (Kobayashi et al., 2011; Macfarlane and Macfarlane, 2011). Also, xylan did not exert a significant influence on apparent absorption and tissue concentrations of the minerals (Jiang, 1996). Thus, mineral balance will not be disturbed, even at high doses of XOS. A few human interventions show that XOS is fermented to SCFAs (acetate, propionate, and butyrate) in the colon, and that it lowers colonic pH (Chung et al., 2007). The SCFAs are associated with improved mineral absorption (Scheppach et al., 2001).

## V. SUMMARY OF THE BASIS FOR THE NOTIFIER'S DETERMINATION THAT XOS IS GRAS

This independent panel of recognized experts, qualified by their scientific training and relevant national and international experience, is well equipped to evaluate the safety of dietary fiber and prebiotic ingredients. Shangdong Longlive asked this panel to determine the GRAS status of XOS intended for use as a multipurpose food ingredient, particularly as a source of dietary fiber. A comprehensive search of the scientific literature was conducted to identify relevant safety studies for this assessment.

Xylooligosaccharides are non-digestible oligosaccharides with DP units of mostly 2-6. A typical analysis shows that Shangdong Longlive's XOS consists of 35% xylobiose and >60% total fiber (DP units of 3-6).

Published studies indicate that XOS are of low toxicity to animals and humans. Xylooligosaccharides and similar molecules (GOS and FOS and other beta-linked carbohydrates) are not digested by human pancreatic or brush border enzymes, and the compounds are not expected to be absorbed intact. Xylooligosaccharides reach the large intestine, where they are fermented by the colonic microflora to SCFAs which promote colon health. Any healthy diet rich in plant-derived fermentable fiber leads to generation, absorption, and excretion of the same metabolites ($H_2$, $CO_2$, SCFA) as those produced upon digestion of XOS. Therefore, the metabolism of XOS does not raise safety concerns and no systemic toxicity is expected to follow ingestion of XOS. In addition, the history of human exposure to xylose-based fibers (as part of fibers from grain sources) indicates that they do not pose a safety concern.

Based on a critical evaluation of the pertinent data and information summarized herein, the Expert Panel members have individually and collectively determined by scientific procedures that the addition of XOS as an ingredient in foods and beverages at a level of 0.2-2.4 grams per serving, meeting appropriate specifications and used according to GMP, is GRAS. Intended uses include milk and milk products, flavored and soy milk, milk desserts, evaporated or condensed milk, powdered milk, cheese, ready-to-drink milk-based meal replacements, and protein; yogurt, puddings, custards, and other milk desserts; gelatin desserts; ready-to-eat cereals, cereal bars, granola bars, protein bars, power bars, and breakfast bars; processed fruits, juice drinks and punch; chewing gums; sports drinks, isotonic beverages, beers and ales; and medical foods. Medical foods are defined as the food that is formulated to be consumed or administered enterally under the supervision of a physician, and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation.

Xylooligosaccharides will be added to foods in the categories listed in the previous section at an average level of 1.3 g per serving. Assuming all the foods will be used for their intended use, the mean and 90$^{th}$ percentile intakes of all users aged 2-99 years are estimated to be 5.1 g per day and 9.8 g per day, respectively (Table 8). Assuming a 60 kg body weight per person, these levels may correspond to mean values of 85 mg/kg BW/day and at the 90th percentile of 163 mg/kg

BW/day. These exposure estimates are very likely to be overestimated since it is not possible that all the foods can contain XOS. Medical foods are not included in the exposure estimates.

These overestimated levels are far below the NOAEL values found from subchronic rat studies (11,510-14,950 mg XOS/kg BW/day). Human intervention trials report health benefits with no adverse effects of XOS. Doses of up to 12 g/day were well tolerated by humans. Also, epidemiological studies indicate that consumption of xylans at up to 13.8 g is not associated with any adverse effects in humans. The IOM recommends increased intakes of total fiber, which is defined as the sum of non-digestible carbohydrates (with a DP of 3 and higher) and lignin. Recommended intakes of total fiber are 25 g per day for adult women and 38 g per day for adult men. Unfortunately, Americans consume only one half of the recommended intakes with an average of 15.9 g per day. Intended use of XOS may help Americans to improve their total fiber intake status.

The information/data provided by Shangdong 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. Thus, the proposed use of XOS is safe.

## VI. CONCLUSIONS

Documentation qualifying a substance as GRAS has been compiled. It 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 Shangdong 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 prepared according to appropriate specifications and GMP.

Therefore, the proposed use of XOS is safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm). Among consensus experts, it is also *Generally Recognized as Safe* (GRAS). In the Expert Panel's opinion and that of other qualified and competent scientists, a review of the same publicly available toxicological and safety information would produce the same conclusion.

## VII. 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.

## SIGNATURES

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

Signature: _____ Date: _____

Kelly Swanson, Ph.D., Associate Professor, University of Illinois, Urbana, IL 61801

Signature: _____ Date: _____

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

Signature: _____ Date: _____

Joanne Slavin, Ph.D., R.D., Professor, University of Minnesota, St Paul, MN

Signature: _____ Date: _____

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## SIGNATURES

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

Signature: (b) (6) Date: 1/20/2013

Kelly Swanson, Ph.D., Associate Professor, University of Illinois, Urbana, IL 61801

Signature: (b) (6) Date: 1/17/13

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

Signature: (b) (6) Date: January 17, 2013

Joanne Slavin, Ph.D., R.D., Professor, University of Minnesota, St Paul, MN

Signature: (b) (6) Date: 1-21-13

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## APPENDIX A: ANALYTICAL RESULTS OF XOS 95P

| | Batch A | Batch B | Batch C |
|---|---|---|---|
| Dry substance, % | 97.36 | 97.75 | 97.86 |
| Moisture, % | 2.64 | 1.91 | 1.96 |
| Ash, % | 0.08 | 0.06 | 0.06 |
| XOS content, %, dry wt. basis | 95.65 | 95.66 | 95.44 |
| Protein, % | <0.1 | <0.1 | <0.1 |
| Fat, % | <0.1 | <0.1 | <0.1 |
| pH | 4.13 | 4.07 | 3.96 |
| Arsenic, ppm | < 0.5 | < 0.5 | < 0.5 |
| Lead, ppm | < 0.3 | < 0.3 | < 0.3 |
| Total plate count, cfu/g | 35 | 15 | <10 |
| Coliform | Negative | Negative | Negative |
| Mold, cfu/g | <10 | <10 | <10 |
| Yeast, cfu/g | <10 | <10 | <10 |
| Pathogens | Negative | Negative | Negative |

## **APPENDIX B: DAILY FOOD SERVINGS BY FOOD GROUPS,** NHANES 2009-2010

NHANES 2009-2010 data were used in all analyses. Day 1 dietary data were used with exclusions for unreliable data and pregnant or lactating females. SAS 9.2 and SUDAAN 11 were used for all calculations. NHANES survey weights, strata, and primary sampling units (PSUs) were used in all calculations.

Food Groups:
The food groups are based on groupings of food codes as designated in the Food Codes column.

Consumers (%):
Percent of a food in the food group within the denoted population.

Servings:
A serving in grams is obtained from FDA Reference Amount Customarily Consumed (RACC). The estimated 90$^{th}$ percentile is shown when greater than zero.

Exposure Source Food Groups

| Food Category | Codes | Short Description | Description |
|---|---|---|---|
| 0 | all | all | all foods |
| 1 | all xos | all_xos | all xos foods |
| 2 | 931 | beer | beers and ales |
| 3 | 9256,9257 | energy_drink | rtd energy, sport, and isotonic beverages |
| 4 | 61,64 | fruit | processed fruits, juice drinks, and punch, cann |
| 5 | 41440100 | meal_repl_nomil | rtd non-milk based meal replacements and protein |
| 6 | 113,115 | soy_bev | rtd soy beverages, chocolate milk, flavored mil |
| 7 | 131 | milk_dessert | milk desserts, frozen |
| 8 | 111,112,118 | milk | liquid milk, milk powder |
| 9 | 14 | cheese | cheese |
| 10 | 116 | meal_repl_milk | rtd milk-based meal replacements and protein |
| 11 | 114,132,915 | yogurt_pudding | yogurt, pudding, and jello |
| 12 | 571-574,535 | rtec | rte cereals, cereal bars, granola bars, protein |
| 13 | 918 | gum | chewing gum |

All Foods

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 100 | 12.7158 | 0.2574 | 18.4635 | 0.6136 |
| 6-12 yr | All | 100 | 15.4112 | 0.2328 | 22.2284 | 0.3397 |
| 13-18 yr | All | 100 | 19.8341 | 0.6086 | 31.5755 | 0.8925 |
| 13-18 yr | Male | 100 | 22.6262 | 0.7256 | 34.8634 | 1.3387 |
| 13-18 yr | Female | 100 | 17.1799 | 0.6594 | 28.1500 | 1.7161 |
| 19-50 yr | All | 100 | 25.6403 | 0.3049 | 40.0369 | 0.6051 |
| 19-50 yr | Male | 100 | 29.1530 | 0.5000 | 45.4059 | 0.9081 |
| 19-50 yr | Female | 100 | 22.0366 | 0.2440 | 33.9877 | 0.7690 |
| 51-99 yr | All | 100 | 23.7955 | 0.3566 | 34.9499 | 1.0118 |
| 51-99 yr | Male | 100 | 26.5581 | 0.6030 | 39.3782 | 1.4324 |
| 51-99 yr | Female | 100 | 21.3491 | 0.3156 | 30.7379 | 0.5811 |
| 2-99 yr | All | 100 | 22.8177 | 0.2231 | 36.1751 | 0.3536 |

All XOS Foods

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 97.5509 | 3.8495 | 0.1030 | 6.6261 | 0.3164 |
| 6-12 yr | All | 95.2655 | 3.5171 | 0.1328 | 6.3702 | 0.2490 |
| 13-18 yr | All | 86.2789 | 3.9163 | 0.1128 | 7.4807 | 0.2054 |
| 13-18 yr | Male | 88.8649 | 4.6133 | 0.1576 | 8.9290 | 0.3990 |
| 13-18 yr | Female | 83.8205 | 3.2139 | 0.1750 | 6.3152 | 0.4001 |
| 19-50 yr | All | 86.7766 | 4.2144 | 0.0770 | 8.6616 | 0.2999 |
| 19-50 yr | Male | 86.9897 | 5.2586 | 0.1592 | 10.8108 | 0.5067 |
| 19-50 yr | Female | 86.5581 | 3.1379 | 0.1031 | 6.2111 | 0.2043 |
| 51-99 yr | All | 88.9277 | 3.5791 | 0.0839 | 7.0903 | 0.1472 |
| 51-99 yr | Male | 89.6047 | 4.2611 | 0.1369 | 8.6241 | 0.4768 |
| 51-99 yr | Female | 88.3282 | 2.9665 | 0.0610 | 5.8797 | 0.1417 |
| 2-99 yr | All | 88.8579 | 3.8940 | 0.0581 | 7.5000 | 0.1026 |

Beer and Ales

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 13-18 yr | Male | 0.7488 | 5.8819 | 1.5360 | 9.2343 | |
| 13-18 yr | Female | 2.2549 | 2.4420 | 0.8147 | 5.1353 | |
| 19-50 yr | All | 17.4684 | 4.9187 | 0.2406 | 10.8865 | 0.6828 |
| 19-50 yr | Male | 25.9304 | 5.5223 | 0.2837 | 11.3459 | 0.8575 |
| 19-50 yr | Female | 8.7875 | 3.0915 | 0.2336 | 5.7212 | 0.4411 |
| 51-99 yr | All | 12.2611 | 3.7474 | 0.1798 | 6.5521 | 0.4489 |
| 51-99 yr | Male | 21.3737 | 3.9926 | 0.2360 | 7.3612 | 0.5102 |
| 51-99 yr | Female | 4.1912 | 2.6400 | 0.1999 | 4.2992 | 0.2631 |
| 2-99 yr | All | 11.7465 | 4.5182 | 0.1931 | 8.9715 | 0.6985 |

rtd energy, sport, and isotonic beverages

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 3.6580 | 1.3525 | 0.3035 | 2.5385 | |
| 6-12 yr | All | 6.6486 | 1.5551 | 0.1025 | 2.5414 | 0.3195 |
| 13-18 yr | All | 10.0358 | 2.8237 | 0.2027 | 4.8562 | 0.5510 |
| 13-18 yr | Male | 13.4908 | 3.3147 | 0.2200 | 5.5994 | 0.5016 |
| 13-18 yr | Female | 6.7513 | 1.8910 | 0.2250 | 2.8155 | 0.2738 |
| 19-50 yr | All | 5.0614 | 2.6428 | 0.1066 | 4.1853 | 0.1309 |
| 19-50 yr | Male | 7.0100 | 2.9619 | 0.1208 | 4.2889 | 0.1995 |
| 19-50 yr | Female | 3.0624 | 1.8934 | 0.2943 | 3.5621 | 0.4280 |
| 51-99 yr | All | 1.3114 | 2.2381 | 0.3413 | 4.1463 | |
| 51-99 yr | Male | 1.9359 | 2.6737 | 0.4131 | | |
| 51-99 yr | Female | 0.7584 | 1.2532 | 0.2173 | | |
| 2-99 yr | All | 4.3956 | 2.4166 | 0.0756 | 4.1723 | 0.1615 |

processed fruits, juice drinks, and punch, canned

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 57.6870 | 1.1547 | 0.0708 | 2.2743 | 0.2337 |
| 6-12 yr | All | 35.7251 | 1.1406 | 0.0340 | 2.0030 | 0.0797 |
| 13-18 yr | All | 25.0888 | 1.8343 | 0.1328 | 3.5670 | 0.4357 |
| 13-18 yr | Male | 27.2382 | 1.9841 | 0.1789 | 4.5177 | 0.7364 |
| 13-18 yr | Female | 23.0455 | 1.6660 | 0.1850 | 2.5795 | 0.3120 |
| 19-50 yr | All | 25.8704 | 1.9545 | 0.0909 | 3.6208 | 0.2051 |
| 19-50 yr | Male | 25.4430 | 2.2189 | 0.1900 | 3.9593 | 0.2303 |
| 19-50 yr | Female | 26.3089 | 1.6921 | 0.0957 | 3.1032 | 0.3209 |
| 51-99 yr | All | 34.2483 | 1.3453 | 0.0465 | 2.5752 | 0.1550 |
| 51-99 yr | Male | 32.0795 | 1.4665 | 0.0622 | 2.6323 | 0.1694 |
| 51-99 yr | Female | 36.1690 | 1.2501 | 0.0656 | 2.3129 | 0.2538 |
| 2-99 yr | All | 31.2001 | 1.5610 | 0.0391 | 3.0464 | 0.0851 |

rtd soy beverages, chocolate milk, flavored milk

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 24.1240 | 1.4031 | 0.1037 | 2.8350 | 0.2338 |
| 6-12 yr | All | 35.7810 | 1.2930 | 0.0573 | 2.0625 | 0.1310 |
| 13-18 yr | All | 16.5341 | 1.5199 | 0.0583 | 2.5012 | 0.0816 |
| 13-18 yr | Male | 19.7721 | 1.5344 | 0.0720 | 2.4999 | 0.0932 |
| 13-18 yr | Female | 13.4559 | 1.4996 | 0.0997 | 2.1875 | 0.4174 |
| 19-50 yr | All | 7.1407 | 1.4634 | 0.1086 | 2.5625 | 0.3746 |
| 19-50 yr | Male | 6.9797 | 1.6539 | 0.1853 | 3.6250 | 0.4234 |
| 19-50 yr | Female | 7.3058 | 1.2766 | 0.0754 | 2.1021 | 0.1967 |
| 51-99 yr | All | 8.4268 | 1.5322 | 0.1353 | 3.5176 | 0.3178 |
| 51-99 yr | Male | 6.6759 | 1.7022 | 0.1705 | 3.5292 | 0.4378 |
| 51-99 yr | Female | 9.9774 | 1.4314 | 0.1389 | 2.7303 | 0.3249 |
| 2-99 yr | All | 12.1531 | 1.4284 | 0.0455 | 2.5408 | 0.1642 |

milk desserts, frozen

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 18.2652 | 1.1634 | 0.0967 | 2.2127 | 0.2871 |
| 6-12 yr | All | 18.7039 | 1.6823 | 0.1109 | 2.9085 | 0.3349 |
| 13-18 yr | All | 16.4821 | 2.0724 | 0.1640 | 4.0312 | 0.6331 |
| 13-18 yr | Male | 18.1307 | 2.3334 | 0.3412 | 4.7434 | 0.9949 |
| 13-18 yr | Female | 14.9148 | 1.7707 | 0.1883 | 2.9989 | 0.4953 |
| 19-50 yr | All | 12.6443 | 1.8509 | 0.0707 | 3.5951 | 0.2652 |
| 19-50 yr | Male | 12.0779 | 2.0178 | 0.1307 | 4.0240 | 0.2501 |
| 19-50 yr | Female | 13.2253 | 1.6945 | 0.0818 | 2.8208 | 0.2540 |
| 51-99 yr | All | 19.4365 | 2.0671 | 0.1131 | 3.8310 | 0.2787 |
| 51-99 yr | Male | 18.8970 | 2.5075 | 0.2022 | 4.8715 | 0.2509 |
| 51-99 yr | Female | 19.9143 | 1.6970 | 0.0745 | 2.9634 | 0.0955 |
| 2-99 yr | All | 16.0168 | 1.8886 | 0.0487 | 3.6064 | 0.2101 |

liquid milk, milk powder

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 77.7154 | 1.6538 | 0.0814 | 3.4872 | 0.2086 |
| 6-12 yr | All | 62.2666 | 1.3790 | 0.0657 | 2.7497 | 0.1564 |
| 13-18 yr | All | 48.7807 | 1.6037 | 0.1212 | 3.1033 | 0.3607 |
| 13-18 yr | Male | 57.2316 | 1.8438 | 0.1539 | 3.6250 | 0.2535 |
| 13-18 yr | Female | 40.7470 | 1.2830 | 0.1229 | 2.7816 | 0.3311 |
| 19-50 yr | All | 41.9586 | 1.3633 | 0.0563 | 2.6756 | 0.1537 |
| 19-50 yr | Male | 41.4537 | 1.5068 | 0.1032 | 3.0667 | 0.2440 |
| 19-50 yr | Female | 42.4765 | 1.2197 | 0.0433 | 2.4503 | 0.1252 |
| 51-99 yr | All | 48.3995 | 1.2163 | 0.0718 | 2.6250 | 0.1781 |
| 51-99 yr | Male | 50.8731 | 1.3730 | 0.1040 | 2.9706 | 0.3390 |
| 51-99 yr | Female | 46.2090 | 1.0636 | 0.0644 | 2.1875 | 0.1597 |
| 2-99 yr | All | 48.5973 | 1.3666 | 0.0322 | 2.7500 | 0.0981 |

cheese

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 47.0202 | 1.1295 | 0.0783 | 2.5841 | 0.2650 |
| 6-12 yr | All | 38.3541 | 1.1842 | 0.0750 | 2.1043 | 0.1556 |
| 13-18 yr | All | 37.8777 | 1.6143 | 0.1283 | 3.6640 | 0.6243 |
| 13-18 yr | Male | 34.5365 | 1.7032 | 0.1127 | 3.6471 | 0.3693 |
| 13-18 yr | Female | 41.0539 | 1.5431 | 0.1962 | 3.4042 | 0.8406 |
| 19-50 yr | All | 43.1263 | 1.6259 | 0.0434 | 3.3342 | 0.1694 |
| 19-50 yr | Male | 43.5835 | 1.8757 | 0.0697 | 3.7771 | 0.1300 |
| 19-50 yr | Female | 42.6573 | 1.3641 | 0.0522 | 2.5854 | 0.1004 |
| 51-99 yr | All | 40.3895 | 1.4441 | 0.0910 | 2.8083 | 0.2067 |
| 51-99 yr | Male | 38.6008 | 1.6189 | 0.1656 | 2.8317 | 0.3942 |
| 51-99 yr | Female | 41.9736 | 1.3017 | 0.0504 | 2.5889 | 0.1188 |
| 2-99 yr | All | 41.5662 | 1.4975 | 0.0457 | 2.9018 | 0.1246 |

rtd milk-based meal replacements and protein

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 0.0000 | | | | |
| 6-12 yr | All | 0.6535 | 1.2766 | 0.1981 | | |
| 13-18 yr | All | 0.0732 | 2.6611 | 0.3168 | | |
| 13-18 yr | Male | 0.0509 | 2.0000 | 0.0000 | | |
| 13-18 yr | Female | 0.0944 | 3.0000 | 0.0000 | | |
| 19-50 yr | All | 0.5538 | 2.1465 | 0.2183 | 2.9432 | |
| 19-50 yr | Male | 0.6963 | 2.1896 | 0.4260 | | |
| 19-50 yr | Female | 0.4076 | 2.0710 | 0.4665 | 2.7253 | |
| 51-99 yr | All | 0.5734 | 1.9218 | 0.3424 | 2.3374 | |
| 51-99 yr | Male | 0.8243 | 2.1813 | 0.2781 | | |
| 51-99 yr | Female | 0.3512 | 1.3823 | 0.1580 | 1.4880 | |
| 2-99 yr | All | 0.4971 | 1.9586 | 0.1842 | 2.8680 | 0.2575 |

yogurt, pudding, and jello

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 21.3236 | 0.7396 | 0.0455 | 1.1925 | 0.1445 |
| 6-12 yr | All | 13.6231 | 0.8554 | 0.0381 | 1.5000 | 0.1282 |
| 13-18 yr | All | 6.0250 | 0.9939 | 0.0900 | 1.5115 | 0.2860 |
| 13-18 yr | Male | 6.5307 | 1.1269 | 0.1151 | 1.4027 | |
| 13-18 yr | Female | 5.5442 | 0.8449 | 0.1264 | 2.1209 | 0.3271 |
| 19-50 yr | All | 9.4961 | 1.0093 | 0.0428 | 1.7500 | 0.2513 |
| 19-50 yr | Male | 7.1481 | 1.1864 | 0.1102 | 2.6250 | 0.2973 |
| 19-50 yr | Female | 11.9048 | 0.9002 | 0.0489 | 1.4849 | 0.1432 |
| 51-99 yr | All | 14.0627 | 0.9587 | 0.0512 | 1.6757 | 0.1181 |
| 51-99 yr | Male | 11.1470 | 1.1359 | 0.0837 | 1.9130 | 0.2751 |
| 51-99 yr | Female | 16.6447 | 0.8536 | 0.0381 | 1.5101 | 0.1382 |
| 2-99 yr | All | 11.7034 | 0.9440 | 0.0245 | 1.7356 | 0.0585 |

rte cereals, cereal bars, granola bars, protein bars, and power bars

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 53.5062 | 0.9211 | 0.0327 | 1.5721 | 0.1010 |
| 6-12 yr | All | 43.3656 | 1.2721 | 0.0426 | 2.1471 | 0.1826 |
| 13-18 yr | All | 31.5476 | 1.5560 | 0.0746 | 2.6247 | 0.2117 |
| 13-18 yr | Male | 32.7045 | 1.7761 | 0.1452 | 2.9848 | |
| 13-18 yr | Female | 30.4478 | 1.3312 | 0.0764 | 2.1468 | 0.1270 |
| 19-50 yr | All | 24.7227 | 1.5494 | 0.0512 | 2.5386 | 0.1215 |
| 19-50 yr | Male | 22.9058 | 1.7733 | 0.0697 | 3.1454 | 0.1974 |
| 19-50 yr | Female | 26.5865 | 1.3516 | 0.0656 | 2.2371 | 0.1023 |
| 51-99 yr | All | 29.9254 | 1.2145 | 0.0358 | 1.8836 | 0.1106 |
| 51-99 yr | Male | 27.5029 | 1.3642 | 0.0691 | 2.2264 | 0.2136 |
| 51-99 yr | Female | 32.0707 | 1.1009 | 0.0245 | 1.7251 | 0.0530 |
| 2-99 yr | All | 30.4147 | 1.3452 | 0.0296 | 2.2467 | 0.0663 |

chewing gum

| Age | Gender | Consumers (%) | Servings | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SE | P90 | SE |
| 2-5 yr | All | 1.9163 | 1.1368 | 0.1348 | 1.5806 | |
| 6-12 yr | All | 4.3365 | 1.6867 | 0.2893 | 2.8000 | 0.9759 |
| 13-18 yr | All | 3.4815 | 1.3328 | 0.1188 | 2.2931 | 0.5371 |
| 13-18 yr | Male | 3.3760 | 1.2603 | 0.1584 | 1.7033 | |
| 13-18 yr | Female | 3.5817 | 1.3978 | 0.1630 | 2.5622 | |
| 19-50 yr | All | 2.7078 | 2.0671 | 0.3926 | 3.9317 | 0.8340 |
| 19-50 yr | Male | 2.3464 | 2.6762 | 0.8105 | 5.0505 | |
| 19-50 yr | Female | 3.0785 | 1.5909 | 0.1466 | 2.5467 | 0.4142 |
| 51-99 yr | All | 1.0109 | 2.0922 | 0.2880 | 3.5094 | |
| 51-99 yr | Male | 0.4901 | 2.4302 | 0.7961 | | |
| 51-99 yr | Female | 1.4721 | 1.9926 | 0.2790 | | |
| 2-99 yr | All | 2.3607 | 1.8652 | 0.2024 | 3.3577 | 0.6226 |

**APPENDIX C: INDIVIDUAL EXPERTS' OPINION ON THE SAFETY OF XOS**
November 4, 2012

GRAS opinion for Xylooligosaccharides

Joanne Slavin, PhD, RD, Professor, University of Minnesota

Xylooligosaccharides (XOS) are hydrolysis products of xylans. Xylans are hemicelluloses (dietary fiber) that are concentrated in cereal grains. Usual intakes of dietary fiber in the United States are less than half the recommended levels, so there is a need to add additional fiber to the diet.

Xylooligosaccharides, the hydrolysis product of xylans, function both as fiber and as prebiotics. XOS are not digested in the upper digestive tract, but are fermented in the large intestine. This fermentation process is considered beneficial, as it lowers gut pH, produces short chain fatty acids, and promotes bifidobacteria and lactobacillus in the gut, both considered healthy microbiota.

The potential adverse effect of XOS, like with all oligosaccharides, is gastrointestinal discomfort. There is no UL for fiber as the IOM Committee considered this option and concluded that vegetarians consume more than 75 grams of fiber per day without ill effect. With fiber intakes as low as they are in the US, we have a need to increase them rather than discourage fiber intake. Human studies find that 10 – 12 grams per day of XOS are well tolerated in healthy human subjects. The fermentation of XOS in the gut increases short chain fatty acids and lowers gut pH, both considered beneficial to digestive health.

Animal data and human clinical studies support that XOS is safe in quantities that may be consumed by the 90$^{th}$ percentile intake of highest projected users.

It is my professional opinion that XOS should be granted GRAS status. Isolated fibers that can be added to popular foods and beverages will help bridge the gap in fiber intakes that exist in the U.S.

**Statement about the GRAS status of xylooligosaccharides (XOS) manufactured by Shangdong Longlive Biotechnology, Ltd.**

**George C. Fahey, Jr.**
**Professor Emeritus of Animal Sciences at the University of Illinois at Urbana-Champaign**
**Kraft Foods Endowed Professor Emeritus of Nutritional Sciences at the University of Illinois at Urbana-Champaign**

My professional opinion is that XOS manufactured by Shangdong Longlive Biotechnology, Ltd. is GRAS. I hold this opinion for the following reasons:

1. XOS are derived from a natural product commonly used in animal nutrition (i.e., corncobs). Corncobs are an excellent source of the dietary fiber constituent, the hemicelluloses.
2. The manufacturing process for preparation of XOS is well-defined, and the chemical, microbiological, and sensory properties of the end-product make it quite suitable as a value-added food ingredient.
3. A very similar fiber byproduct, XOS, is composed mostly of XOS and arabinoXOS and already is listed as a GRAS ingredient.
4. Animal safety studies indicate no adverse effects with XOS.
5. Human clinical studies show no adverse effects of XOS.
6. Estimated daily intake levels are much below those levels that might result in a problem for the human. Any problem would be manifested in the form of gastrointestinal upset as would occur upon overconsumption of most dietary fibers.

These 6 items lead me to the conclusion that this particular XOS ingredient is GRAS.

**GRAS notification for Shangdong Longlive Biotechnology, Ltd.'s Xylooligosaccharides**
**Expert opinion: Kelly Swanson, PhD**

This opinion statement is in support of the GRAS notification for a xylooligosaccharide (XOS) product produced by Shangdong Longlive Biotechnology, Ltd. Based on the published literature, I believe that XOS are safe for consumption at the dose suggested in the notification (0.3 to 2.4 g/serving). A brief justification for this is provided below.

Americans only consume approximately half of the recommended level of dietary fiber. Products such as XOS can boost daily fiber intake and provide benefits as they pertain to gastrointestinal health. As with other fermentable fibers, the microbial fermentation of XOS in the large bowel produces short-chain fatty acids, which supply energy to colonocytes, maintain an acidic pH that limits pathogen growth, and provides a substrate for commensal bacteria. XOS is also known to promote laxation, reduce fecal putrefactive compounds, reduce blood lipids, and are a recognized source of 'prebiotics' or food components conferring a health benefit on the host by modulating specific gut microbiota.

The XOS are an enzymatic hydrolysis product of corncobs and are quite similar to other GRAS products, including XOS (GRN 343) that is composed of XOS, arabinoxylooligosaccharides (AXOS), xylose, and xylitol. Xylitol and xylose are listed as GRAS and included on the "Everything" Added to Food in the United States (EAFUS) list. A xylanase preparation, the enzyme used to product XOS, is also GRAS (GRN 54). Such products have been used in the US and Asia (Japan) for many years.

XOS are not mutagenic or genotoxic, even at very high doses. Using NHANES data, the high-end (90$^{th}$ percentile) consumer would consume 9.8 g XOS/day (or 163 mg/kg BW/day). The literature has demonstrated that this dose is much lower than the No-observed-adverse-effect-level (NOAEL) from a study in rats, and that XOS is well tolerated by humans at doses of up to 12 g/day.

To conclude, given the published research on the topic, I believe that the XOS product produced by Shangdong Longlive Biotechnology, Ltd. should be considered GRAS at the suggested dose (0.2 to 2.4g/serving).

Sincerely,

(b) (6)

Kelly Swanson
Associate Professor
Department of Animal Sciences
University of Illinois, Urbana, IL 61801

Dr. Susan Cho

The $LD_{50}$ value of XOS is reported to be > 10 g/kg BW in rats and >32 g/kg BW in mice. The data indicate that XOS belongs to the group with the lowest toxicity rating. Subchronic toxicity studies that administer XOS via diet have been unremarkable; NOAEL of 11,510 -14,950 mg/kg BW/day in rats. In addition, various studies showed no mutagenic, teratogenic, or genotoxicity effects of XOS. Human clinical trials reported that intakes of XOS up to 12 g per day were well tolerated without adverse events. Also, xylose, a complete hydrolysis product of XOS as well as xylans (a polymer of xylose or XOS), did not reveal any safety concerns. In the Finnish population, whose grain fiber consumption levels are almost 2 times higher than Americans, daily xylan intakes are estimated to be 13.8 g at the 99$^{th}$ percentile. Also, grain fiber intake and resulting xylan consumption are inversely associated with reduced risk of type 2 diabetes without having any adverse effects. The data indicate that high intakes of grain fiber and xylans (as high as 13.8 g per day) are not only safe, but also offer health benefits. Xylans and XOS are metabolized in a similar way to produce SCFA, $H_{2,}$ and $CO_2$ in the colon. This does not raise safety concerns.

To calculate an estimated daily intake (EDI; estimated exposure) for XOS, we considered intended use levels of the ingredient per serving as well as number of servings to be consumed daily. The mean and 90$^{th}$ percentile intakes including XOS by all users (aged 2-99 years) from all GRAS-proposed use categories are 3.9 and 7.5 servings per day, respectively. Xylooligosaccharides will be added to foods in the categories listed in the previous section at an average level of 1.4 g per serving. Assuming all the foods will be used under the intended use, mean and the 90$^{th}$ percentile intakes of all users aged 2-99 years are estimated to be 5.1 and 9.8 g per day, respectively. Assuming a 60 kg body weight per person, these levels may correspond to a mean of 85 mg/kg BW/day and the 90th percentile intake of 163 mg/kg BW/day. These exposure projections are very likely to be overestimated since it is not possible that all the foods can contain XOS. Medical foods are not included in the exposure estimates. These overestimated levels are far below the NOAEL values found in subchronic rat studies (11,510-14,950 mg XOS/kg BW/day).

The only side effects that may be associated with XOS consumption, when eaten in excessively large quantities, might be gastrointestinal discomfort, which is a transient symptom. The IOM (IOM, 2002) states that “while occasional adverse gastrointestinal symptoms are observed when consuming one of the above isolated or synthetic fibers, serious chronic adverse effects have not been observed. Furthermore, due to bulking effects of fibers, excess consumption is likely to be self-limiting. Therefore, an UL (Tolerable Upper Intake Level) was not set for these individual fibers.” The UL is defined as a safe upper level for consumption over a lifetime or the highest level of a daily nutrient intake that will most likely present no risk of adverse health effects in almost all individuals in the general population. Also, the American Dietetic Association’s position paper (Marlett et al., 2002) has found no serious health hazard related to fiber intakes in the American population. Consistent with the IOM, the FDA did not establish the maximum daily dosage levels of dietary fiber when it issued the final rule of health claims related to fiber containing grain products.

Thus, proposed use of XOS is considered safe.

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SUBMISSION END