AIBMR Life Sciences, Inc.

September 27, 2018

Susan Carlson, PhD
Division Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Department of Health and Human Services
5001 Campus Drive
College Park, MD 20740

Dear Dr. Carlson:

In accordance with regulation 21 CFR Part 170 Subpart E (Generally Recognized as Safe (GRAS) Notice), on behalf of Prenexus Health, Inc. (the notifier), the undersigned, Timothy S. Murbach, submits, for FDA review, the enclosed notice that xylooligosaccharides, derived from sugarcane, are GRAS for use in foods.

Should you have any questions or concerns regarding this notice, please contact me at 253-286-2888 or tim@aibmr.com

Sincerely,

Timothy S. Murbach, ND, DABT (agent of the notifier)
Senior Scientific & Regulatory Consultant
AIBMR Life Sciences, Inc. ("AIBMR")

Notice to US Food and Drug Administration of the Conclusion that the Intended Use of Prenexus Xylooligosaccharides (XOS), Derived from Sugarcane (XOS95®) is Generally Recognized as Safe

Submitted by the Notifier:

Prenexus Health, Inc.
1343 N Colorado Street
Gilbert, AZ 85233

Prepared by the Agent of the Notifier:

AIBMR Life Sciences, Inc
2800 E. Madison, Suite 202
Seattle WA 98112

September 27, 2018

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Part 1: Signed Statements and Certification

1.1 Submission of GRAS Notice

Prenexus Health, Inc. (the notifier) is submitting a new GRAS notice in accordance with Title 21 of the U.S. Code of Federal Regulations (CFR), Chapter I, Subchapter B, Part 170, Subpart E, regarding the conclusion that xylooligosaccharides, derived from sugarcane, are Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act.

1.2 Name and Address of the Notifier and Agent of the Notifier

Notifier

Timothy R. Brummels
Chief Executive Officer/President
Prenexus Health, Inc.
1343 N Colorado Street
Gilbert, AZ 85233
Tel: (402) 452-6795
tbrummels@prenexushealth.com

Agent of the Notifier

Timothy S. Murbach, ND, DABT
Senior Scientific & Regulatory Consultant
AIBMR Life Sciences, Inc.
2800 E. Madison, Suite 202
Seattle, WA 98112
Tel: (253) 286-2888
tim@aibmr.com

1.3 Name of the Substance

Xylooligosaccharides (XOS) derived from sugarcane (note, in this document the phrase "Prenexus XOS derived from sugarcane" will be used when referring specifically to the notified substance).

Tradename: XOS95®

1.4 Intended Conditions of Use

Prenexus XOS derived from sugarcane is intended to be used as a texturizer and food ingredient in a variety of food categories and addition concentrations ranging from 0.31 (cooked cereals) to 100 (sugar substitutes) g/100 g as shown in Table 3 in Subpart 3.1 below.

Prenexus XOS derived from sugarcane is not intended for use in foods where standards of identity would preclude such use, infant formula, or any products that would require additional regulatory review by USDA.

1.5 Statutory Basis for GRAS Conclusion

The conclusion of GRAS status of Prenexus XOS derived from sugarcane for its intended conditions of use, stated in Part 1.4 of this notice, has been made based on scientific procedures.

1.6 Not Subject to Premarket approval

We have concluded that Prenexus XOS derived from sugarcane is GRAS for its intended conditions of use, stated in Part 1.4 of this notice, and, therefore, such use of Prenexus XOS derived from sugarcane is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.7 Data and Information Availability Statement

The data and information that serve as the basis for this GRAS conclusion will be available for review and copying during customary business hours at the office of Timothy R. Brummels, Chief Executive Officer/President, Prenexus Health, Inc., 1343 N Colorado Street, Gilbert, AZ 85233, telephone: (402) 452-6795, email: tbrummels@prenexushealth.com or will be sent to FDA upon request.

1.8 Exemption from Disclosure under the Freedom of Information Act

None of the data and information in Parts 2 through 7 of this GRAS notice are considered exempt from disclosure under the Freedom of Information Act (FOIA) as trade secret or commercial or financial information that is privileged or confidential.

1.9 Certification of Completion

We hereby certify that, to the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of Prenexus xylooligosaccharides derived from sugarcane.

[redacted] 09/27/2018

Timothy R. Brummels Date
Chief Executive Officer/President
Notifier

Part 2: Identity, Manufacture, Specifications, and Physical or Technical Effect

2.1 Identification

2.1.1 XOS

XOS (syn: 1,4-β-D-xylooligosaccharides) are a hydrolysis product of the xylan fiber fraction of plant cell walls that is abundant in many commonly consumed foods.[1-3] In fact, xylans are the most abundant of the hemicelluloses, which in turn are the second most abundant naturally occurring polysaccharides.[2, 4] XOS are comprised of a mixture of water soluble non-digestible polymers of D-xylopyranosyl (xylose) residues linked by β-(1→4) glycosidic bonds.[2, 3, 5] The polymers vary in length with degrees of polymerization (DP) typically ranging from 2 to 20 xylose residues. XOS substituents are representative of the xylan source from which the XOS are derived. The monomeric unit of the XOS backbone, xylose, is a five-carbon sugar (i.e., a pentose) with a molecular formula of $C_5H_{10}O_5$ and molecular weight of 150.13 g/mol. The structural formula of XOS is shown in Figure 1 below; the molecular formula is $C_5{}^nH_8{}^n{}_{+2}O_4{}^n{}_{+1}$, where n = DP; the molecular weight varies with the DP.

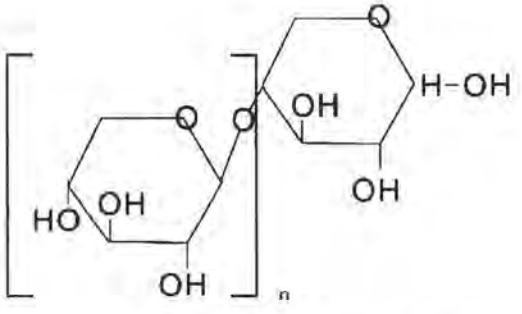

Figure 1. Structural Formula of XOS

2.1.2 Prenexus XOS derived from sugarcane

Prenexus XOS derived from sugarcane from Prenexus Health, Inc. (hereinafter, Prenexus) is an off white to tan powder with a characteristic odor, slightly sweet taste, and average DP range of 3–12 and is derived from USDA high-fiber hybrid sugarcane (*Saccharum* spp.) xylan. Prenexus XOS derived from sugarcane is concentrated to provide a XOS content of no less than 75%, and the remaining solid fraction is comprised of other oligosaccharides (i.e., depending on natural variation of the source material may include arabinoxylo-, arabino-, galacto-, mannan-, and

glucooligosaccharides), mono- and disaccharides, (including xylose (<1%) and primarily glucose, fructose, and sucrose), and small amounts ash, polyphenolic compounds, and organic acids. Moisture content makes up the remaining fraction; thus, characterization of the ingredient is approximately 100% (see Tables 1 and 2; Specifications and Batch analyses, respectively) with a typical XOS content of 81% (note, with respect to the mono- and disaccharide content, the specifications and batch analyses for 'sugars' pertains to "non-oligosaccharide sugars," as typical practice for food applications includes small amounts of disaccharides (e.g., xylobiose; DP 2 oligosaccharides) in the XOS and other oligosaccharide fractions[1]).

2.1.3 Source Material

Sugarcane is a member of the Poaceae (alt., Gramineae) family (common name, grass family), which also contains common edible grains, such as wheat, barley, rye, corn, oat, rice, and sorghum. Sugarcane is a common source of sucrose (sugar) and aconitic acid (which are GRAS for direct food use pursuant to 21 CFR Parts 184.1854 and 184.1007, respectively) in the diet, and sugarcane fiber is considered to be among the "mixed plant wall fibers" that FDA considers as included in "a general category of isolated non-digestible carbohydrates" that the Agency intends to propose for addition to its list of non-digestible carbohydrates that are dietary fiber for purposes of nutrient declarations pursuant to 21 CFR § 101.9(c)(6)(i) and for which the Agency is currently exercising enforcement discretion for such declaration during the rule making process.[6]

Sugarcane is composed of stem (a.k.a., stalk) and straw (i.e., leaves and tops).[7] Air-dry "millable" sugarcane stem, which represents approximately 75% of the whole plant, is comprised of approximately 11–16% fiber, 12-16% sugars, 2-3% non-carbohydrate solids, and 63–71% water (note, although the authors state the composition represents dried stalk, the water content reported suggests green stalk composition). Meal (produced for pig feed) derived from grinding cleaned and dried stalks contains 26.0% crude fiber and 66.0% total sugars with hemicellulose representing 45% of the plant cell walls.[8] In a more recent analysis, de Carvalho reported that sugarcane bagasse (i.e., the remaining fibrous stem material following milling to obtain cane juice for sugar production[7]) is comprised of 82.7% carbohydrate and lignin, 15.5% extractives, and 2.3% ash on a dry basis, and sugarcane straw is comprised of 79.9, 12.2, and 7.9% carbohydrate and lignin, extractives, and ash on a dry basis, respectively.[9] The major sugar in both bagasse and straw was glucose (36.0 and 36.3%, respectively) followed by xylose (21.4 and 22.8%, respectively; note, xylose is present in sugarcane as xylan, and xylan content

can be estimated based on xylose content). Together, glucose and xylose accounted for approximately 89% of total sugar content of both bagasse and straw.

Early, mostly qualitative, investigations estimated the fiber component of sugarcane is comprised of approximately 55% cellulose, 20% xylan (from which XOS is isolated), 4% arabinan, and 15% lignin.[10] Sugarcane bagasse was reported to contain approximately 40% cellulose, 24% hemicellulose, and 25% lignin on a dry basis.[4] Canilha et al., 2012 reported similar values from studies on Brazilian sugarcane bagasse (38.4–45.5% cellulose, 22.7–27.0% hemicellulose, and 19.1–32.4% lignin), and Brazilian sugarcane straw contained 33.3–36.1% cellulose, 18.4–28.9% hemicellulose, and 25.8–40.7% lignin.[7] In her 2015 thesis, de Carvalho reported the need to correct for the high silica content of sugarcane and determined the hemicellulose content of sugarcane bagasse and straw as 28.7 and 29.8%, respectively, while the lignin contents were 18.0 and 13.9%, respectively.[9] In addition to cellulose, xylan, and lignin, based on sugar composition, other minor polysaccharides likely present in sugarcane are arabinan, arabinogalactan, and glucans.

In general, xylans are heteropolysaccharides with a homopolymeric β-1,4-linked xylose backbone and have a DP ranging from 70–200.[4] Arabinose, galactose, glucose, mannose, and acetic, *p*-coumaric, ferulic, and glucuronic (or its 4-*O*-methyl ester) acids are common constituents in addition to xylose, which is the most abundant constituent, typically comprising 50% or more of the compound. The xylose backbone may be substituted with *O*-acetyl, α-L-arabinofuranosyl, α-1,2-linked glucuronic acid, or 4-*O*-methylglucuronic acid groups; however, both substituted (arabinoxylan, glucuronoxylan, and glucuronoarabinoxylan) and unsubstituted (linear homoxylan) xylans have been isolated from plants, and there is a wide degree of variability in terms of composition and frequency (or absence) of branches.

The structures of sugarcane bagasse and straw xylans have been studied, and acetylated glucuronoarabinoxylan (GAX), comprised of a linear (1→4)-linked β-D-xylopyranosyl backbone with *O*-linked α-L-arabinofuranose (ARA*f*), 4-*O*-methyl D-glucuronosyl (4-*O*-MeGl*p*A), and acetyl group partial substitutions, is the major hemicellulose present.[11] Acetylated GAX structures of the bagasse and straw were similar with slightly lower glycosyl substitution in bagasse (10 xylose:0.5 ARA*f* and 10 xylose:0.1 4-*O*-MeGl*p*A) versus straw (10 xylose:0.8 ARA*f* and 10 xylose:0.1 4-*O*-MeGl*p*A) and a slighty higher degree of acetylation (0.33 and 0.10, respectively). ARA*f* substitutions occurred at the C3 position of the xylose backbone residues and 4-*O*-MeGl*p*A substitutions occurred at the C2 position while acetyl group substitutions occurred at the C2 and/or C3 positions. Structural formulae of sugarcane bagasse and straw xylans are shown in Figure 2.

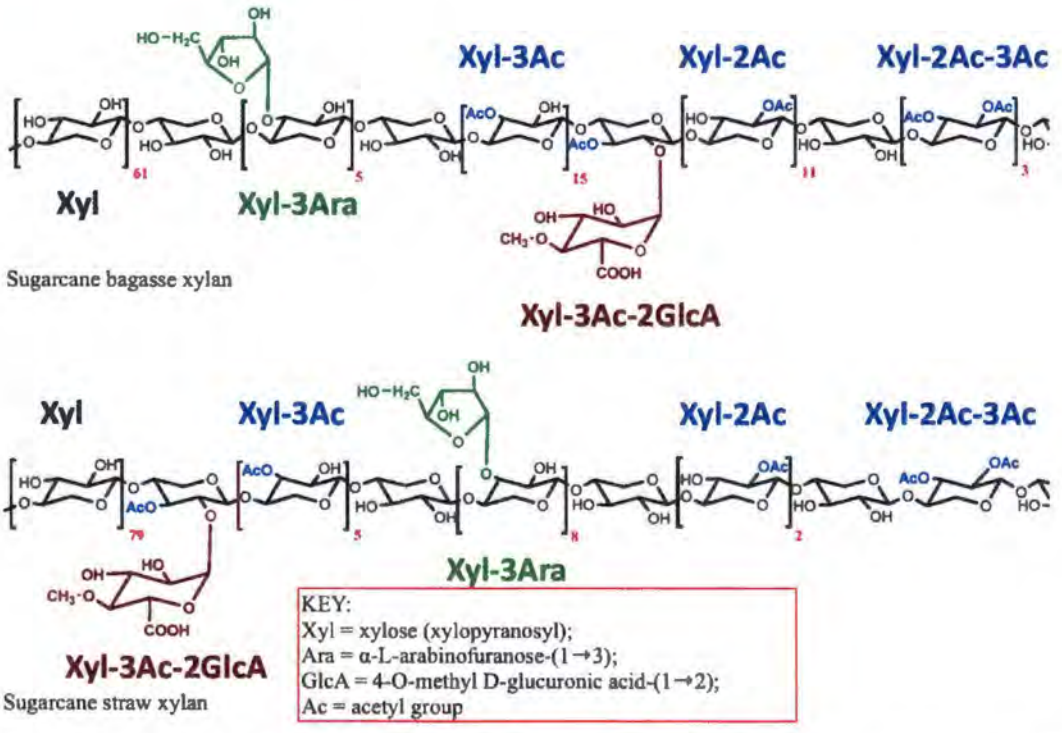

Figure 2. Structural Formulas of Sugarcane Xylan (adapted with permission from Carvalho et. al., 2017)

2.2 Manufacturing

2.2.1 Manufacturing Overview

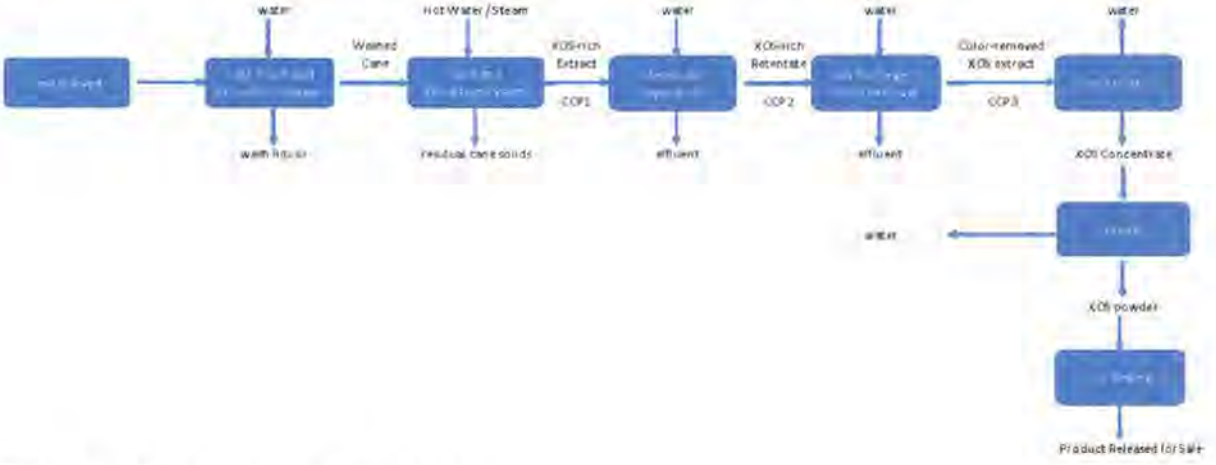

Figure 3. Manufacturing Flowchart

Harvest and Transport of Sugarcane: Eight high fiber hybrid sugarcane varieties originally sourced from USDA as greenhouse grown plantlets are planted, grown, and harvested under organic certification and shipped to the manufacturing facility by Prenexus; thus, lineage and identity control of the USDA-developed varieties remains under control of Prenexus from cultivation through manufacturing.

Cane Shredding: Raw sugarcane is mechanically shredded. Shredding may occur at the manufacturing facility or prior to shipping. Intact or shredded cane may be, optionally, frozen and thawed prior to or after shipping before further processing.

Crush and Extraction System: The shredded sugarcane is washed with water to remove dirt, fines, and some sucrose. Liquid is removed using a screw press to recover fiber solids. The wash and press cycle is repeated multiple times in separate sets of tanks.

Cook and Extraction System: In a reaction vessel, hot water is circulated through the washed fiber under pressure in order to extract and separate soluble XOS, sugars, and polyphenols from the lignin and fiber components of the cane resulting in an XOS-rich extract that is separated from the insoluble fiber using a screw/filter press.

Filtration/Purification and Decolorization: The XOS-rich extract is further processed through multiple filtration steps in order to remove residual fines, sugars,

soluble lignin-derived compounds (e.g., phenolics), organic acids, and other trace impurities. An ion exchange (or equivalent non-solvent method) step removes additional polyphenols and residual pigment compounds.

Concentration and Drying: The resulting purified and decolorized extract is concentrated via reverse osmosis and dried resulting in a finished product that is an off white to tan powder comprised of >87% oligosaccharides and small quantities of sugars, acetic acid, and polyphenolic compounds (see Table 2—Specifications).

Quality Control Testing and Release: Samples of permeate and retentate streams from each separation step, the liquid extract from the ion exchange step, and the final dry product are assayed for compliance with interim (CCP 1–3) and final product specifications. Final product meeting specifications is released for packaging.

Packaging and Storage: The finished product is packaged in mylar, HDPE, or LDPE bags of suitable thickness to act as a moisture barrier and stored in an air tight 25 kg fiber or poly drums, to protect from light and moisture, in a dry environment at room temperature.

2.2.2 Good Manufacturing Practice

Prenexus XOS derived from sugarcane is produced according to ISO 22000:2005; 9001:2008 requirements under strict adherence to current GMP standards set to comply with Title 21 of the U.S. CFR part 110 and the company's HACCP plan.

2.2.3 Raw Materials

Raw materials used in the production of Prenexus XOS derived from sugarcane are of appropriate food grade. The sugarcane used as the starting raw material is USDA certified organic high fiber non-GMO *Saccharum* spp. hybrids grown in the U.S. Prenexus XOS derived from sugarcane is non-GMO, and no material of animal origin is used in its production.

2.3 Specifications

The specifications for the food-grade product Prenexus XOS derived from sugarcane, along with the specification methods, are listed in Table 1 below.

Table 1. Prenexus XOS derived from sugarcane Specifications

Test Parameters	Specification	Method
Physical Characteristics		
Appearance	Free flowing powder	Visual
Color	Off white to light tan	Visual, ICUMSA
Chemical Tests		
Total solids (dry)	>93% (w/w)	Internal (HMA)
Total oligosaccharides	>87% (w/w)	NREL/TP-510-42618 (HPLC)
XOS	>75% (w/w)	NREL/TP-510-42618 (HPLC)
Sugars*	<12% (w/w)	NREL/TP-510-42618 (HPLC)
Other (polyphenols, organic acids)	<3.0% (w/w)	NREL/TP-510-42618 (HPLC)
Moisture	<7%	Internal (HMA)
Heavy Metals		
Arsenic	<0.2 ppm	AOAC 2011:19 & 993.14
Cadmium	<0.2 ppm	AOAC 2011:19 & 993.14
Lead	<0.2 ppm	AOAC 2011:19 & 993.14
Mercury	<0.2 ppm	AOAC 2011:19 & 993.14
Microbiological Tests		
Aerobic Plate Count	<3,000 cfu/g	USP C2021.7
Total Yeast	<1,000 cfu/g	AOAC and USP M2021:7
Total Mold	<1,000 cfu/g	AOAC and USP M2021:7
Total coliforms	<10 cfu/g	AOAC 989.10 & 986.33
Salmonella spp.	Absent/10 g	USP S2022:6
Pseudomonas spp.	Absent/10 g	USP U2022:6
Staphylococcus spp.	Absent/10 g	USP A2022:7
Listeria spp.	Absent/10 g	AOAC RI 030502

Abbreviations: cfu, colony forming units; HPLC, high-performance liquid chromatography; HMA, halogen moisture analyzer; ICUMSA, International Commission for Uniform Methods of Sugar Analysis; NREL, National Renewable Energy Laboratory; USP, United States Pharmacopeia; w/w, weight/weight.

*Non-oligosaccharide component sugars (e.g., glucose, fructose, xylose, sucrose)

2.3.1 Batch Analysis

Production conformity and consistency of Prenexus XOS derived from sugarcane are tested in production lots. Batch analyses of three non-consecutive lots are shown below and are reasonably consistent and met the product specifications (see Table 2).

Table 2. Prenexus XOS derived from sugarcane Batch Analyses

Test Parameters	Specification	Lot No./Date of Manufacture		
		180713 07/13/2018	160803 08/03/2016	160711 07/11/2016
Physical Characteristics				
Appearance	Free flowing powder	conforms	conforms	conforms
Color	Off white to light tan	conforms	conforms	conforms
Chemical Tests				
Total solids (dry)	>93% (w/w)	96%	98%	98%
Total oligosaccharides	>87% (w/w)	91%	93%	89%
XOS	>75% (w/w)	84%	80%	81%
Sugars*	<12% (w/w)	6.3%	5.6%	9.5%
Other (polyphenols & organic acids)	<3.0% (w/w)	1.6%	1.3%	1.9%
Moisture	<7%	4%	2%	2%
Heavy Metals				
Arsenic	<0.2 ppm	<0.01 ppm	0.045 ppm	0.051 ppm
Cadmium	<0.2 ppm	<0.005	<0.005	<0.005
Lead	<0.2 ppm	0.0318	0.0247	0.047
Mercury	<0.2 ppm	<0.005	<0.005	<0.005
Microbiological Tests				
Aerobic Plate Count	<3,000 cfu/g	100 cfu/g	200 cfu/g	1100 cfu/g
Total Yeast	<1,000 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Total Mold	<1,000 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Total coliforms	<10 cfu/g	conforms	conforms	conforms
Salmonella spp.	Absent/10 g	conforms	conforms	conforms
Pseudomonas spp.	Absent/10 g	conforms	conforms	conforms
Staphylococcus spp.	Absent/10 g	conforms	conforms	conforms
Listeria spp.	Absent/10 g	conforms	conforms	conforms

Abbreviations: cfu, colony forming units; w/w, weight/weight.
*Non-oligosaccharide component sugars (e.g., glucose, fructose, xylose, sucrose)

2.3.2 Residual Solvent Analysis

Water is the only solvent used in the manufacture of Prenexus XOS derived from sugarcane; hence residual solvent analysis is not necessary and is not performed.

2.3.3 Residual Pesticide Analysis

In accordance with standard operating procedures, Prenexus is committed to periodic 3rd party testing of its Prenexus XOS derived from sugarcane (certified organic) for pesticide residues in accordance with US and European Pharmacopoeia standards (USP 34 NF29:561 & EP 07&/2008:2081). All lots tested to date were found to comply.

2.4 Physical or Technical Effect

Prenexus XOS derived from sugarcane is not intended to produce any physical or other technical effects that are relevant to the safety of the ingredient.

Part 3: Dietary Exposure

3.1 Intended Use

Prenexus XOS derived from sugarcane is intended to be used as an ingredient in the food categories and addition concentrations shown in Table 3 below. Prenexus XOS derived from sugarcane is not intended for use in foods where standards of identity would preclude such use, infant formula, or any products that would require additional regulatory review by USDA.

Table 3. Intended use of Prenexus XOS derived from sugarcane

Food Category	Maximum* Intended Use Addition Concentrations (g/100 g)
Bars	2.82–6.00
Crackers and Salty snacks from grain products	2.88–8.00
Chewing gums	27.98–43.33
Breads, Grains, and Pastas†	1.28–4.80
Coconut beverages, Juices, and Nectars†	0.67–1.00
Bottled and canned coffees and Prepared teas	0.10–0.67
Fruit drinks and Nonfruit beverages	0.46–1.00
Beverage concentrates, dry, not reconstituted	10–15.00
Nutrition drinks	0.23–0.67
Nutrition powders	3.16–26.67
Energy and Sports drinks, Fluid replacements, and Other functional beverages	0.24–0.67
Cookies	2.91–8.00
Gelatin desserts or salads	1.69–2.03
Cereal grains, not cooked	5.33
Pancakes and Waffles	2.66–6.00
Cooked cereals	0.31–4.36
Ready-to-Eat cereals†	3.01–16.00
Cheese and Imitation cheese†	0.71–8.00
Imitation milk, fluid	0.77–1.00
Yogurt†	0.80–1.52
Flavored milk and milk drinks, fluid	0.64–1.00
Mild, dry, and powdered mixes with dry milk, not reconstituted	10.43
Cream substitutes†	6.41–16.00
Milk desserts, frozen	1.20–1.52
Puddings, custards, and other milk desserts	1.23–4.44
Cheese soups	0.98
Nut butters	7.37–7.50
Sugar substitutes†	50–100

*Maximum addition concentrations vary depending on specific individual food codes within food categories.
†Intended use includes only some subcategories within the main category and/or only specific foods within the food category.

3.2 Exposure Estimates

Exposure estimates combine data on the quantity of a particular food category that is consumed with the intended concentration level of an ingredient to be added to that food category. Exposure to XOS from Prenexus XOS derived from sugarcane based on its intended uses was estimated for the U.S population using food consumption data from the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES). The most recent data available at the time of this writing (2013–2014) was analyzed using Creme Food Safety software 3.6 (www.cremeglobal.com). These data were obtained from 7,574 individuals that underwent two non-consecutive 24-hour dietary recall interviews (the first was collected in-person, the second by phone 3–10 days later). WWEIA food codes that were considered most similar to the intended use categories were utilized in the assessment and were assigned the relevant intended use concentrations.

Background exposure to XOS was calculated using data from GRAS notices 458 and 343. Intended use data for GRN 458 were presented on pages 15 and 16 (and in particular, Table 6), section III.B, and pages 43–50 (and in particular the table of Exposure Source Food Groups), Appendix B of the notice, which are incorporated herein by reference.[12] Intended use data for GRN 343 were presented beginning on page 61 of 61, section 8 (Appendix II), and in particular, Appendix II pages 6 and 7, section 3.0, Table 3.1, of the notice, which are incorporated herein by reference.[13] Food group/food code data from GRNs 458 and 343 were mapped to the most recent NHANES data (2013–2014) for use within the Creme Food Safety software. Because the XOS concentrations of Prenexus XOS derived from sugarcane and each of the respective ingredients that were the subjects of GRNs 458 and 343 varied among the three ingredients, and non-XOS constituents also varied according to the source material and XOS content, but were comprised of varying amounts of other non-XOS oligosaccharides and common constituents of edible plant materials, such as sugars, the addition concentrations of each ingredient used in the current Creme Food Safety software analyses was determined as the XOS fraction of the ingredient in order to provide a standardized exposure estimate across the ingredients. When identical food codes were mapped from both GRNs 458 and 343, the higher of the two concentrations were applied in calculations for background exposure estimates.

Creme Food Safety software is a probabilistic modeling tool that uses high-performance computing to predict intake (including total aggregate exposure) of food groups and/or individual food ingredients. Creme Food Safety performs calculations using large-scale food consumption data sets. It bases the calculated estimates on each individual's body weight from the survey, as opposed to averaged body weights. In other words, tabulated results for absolute exposure (mg/day) and

exposure relative to body weight (mg/kg bw/day) cannot be compared using a standard (e.g., 60 kg) weight factor. Calculations also incorporated the NHANES assigned sample weights for each individual in the survey, which measure the number of people in the population represented by that specific subject and help to ensure that the results statistically represent the entire U.S. population. Sample weights for NHANES participants incorporate adjustments for unequal selection probabilities and certain types of non-response, as well as an adjustment to independent estimates of population sizes for specific age, sex, and race/ethnicity categories. The data are shown for "food consumers" (which includes only data from individuals who reported consuming one or more food/beverage categories intended to contain Prenexus XOS derived from sugarcane over the two-day survey period, as opposed to the whole population). Results are given as both absolute exposure (mg/day), as well as exposure relative to body weight (mg/kg bw/day).

The relative standard error (RSE; calculated by dividing the standard error of the estimate by the estimate itself and multiplying by 100) is a statistical criterion that can be used to determine the reliability of estimates as pertains to the population (the larger the RSE the less reliable the estimate).[14] RSE values greater than 25–30% are often considered reasonable cut-offs by which to consider a value unreliable.[14, 15] For the purpose of this GRAS conclusion, an RSE value of greater than 25% was used to indicate that the estimated value was unreliable with regard to representing the population. RSE values are shown in the tables below for the 90th percentile values only, as the 90th percentile values are the most pertinent for the exposure estimates. All of the values in the tables were considered reliable using the 25% cut-off.

Data estimated directly from the NHANES short 2-day survey do not necessarily adequately represent individual usual long-term intake due to the large amount of random error. This is because it may not correctly capture infrequent consumers. It assumes that subjects who consumed a product on a survey day consume it every day of the year, and it does not adjust for potential day-to-day variation in intake (i.e., intra-individual variation over time is not accounted for). Thus estimation of "usual" or "lifetime" exposure was also added to the model based on methodologies developed by Nusser et al., 1996, at Iowa State University.[16] These lifetime data are considered the most relevant data, as GRAS exposure estimates should be based on expected regular exposure over the lifespan. The technique of estimating usual/lifetime intakes relies on the ability to transform the input daily average data (from food consumers) into normality, which is tested using the Anderson-Darling test statistic within the Creme Global software. Occasionally the Creme software determined that lifetime intake estimates required warnings or were not possible due

to issues with the original data set; such issues are noted with asterisks and are explained below the tables.

It should also be noted that these types of exposure estimates are extremely conservative as they assume that 100% of the foods in the marketplace from the intended food categories contain the maximum addition levels of Prenexus XOS derived from sugarcane. While food labels will list the Prenexus XOS derived from sugarcane as an ingredient and may even highlight it occasionally in marketing, it is assumed that many consumers will not always realize that it is present in the food. In other words, Prenexus XOS derived from sugarcane may be an "invisible" ingredient to many consumers, which decreases the chance that only food products that contain it will be chosen by consumers. Additionally, there will be cost and market share limitations of adding Prenexus XOS derived from sugarcane to foods in general, making it even less likely that an individual would consume them in all of the intended use food groups consumed daily.

In order to calculate more realistic exposure estimations for XOS from Prenexus XOS derived from sugarcane from the proposed food uses, additional Creme exposure assessments were performed that assumed a presence probability of 20% Prenexus XOS derived from sugarcane in the proposed food categories, combined with background dietary exposure presence probability factors of either 100% or 20%. The 20% presence probability factor was intended to represent an approximate 20% market share of the ingredient in foods from each of the intended use categories, which is still considered a highly conservative, yet more realistic, assumption. The maximum addition level for each food category was still utilized.

Results of the four Creme assessments (background only at 100% presence probability, background plus new intended uses at 100% presence probability, background at 100% presence probability and new uses at 20% presence probability, and both background and new uses at 20% presence probability) are shown in Tables 4 and 5 below.

Table 4. Total (Aggregate) Absolute Exposure to XOS by Proposed Use Food Consumers Using NHANES 2013–14 data (mg/day)

Data Set	Presence Probability (%)	N (% of total population)	Aggregate absolute consumption of XOS by food consumers (ages 2+) Daily Average (mg/day)					Lifetime 90th% Exposure Estimates (mg/day)
			Mean	Mean std err	90th%	90th% std err	90th% RSE Value	
Background uses[a]	100	6847 (97.6)	4814	78.0	9352	183.5	2.0	7647*
Background uses plus intended uses[b]	100	7046 (99.7)	12589	116.7	20738	241.6	1.2	18424*
Background uses plus intended uses[c]	100 (bkgd) 20 (uses)	6949 (98.7)	7206	87.0	13234	192.2	1.5	10468*
Background uses plus intended uses[d]	20	5883 (83.4)	2987	49.5	5882	120.0	2.0	3845*

[a]Creme run #308; [b]Creme run #392; [c]Creme run #394; [d]Creme run #395

*Creme Warning -2048 "Number of days per person should be consistent for a foods calculation"; data can still be used.

Table 5. Total (Aggregate) Exposure to XOS by Proposed Use Food Consumers Relative to Body Weight Using NHANES 2013–14 data (mg/kg bw/day)

Data Set	Presence Probability (%)	N (% of total population)	Aggregate consumption of XOS by food consumers (ages 2+) relative to body weight Daily Average (mg/kg bw/day)					Lifetime 90th% Exposure Estimates (mg/kg bw/day)
			Mean	Mean std err	90th%	90th% std err	90th% RSE Value	
Background uses[a]	100	6847 (97.6)	84.6	1.5	184.3	4.3	2.3	160.2*
Background uses plus intended uses[b]	100	7046 (99.7)	214.4	3.0	415.0	11.3	2.7	395.1*
Background uses plus intended uses[c]	100 (bkgd) 20 (uses)	6949 (98.7)	126.8	2.1	264.9	7.5	2.8	232.7*
Background uses plus intended uses[d]	20	5883 (83.4)	51.0	1.0	107.1	3.8	3.5	84.9*

[a]Creme run #308; [b]Creme run #392; [c]Creme run #394; [d]Creme run #395

*Creme Warning -2048 "Number of days per person should be consistent for a foods calculation"; data can still be used.

The exposure tables show the results of the various exposure assessments related to background XOS exposure and new uses of XOS proposed by Prenexus. They suggest that approximately 83.4–99.7% of the population may be exposed to XOS

from the various food categories, depending on the presence probability factor that was utilized. Dietary background XOS exposure estimates suggest 97.6% of the population is exposed when using a 100% presence probability. The 90th percentile lifetime background dietary XOS exposure estimates for the total population of consumers (ages 2+) was approximately 7647 mg/day or 160.2 mg/kg bw/day. When the additional intended uses for Prenexus XOS derived from sugarcane were added to the background exposure estimate, the 90th percentile lifetime exposure to XOS went up to 18,424 mg/day or 395.1 mg/kg bw/day. Again, these results are extremely conservative as they assume that 100% of the foods in the marketplace from the background food categories contain the maximum addition levels of XOS from previous GRAS conclusions and from the current intended uses for Prenexus XOS derived from sugarcane. When background exposure levels were left at 100% presence probability and the new intended uses were assigned a 20% presence probability, the exposure estimate dropped to 10,468 mg/day or 232.7 mg/kg bw/day. When both the background exposures and the new intended use concentrations were assigned a 20% presence probability factor, the resulting lifetime 90th percentile exposure was 2969 mg/day, or 84.9 mg/kg bw/day. This lower exposure data (20% presence probability for both background and new uses) is considered likely the most realistic of the assessments, yet it is still considered highly conservative, as to reach 20% of the market share of even a single food category (i.e., for XOS be present in 20% of the foods within any single category) within the total intended use was considered unlikely even when anticipating total XOS exposure from all current or future sources.

Given a typical XOS content of Prenexus XOS derived from sugarcane of approximately 81%, exposure to 3845 mg/day, or 84.9 mg/kg bw/day, XOS is equivalent to approximately 4747 mg/day, or 104.8 mg/kg bw/day, of Prenexus XOS derived from sugarcane.

Part 4: Self-limiting Levels of Use

There are no known inherent self-limiting levels of use.

Part 5: Experience Based on Common Use in Food Prior to 1958

The GRAS conclusion for Prenexus XOS derived from sugarcane is based on scientific procedures, and thus, experience based on common use in food prior to 1958 is not considered pivotal information. While xylan and other plant fibers, from which XOS may be isolated, have been commonly consumed by humans, to the best of our knowledge, isolated XOS was not commonly used in foods prior to 1958.

Part 6: Narrative

6.1 Current Regulatory Status of XOS

A thorough search for the current regulatory status of XOS, relevant to their use in food in the United States, was conducted. A summary of the pertinent search results is shown below:

- An FDA GRAS notice (GRN No. 000458) for XOS was found in the FDA GRAS Notice Inventory database. GRN 458 received FDA's "no questions" response letter on August 23, 2013 indicating no current challenge by the agency to the safety of the ingredient for its intended use "as a bulking agent in beers and ales at a level of 0.5 grams per serving (g/serving); and, as an ingredient in breakfast bars, cereal bars, cheese, chewing gums, custards, flavored and soy milk, gelatin desserts and salads, medical foods, milk and milk products, isotonic beverages, milk desserts, ready-to-drink milk-based meal replacements, power bars, puddings, protein bars, processed fruits, juice drinks and punch, ready-to-eat cereals, sports drinks, and yogurt at levels ranging from 0.2 to 2.4 g/serving".[12] The notice described the ingredient as ≥95% XOS derived by acid pre-treatment followed by enzymatic hydrolysis of corncob and comprised of oligomers ranging in DP from 2–6 β-1,4-linked xylose residues, of which 35% (typical) are xylobiose (i.e., DP = 2). The ingredient is produced in multiple product formulations, with the 95% material adjusted using a maltodextrin carrier, to provide three additional finished products with XOS concentrations of 20, 35, or 70%.

- An FDA GRAS notice (GRN No. 000343) for wheat bran extract (WBE) composed primarily of xylo- and arabinoxylo-oligosaccharides (collectively abbreviated as AXOS) was found in the FDA GRAS Notice Inventory database. GRN 343 received FDA's "no questions" response letter on November 22, 2010 indicating no current challenge by the agency to the safety of the ingredient for its intended use "as an ingredient in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, frozen dairy desserts, gelatin and puddings, grain products and pastas, jams and jellies, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and snack foods at concentrations ranging from 2.4 to 3.2 grams of wheat bran extract per serving".[13] The notice described the ingredient as wheat bran extract comprised of not less than 70% AXOS (with a β-1,4-linked xylose

backbone and an average range of DP of 3–8 and *O*-linked α-L-arabinofuranosyl substituents at the C2 and C3 positions of some xylose residues) on a dry matter basis, not less than 90% total poly- and oligosaccharides, not less than 7% beta-glucan, 1–3% ferulic acid, not more than 2% each of protein and ash, and not less than 94% total dry matter. Based on the mean of five batch compositional analyses provided in the notice, typical $XOS_{(DP\ 2-9)}$ content of WBE is 40.2% on a dry matter basis, of which 26.6% is present as xylobiose. The extract is manufactured as a partial enzymatic depolymerization of the water insoluble arabinoxylan component of the bran beginning with removal of starch followed by solubilization of xylo- and arabinoxylo-oligosaccharides.

6.2 Absorption, distribution, metabolism, and excretion (ADME)

Only a very few di-, oligo, and polysaccharides can be digested by humans and other mammals due to bond and substrate specificity of enzymes found in the gastrointestinal tract.[17, 18] α-Amylase secreted in saliva and pancreatic juices in the mouth and small intestine, respectively, and maltase present in the small intestinal brush border, cleave only α-(1→4) glycosidic bonds between two glucose residues. Three additional brush border enzymes cleave other glycosidic bonds as follows: sucrase cleaves an α-(1→2) glycosidic bond between a glucose residue and fructose residue, isomaltase cleaves an α-(1→6) glycosidic bond between two glucose residues, and lactase cleaves a β-(1→4) glycosidic bond between a galactose residue and a glucose residue. Any other linkages in carbohydrates are not acted upon by these enzymes and, therefore, such carbohydrates, with different linkages and/or monomeric units, are classified as non-digestible carbohydrates.

XOS is classified as a non-digestible carbohydrate due to its composition of xylose residues linked by β-(1→4) glycosidic bonds. Such classification is supported experimentally as saliva, pancreatin, gastric juice, and intestinal homogenate all failed to hydrolyze xylobiose in vitro.[19] Additionally, in vivo studies in ileostomized humans[20] and pigs cannulated at the terminal ileum[21] demonstrated that non-starch carbohydrates containing large fractions of arabinoxylans (from which XOS is derived) are not appreciably digested in the small intestine when ingested in foods. Stability testing of XOS under conditions of temperature and pH similar to that of the human stomach also suggest a low occurrence of non-enzymatic hydrolysis as XOS comprised chiefly of xylobiose and xylotriose was stable for 60 days at 37 °C over a pH range from 2.0 to 7.0.[22] Some small loss of glycosidic linkages was observed at pH 2.0 beginning on day 3 of the experiment with 2, 4, 8, and 12% bond

hydrolysis observed following 3, 7, 28, and 56 days, respectively, at 37 °C. However, as transit through the acidic gastric environment is measured in hours rather than days, and a bolus of food typically raises gastric pH,[23] these results suggest stability of XOS during normal gastric transit. Other oligosaccharides present in minor amounts in Prenexus XOS derived from sugarcane (e.g., arabinoxylo-, arabino-, galacto-, and glucooligosaccharides) are also considered non-digestible carbohydrates.

As with the intestinal enzyme specificity for only a few types of glycosidic linkages, only a few carbohydrate monomers (i.e., monosaccharides) are known to be readily absorbed in the human/mammalian small intestine (and little, if any disaccharides are absorbed).[17, 18] Thus, intact non-digestible oligosaccharides, including XOS, are not expected to be appreciably absorbed across the intestinal border. Because XOS and other minor oligosaccharides present in Prenexus XOS derived from sugarcane are not appreciably absorbed, they are not subjected to processes of tissue distribution, metabolism, and excretion within humans and other mammals. The digestion of the small amounts of sucrose and the pharmacokinetics of small amounts of free monosaccharides present in Prenexus XOS derived from sugarcane are well-known, although processes by which the human body works on xylose are somewhat less well elucidated in comparison to the more common dietary monosaccharides (e.g., glucose, fructose, galactose). Nonetheless, xylose is a small component of the typical human diet acted on by normal physiological processes, and its pharmacokinetics have been studied.[24-32] Due to their very small presence in the finished ingredient, the pharmacokinetics of these mono- and disaccharides are not further discussed.

The fate of XOS within digestive tract is likely similar to that of other non-digestible oligo- and polysaccharides. Four different XOS preparations (acetylated, linear (the major component of Prenexus XOS derived from sugarcane), and nonacetylated 4-*O*-methyl D-glucuronosyl and/or galactosyl substituted XOSs (AcXOS, nXOS, and GlcAmeXOS, respectively) derived from Eucalyptus with a DP range of 3–15 and AXOS derived from spent grain with a DP range of 3–11) were fermented using fecal inocula (FI) derived from four (V1–V4) human volunteers.[33] FI cell growth corresponded with decreases in XOS and production of short chain fatty acids (SCFA) and lactate. All four XOS preparations were fermentable, but specific patterns of degradation and SCFA and lactate production were dependent on both the particular XOS structures and the particular FI (i.e., inter-individual variation occurred). In general, the nXOS was highly susceptible to fermentation. Fermentation was more rapid with nXOS and AXOS than the others during the first 20 hours after which GlcAmeXOS matched their rate. XOS substituents were not easily degraded, resulting in preferential fermentation of lower substituted XOS

relative to higher substitution; thus, as fermentation of GlcAmeXOS proceeded, accumulation of relatively highly substituted XOS occurred, resulting in delayed or completely interrupted fermentation. In general, lower DP nXOS was fermented more rapidly relative to higher DP nXOS; however, large inter-individual variation was observed with FI from V4 fermenting DPs, even above 10, very rapidly. Fermentation occurred in two stages. During the first state, nXOS produced mainly acetate and lactate in equal amounts while during the second state production of propionate and butyrate also occurred and the amount of lactate, and to a lesser extent, acetate, decreased. This was also subject to inter-individual variation as no propionate or butyrate were produced with FI from V1. Because butyrate was produced late, when most XOS was already degraded, with a concomitant decrease in lactate, it is likely that that butyrate production was the result of substantial secondary fermentation of lactate.

Fermentation by colonic microflora of XOS to SCFA is consistent with results observed in human studies in which ingestion of XOS for three to four weeks promoted growth of bifidobacteria and lowered fecal pH.[34-36] Further, no xylobiose was excreted in feces or urine of Sprague-Dawley rats during 24 hours following oral administration of 100 or 300 mg/kg bw.[19] Given the in vitro results and background literature establishing that XOS is not digested, this result supports the likelihood that XOS is not absorbed and is completely fermented by colonic microflora to SCFA and lactate. SCFA represent a major soluble fraction of fecal water and are also readily absorbed in the colon (regardless of whether produced as a carbohydrate fermentation product of colonic microflora or ingested in the diet).[37, 38] Once absorbed, SCFA represent an important energy source for colonocytes (particularly butyrate) and may be transported to more distant peripheral tissues for energy production.[39, 40]

6. 3 Toxicology Studies

6.3.1 Bacterial Reverse Mutation Assays

Results of two bacterial reverse mutation assays were reported on page 25, section IV.D of GRN 458, which is incorporated herein by reference.[12] In a study conducted by Oh, et al.,[41] and summarized by Fu et al.[42] XOS derived from corncob xylan was not mutagenic in tester strains *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 *uvrA* with or without S9 metabolic activation at five concentrations up to 5000 µg/plate. Based on the data tables reviewed, we do not question the authors' conclusion. The second bacterial reverse mutation assay was an unpublished study summarized by Fu et al.[42] in which two experiments were

conducted in *S. typhimurium* tester strains TA97, TA98, TA100, and TA102. No biologically relevant or dose-related increases in revertant colonies were observed following treatment with XOS with and without S9 at five concentrations up to 5000 µg/plate.

Another bacterial reverse mutation assay was reported on page 36 of 61, section 2.3.4.1 of GRN 343, which is incorporated herein by reference.[13] As part of a battery of toxicological investigations, Francois et al. evaluated the mutagenicity of wheat bran extract highly-enriched in AXOS (WBE) in S. *typhimurium* TA98, TA100, TA1535, and TA1537 and *E. coli* WP2 *uvrA* tester strains in two independent experiments (plate incorporation and pre-incubation methods) with and without metabolic activation at seven concentrations up to 5000 µg/plate.[43] The test item was provided by the notifier of GRN 343 and its composition was consistent with the batch analyses provided in the GRN; thus, XOS content of the test item was approximately 40%. The assay was conducted in compliance with GLP and in accordance with test guidelines OECD 471, ICH S2A and S2B, and EPA OPPTS 870.5100. No significant increases in mean numbers of revertant colonies were observed following treatment with the test item under any experimental conditions. We agree with the authors' conclusion that WBE was not mutagenic under the applied conditions.

6.3.2 In vitro Chromosomal Aberration Assay

A chromosomal aberration assay was reported on page 37 of 61, section 2.3.4.1 of GRN 343, which is incorporated herein by reference.[13] In continuing the toxicological evaluation of WBE, based on the results of a preliminary cytotoxicity test, Francois et al. conducted two independent experiments (A & B, each in duplicate) exposing V79 Chinese hamster lung cells to WBE concentrations of 1250, 2500, and 5000 µg/mL in the presence or and absence of S9 metabolic activation for 3 hours with a 20 hour sampling time in experiment A or to WBE concentrations of 56.25, 312.5, 625, 1250, and 2500 mg/mL for 3 hours in the presence of S9 with a 28 hour sampling time in experiments B. The assay was conducted in compliance with GLP and in accordance with test guidelines OECD 473, ICH S2A and S2B, and EPA OPPTS 870.5375. Slight cytotoxicity observed at the high dose indicated the test item reached the target tissue. Two hundred well-spread metaphase cells were scored per concentration, and no statistically significant differences compared to negative controls in numbers of chromatid or chromosome aberrations or in the rate of polyploid and endoreduplicated metaphases were observed after treatment with the different concentrations of WBE with or without metabolic activation in either experiment. No dose-response

relationships were noted. We agree with the authors' conclusion that WBE was not clastogenic under the applied conditions.

6.3.3 In vivo Micronucleus Study

The results of an unpublished bone marrow micronucleus study in mice summarized by Fu et al.[42] were reported on page 25, section IV.D of GRN 458, which is incorporated herein by reference.[12] No significant increases in the rate of micronucleated polychromatic erythrocytes were observed in bone marrow slides of mice treated with XOS up at doses of 2.5, 5.0, and 10.0 g/kg bw compared to controls.

6.3.4 Acute Oral Toxicity Studies

Results of two acute oral toxicity studies were reported on pages 20–22, sections IV.B.1 (Park et al., page 20) and IV.B.2 (Gao et al., pages 20–21) of GRN 458, which are incorporated herein by reference.[12]

In the study conducted by Park, et al.,[44] and summarized by Fu et al.,[42] XOS derived from corncob xylan was administered orally to 15 SPF Sprague Dawley rats/sex/group at single bolus doses of 0, 5000, and 10000 mg/kg. No mortality or clinical signs of toxicity were observed during the 14-day observation period, and no gross abnormalities were observed at necropsy. Based on the data tables presented by Park et al., we do not question the authors' conclusion of an oral LD_{50} in rats > 10000 mg/kg bw.

The second study reported was conducted by Gao et al. in 10 Kunming mice/sex/group at single gavage doses 0 or 32 g/kg bw XOS.[45] The study was conducted in compliance with China State Food and Drug Administration GLP, National Institutes of Health Guide of the Care and Use of Laboratory Animals, and Technical Standards for Testing & Assessment of Health Food (Ministry of Health PR China, 2003). XOS was provided by the notifier of GRN 458 and described in the notice on page 20, section IV.B.2 as the 95% product; however, characterization of the test item by Gao et al. indicated an 87% XOS. No mortality or clinical signs of toxicity were observed during the 14-day observation period, and no gross abnormalities were observed at necropsy. While no tabular data was presented, based on the briefly reported results, we do not question the authors' conclusion of an oral LD_{50} in mice > 32 g/kg bw.

6.3.5 Fourteen-day Repeated-Dose Oral Toxicity Study

GRN 343 summarized a 14-day dose-range finding study of WBE conducted by Francois et al.[43] on pages 34–35 of 61, section 2.3.2, which are incorporated herein by reference.[13] Five rats/sex/group were administered WBE in the diet (at the expense of pregelatinized corn starch) at concentrations of 5, and 10% while the control group received the unaltered basal diet. WBE was well tolerated with no adverse effects observed. We agree with the conclusion in GRN 343 that cecal enlargement observed in the WBE groups is a known physiological effect observed with fermentable non-digestible carbohydrates.

6.3.6 Subchronic Repeated-Dose Oral Toxicity Studies

Results of two 13-week repeated-dose oral toxicity studies were reported on pages 21–23, section IV.B.4 (Park et al., page 21 and Gao et al., pages 21–23) of GRN 458, which are incorporated herein by reference.[12]

In the study conducted by Park, et al.,[44] and summarized by Fu et al.,[42] XOS derived from corncob xylan was administered orally to 10 Sprague Dawley rats/sex/group at doses of 0, 333, 1000, and 3000 mg/kg bw/day for 13 weeks; two additional satellite groups of 10 control and high-dose rats/sex were included for 4 weeks of observation after the treatment period. No mortality or adverse clinical signs were observed, and no effects on body weight, food or water consumption, clinical pathology, gross pathology, absolute or relative organ weights, or histopathology that could be attributed to administration of the test item were observed. Based on the data tables presented by Park et al., we do not question the reported determination of a NOAEL of 3000 mg/kg bw/day XOS, the highest dose tested, in male and female Sprague Dawley rats.

In the study conducted by Gao et al., 10 Wistar rats/sex/group were administered diets containing 0, 0.9, 2.9, 9.8, or 10% XOS for 13 weeks.[45] The study was conducted in compliance with China State Food and Drug Administration GLP, National Institutes of Health Guide of the Care and Use of Laboratory Animals, and Technical Standards for Testing & Assessment of Health Food (Ministry of Health PR China, 2003). XOS was provided by the notifier of GRN 458 and described in the notice on page 21, section IV.B.4 as the 95% product; however, characterization of the test item by Gao et al. indicated only 87% XOS. No mortality, ophthalmological abnormalities, or adverse clinical signs were observed. No adverse effects on body weight, food consumption and feed efficiency, clinical pathology, or absolute or relative organ weights were observed (we note that intestinal weights, including the weight of the cecum were not determined). No gross or microscopic lesions attributable to administration of the test item were

observed. As such, we agree with the concluded NOAEL as 10% XOS in the diet (equivalent to 14.95 and 11.51 g/kg bw/day in males and females, respectively) in Wistar rats, the highest dose tested.

GRN 343 summarized a 90-day feeding study of WBE conducted by Francois et al.[43] on pages 35–36 of 61, section 2.3.3, which are incorporated herein by reference.[13] Ten SPF Crl:(WI)BR Wistar rats/sex/group were administered WBE in the diet (at the expense of pregelatinized corn starch) at concentrations of 0.3, 1.5, or 7.5%. The high dose was selected based on the results of the above range-finding study in order to minimize physiological cecal effects. Three control groups of 10 rats/sex/group received either the basal diet or diets substituted with 7.5% inulin or wheat bran (the source of WBE). The GLP study was conducted in accordance with OECD test guideline 408. No mortality was observed during the study, and no treatment-related clinical signs or effects on behavior or motor activity or other functional observations were observed. No adverse effects on body weight and body weight gain, food consumption and feed efficiency, ophthalmoscopy, organ weights, or clinical, gross, and histopathology were observed. Non-adverse treatment-related effects were observed for increased water intake; serum calcium, phosphate, and potassium (females only); and absolute and relative full cecal weights and the histological finding of minimal bilateral hypertrophy of renal cortical tubules without associated degenerative changes. These effects were also observed in the inulin and/or wheat bran controls groups, mostly with greater magnitude. We agree with the conclusions that increased water intake and increased absorption of electrolytes are generally associated with increased ingestion of fermentable non-digestible carbohydrates and that increased cecal weight is a physiologic adaption to their ingestion. We also agree that the histological renal changes observed are likely an adaptive response to the increased serum electrolytes observed and that all of these changes are not of toxicological concern. Therefore, we agree with the determination of the NOAEL as 7.5% WBE, equivalent to a combined mean of 4354 mg/kg bw/day (approximately equivalent to 1750 mg/kg bw/day XOS), in the diet of male and female Wistar rats.

6.3.7 Chronic Repeated-Dose Oral Toxicity Study

In addition to the studies summarized in GRNs 458 and 343, a 26-week repeated-dose oral toxicity study (with a 4-week recovery period) in dogs was recently published in order to add to the body of toxicological data evaluating the potential health hazards, including identification of toxic effects and target organs, of repeated oral exposure to XOS.[46]

Methods: The test item was provided by Shandong Longlive Biotechnology Co., Ltd (the notifier of GRN 458), and was described as XOS from corncob comprised of 29, 30, 16, 10, 4, and 6% xylobiose, xylotriose, xylotetraose, xylopentaose, xylohexanose, and xyloglucan, respectively, and 5% monosaccharides, such as glucose, arabinose, and xylose. The study was conducted in accordance with the Guidelines for Repeated Dose Toxicity Tests of Natural Medicine of the State Food and Drug Administration of China and under the approval of the Animal Ethics Committees of Shandong Institute of pharmaceutical industry.

Four purebred Beagle dogs/sex/group were administered XOS by gavage (vehicle and dose volume not reported) at doses of 0 (control), 1250, 2500, and 5000 mg/kg bw/day XOS for up to 26 weeks. One animal/sex/group was sacrificed following 13-weeks exposure, two animals/sex/group were sacrificed at the end of the dosing period following 26-weeks exposure, and the remaining one animal/sex/group was sacrificed following a 28-day recovery period.

The following tests and examinations were conducted during the study:

- Observations of mortality, behavior, and clinical signs were made daily.
- Measurements of body weight and temperature were made prior to dosing and at weekly intervals thereafter; food intake was also measured.
- Clinical pathology parameters were evaluated prior to dosing and at weeks 13, 26, and 30 as follows:
    - Hematology: white blood cell counts (WBC), red blood cell counts (RBC), platelet counts (PLT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrations (MCHC), hemoglobin concentrations (HGB), mean corpuscular volume (MCV), hematocrit (HCT), thrombin time (TT), prothrombin time (PT), and activated partial thromboplastin time (APTT);
    - Clinical chemistry: triglycerides (TG), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (GGT), creatinine (CRE), glucose (GLU), total bilirubin (T-BILI), blood urea nitrogen (BUN), total protein (TP), creatinine kinase (CK), and albumin (ALB);
    - Urinalysis: protein, specific gravity, nitrite, leukocytes, pH, ketones, glucose, urobilinogen, occult blood, hemoglobin, and bilirubin.
- Ophthalmological examinations and electrocardiograms (ECG) were conducted prior to dosing and at weeks 13, 26, and 30.

- Measurements of organ weights (absolute and relative; including brain, liver, heart, testes, epididymis, kidney, thymus, adrenal glands, lungs, spleen, ovaries and uterus) and gross pathological examinations were conducted on all animals at necropsy at weeks 13, 26, and 30.
- Blinded histopathological examinations were conducted on the following preserved organs and tissues of all animals at each sacrifice period: kidneys, heart, testes with epididymides, urinary bladder, salivary glands, ovaries, spleen, thymus, brain, liver, adrenal glands, peripheral nerve, pituitary gland, intestine, prostate gland, uterus, gall bladder, pancreas, thyroid gland (with parathyroid gland), lungs, bone marrow, stomach, lymph nodes, spinal cord, trachea, and skin.
- All data were subjected to statistical analysis.

Results: No mortality occurred, and animals exhibited good health throughout. Transient diarrhea was observed in mid-dose animals during weeks 1 and 2, and diarrhea and vomiting was observed in all high-dose animals throughout the dosing period but resolved during the recovery period. A trend towards lower body weights was observed in high-dose males during the study and in high-dose females from weeks 22–26; however, the differences were not statistically significant compared to the respective controls. No effects were observed on body temperature, food consumption, ECG results, or ophthalmological examinations. The authors considered the observed diarrhea incidental due to its resolution following week 2 in mid-dose animals and its gradually declining occurrence and resolution during the recovery period in high-dose animals. Diarrhea was likely due to physiological effects of the large amounts of non-digestible carbohydrates ingested at the mid- and high-dose levels with early adaption at the mid-dose level. This is consistent with observations in studies in animals and humans with other poorly digestible carbohydrates. While generally mild gastrointestinal symptoms have also been associated with poorly and non-digestible carbohydrates, it is unclear whether the vomiting observed in high-dose animals can be attributed to such effects. As dosing volumes were not reported, it is possible, but unknown, whether excessive dosing volume could have contributed to the observed effects.

There were no statistically significant differences in clinical pathological parameters among the groups at the start of the study. Statistically significant differences compared to controls were observed in hematological parameters at the week 13 evaluation for MCH, MCHC, and PT in female animals and at the week 26 evaluation for MPV in female animals and MCH and MCHC in male animals. With the possible exception of the increased MPV in females at week 26, the observed

statistically significant differences were not dose-related, lacked clinical correlations in other study parameters, and remained within the historical control ranges of the laboratory. The increase in MPV could not be ruled out as dose-related at the high-dose level but was of low magnitude and lacked clinical correlations, including effects on other coagulation parameters. Therefore, all observed hematological effects were considered incidental spontaneous occurrences without toxicological relevance (see Table 6). As no data were shown for the week 30 evaluation at the end of the recovery period, it is unknown whether any of the observed alterations persisted; however, the authors report that no treatment-related abnormalities were observed.

Table 6. Summary of Statistically Significant Hematology (Gao 2017)

Group (mg/kg bw/day)	MCH [pg]		MCHC [g/L]		MPV [pg]		PT [s]	
	Wk 13	Wk 26	Wk 13	Wk 26	Wk 13	Wk 26	Wk 13	Wk 26
Male								
0 (Control)	6.05 ± 0.68	21.23 ± 0.32	8.89 ± 1.11	31.73 ± 0.47	12.28 ± 0.70	9.67 ± 0.76	5.03 ± 0.39	6.60 ± 0.62
1250	6.45 ± 0.58	20.33 ± 0.21*	9.53 ± 1.27	30.03 ± 0.47*	12.65 ± 0.86	8.57 ± 0.71	4.83 ± 0.62	6.20 ± 0.17
2500	6.93 ± 0.75	21.03 ± 1.12	10.08 ± 1.30	30.30 ± 1.05	12.58 ± 1.93	8.60 ± 1.00	5.68 ± 1.12	6.63 ± 1.04
5000	5.95 ± 1.07	21.03 ± 0.45	9.50 ± 1.89	31.13 ± 0.71	11.68 ± 1.71	9.03 ± 0.29	4.85 ± 0.34	6.30 ± 1.57
Female								
0 (Control)	4.50 ± 0.91	21.17 ± 0.90	7.28 ± 0.68	31.80 ± 2.55	10.60 ± 0.88	8.83 ± 0.06	6.28 ± 0.64	6.27 ± 1.36
1250	5.83 ± 0.48	20.80 ± 0.72	8.93 ± 0.69	31.97 ± 2.52	11.38 ± 2.30	9.33 ± 0.29*	5.53 ± 0.40	5.93 ± 1.27
2500	5.50 ± 0.68*	20.00 ± 0.17	8.15 ± 1.19*	30.53 ± 1.29	10.48 ± 1.72	9.30 ± 0.26*	5.15 ± 0.47*	4.97 ± 0.55
5000	4.98 ± 1.79	21.00 ± 1.40	7.03 ± 3.08	31.20 ± 2.98	11.50 ± 1.16	9.53 ± 0.32*	6.20 ± 1.01	6.23 ± 0.29

Abbreviations: MCH, mean corpuscular hemoglogin; MCHC, mean corpuscular hemoglobin concentration; MPV, mean platelet volume; PT, prothrombin time.
*p < 0.05

Statistically significant dose-related increases in TC were observed in male dogs at both the week 13 and week 26 evaluations; however, the magnitude of increases were small, remaining within the historical control range of the laboratory, were not seen in female animals, and have not been observed in human studies[35, 36, 47, 48]; therefore, the changes were not considered toxicologically relevant. As with the hematological evaluation, no data were shown for the week 30 evaluation of clinical chemistry parameters; therefore, while the authors reported that no treatment-related abnormalities were observed, it is unknown whether the observed alterations in TC persisted in males at the end of the recovery period. No other statistically significant differences in clinical chemistry parameters were observed at the week 26 evaluation and the few other statistically significant differences observed among the groups and sexes at the week 13 evaluation occurred spontaneously without dose-relation and remained within the historical ranges of the laboratory (see Table 7). Additionally, the authors reported that no treatment-related effects were observed

on urinalysis parameters and that urine volume, specific gravity, and pH were in normal ranges (data not shown).

Table 7. Summary of Statistically Significant Clinical Chemistry (Gao 2017)

Group (mg/kg bw/day)	ALP [U/L]		BUN [mmol/L]		TC [U/L]		ALB [g/L]	
	Wk 13	Wk 26	Wk 13	Wk 26	Wk 13	Wk 26	Wk 13	Wk 26
Male								
0 (Control)	95.77 ± 12.74	79.91 ± 22.22	8.25 ± 0.99	4.53 ± 0.81	2.60 ± 0.52	2.90 ± 0.10	30.70 ± 3.21	35.80 ± 1.48
1250	97.67 ± 4.45	60.37 ± 14.51	7.73 ± 1.05	5.40 ± 0.60	2.98 ± 0.18	2.85 ± 0.32	30.29 ± 1.56	35.01 ± 1.46
2500	125.51 ± 13.86**	114.82 ± 1.99	7.66 ± 1.18	4.53 ± 0.56	3.44 ± 0.36*	3.30 ± 0.28	28.24 ± 1.80	33.97 ± 1.37
5000	109.50 ± 37.57	86.60 ± 19.66	7.18 ± 1.31	4.74 ± 0.30	3.56 ± 0.55*	3.63 ± 0.37*	30.19 ± 1.38	36.09 ± 1.58
Female								
0 (Control)	106.49 ± 36.72	92.96 ± 36.52	8.34 ± 1.15	5.05 ± 1.13	3.82 ± 0.79	3.07 ± 0.09	30.11 ± 1.04	36.58 ± 1.83
1250	100.97 ± 23.13	81.27 ± 19.07	10.65 ± 1.16*	5.16 ± 0.35	2.98 ± 0.54	3.42 ± 0.96	28.40 ± 0.33*	34.89 ± 0.79
2500	107.55 ± 23.76	85.42 ± 27.16	9.27 ± 1.67	4.82 ± 0.75	3.33 ± 0.55	3.12 ± 0.58	29.46 ± 0.88	36.32 ± 1.77
5000	123.60 ± 34.98	105.39 ± 18.42	7.42 ± 1.49	4.36 ± 1.00	3.29 ± 0.55	2.93 ± 0.65	30.85 ± 0.66	37.12 ± 0.96

Abbreviations: ALB, albumin; ALP, alkaline phosphatase; BUN, blood urea nitrogen; TC, total cholesterol.
*p < 0.05; **p < 0.01

Absolute and relative organ weights of all animals at the 13, 26, and 30 week necropsies were within historical control ranges of the laboratory. The authors did not report whether any statistically significant differences compared to controls were observed for any absolute or relative organ weights (data not shown); in rodent studies increased cecal weights (a physiological response to ingestion of poorly and non-digestible carbohydrates) have been observed; however, it is unclear whether cecal weights were among the organ weights obtained in this study. No treatment-related gross or histopathological changes were observed in the organs and tissues, including intestinal tissues where direct XOS exposure was assumed, of any animals (data not shown).

Conclusions: Repeated administration by gavage of 1250, 2500, and 5000 mg/kg bw/day XOS for up to 26 weeks resulted in vomiting in all animals of the high-dose group over the course of treatment. Vomiting resolved completely during the recovery period, and the authors considered it a treatment-related adverse effect for purposes of estimating a NOAEL. Since rodents are not able to vomit, it is not possible to look to the previous studies conducted and reported above for potential explanations. Additionally, we cannot rule out factors such as excessive dose volume, vehicle effects, and viscosity as potential causes of vomiting because no information in these respects were reported. For these reasons, we do not disagree with the authors' conclusion. Diarrhea was also observed but is a known physiological response to excessive amounts of poorly and non-digestible carbohydrates that does not result in degenerative changes or changes that persist

up on cessation of dosing. No other treatment-related adverse effects or signs of toxicity in male or female purebred Beagle dogs were observed. Thus, the NOAEL was determined to be 2500 mg/kg bw/day.

6.4 Additional Scientific Studies

6.4.1 Human Studies

Twenty clinical trials investigating various effects of XOS or AXOS (of which XOS is a component, accounting for approximately 40%) in human subjects were identified in our literature searches. Of these thirteen[35, 47-58] reported specific procedures for monitoring adverse events (AE), six[49, 52-55, 59] reported the occurrence of any AEs during treatment with XOS/AXOS, and nine[35, 47, 48, 50, 51, 56-58, 60] specifically reported the absence of AEs while the remaining five studies located made no mention of AEs.[34, 36, 61-63]

Five[35, 47, 49, 50, 53] of the 20 clinical trials located were summarized in GRN 458 on pages 27–28, section IV.G.1, which are incorporated herein by reference (an additional trial on AXOS was summarized in GRN 458 and was also summarized in GRN 343 and is discussed in the next paragraph).[12] XOS was well-tolerated at doses up to 12 g daily for 1 week and up to 4 g daily for up to 8 weeks with only an 18% incidence of transient diarrhea on the first day of administration followed by adaptation occurring at high doses (10–12 g) and no gastrointestinal symptoms occurring at doses below 8 g daily. No other AEs were reported, and XOS consumption did not adversely affect nutritional status or laboratory parameters.

One[52] of the 20 clinical trials located, as well as a prepublication version of Francois et al., 2012,[55] were summarized in GRN 343 on pages 37–41 of 61, section 2.4, which are incorporated herein by reference.[13] AXOS was well-tolerated at doses up to 10 g daily (13.9 g of total ingredient; ~5.6 g XOS) for 3 weeks. Frequency of minimally to mildly disturbing flatulence following ingestion of 10 g (but not 3 g) AXOS daily was increased compared to placebo, but the increase was not statistically significant. No other gastrointestinal effects or other AEs different than placebo occurred, and no biologically relevant alterations in laboratory parameters compared to placebo were observed.

The remaining eight trials that either included specific procedures for monitoring AEs and/or that specifically reported the presence or absence of AEs are summarized with respect to safety relevant procedures and results below.

In a randomized double-blinded placebo-controlled trial 16 healthy and 13 pre-diabetic adult subjects received either 2 g XOS or placebo capsules daily for 8

weeks.[60] XOS used in the study was manufactured by the notifier of GRN 458 and described as 2.8 g of 70% XOS. No adverse effects were reported by the subjects during the trial, and XOS did not adversely affect fasting glucose or glucose or insulin responses to an oral glucose challenge or other biomarkers associated with pre-diabetes.

The effects of XOS alone or in combination with a probiotic (*Bifidobacterium animalis* subsp. *lactis*) were investigated in a randomized double-blinded placebo-controlled cross-over study.[48] Forty-four healthy adult subjects entered the trial and received either 8 g XOS + maltodextrin, 8 g XOS + probiotic (109 cfu), probiotic + maltodextrin (109 cfu), or maltodextrin + maltodextrin (as the placebo) daily for 21 days. XOS used in the study was manufactured by the notifier of GRN 458 but was not otherwise described; all treatments were administered as sachets of powder that the subjects were instructed to mix in a beverage. There was a 28-day washout period between each treatment and the treatment phases were administered in random order. Subjects were asked to record bowel habits, mood, medication use, and adverse events daily during active treatment phases and washout periods, and blood lipids were monitored. Three subjects dropped out during the trial, one due to pregnancy, one due to vasal vagal reaction during the study blood draw, and one due to headache, abdominal pain, bloating, and increased flatulence when receiving the probiotic only treatment. Compared to placebo, there was a statistically significant, but small, increase in number of bowel movements per day (1.4 ± 0.6 vs 1.5 ± 0.6; $P = 0.005$) during the XOS only treatment; however, there were no statistically significant differences among the groups in incidence or duration of AEs, and no adverse effects on blood lipids were observed.

Finegold et al. investigated the tolerance to XOS in a randomized double-blinded placebo-controlled trial in which 32 healthy adult subjects received 1.4 or 2.8 g XOS (note: XOS was manufactured by the notifier of GRN 458 and the 1.4 and 2.8 g capsules were reported as containing 175 and 350 mg XOS, respectively) or placebo (maltodextrin) in capsules daily for 8 weeks.[57] Subjects were provided symptom charts to be completed daily using a scale of 0 (no symptom) to 3 (severe symptom) and also recorded stool frequency and consistency. One subject from the placebo group and one subject from the low-dose group withdrew from the study due to non-specific gastrointestinal complaints, and one placebo subject and two subjects from each of the low- and high-dose groups were excluded from analyses due to compromised stool specimen quality (it is unclear from the study whether this exclusion included exclusion of analysis of the adverse event symptom chart scores of these subjects). No diarrhea or severe symptoms were reported by any of the study subjects, and there were no statistically significant differences in AEs in the treatment groups compared to placebo.

Sixty young healthy adults were administered XOS, either alone (5 g/day) or in combination with inulin (1 g XOS + 3 g inulin), or placebo (wheat maltodextrins) for 4 weeks in a randomized double-blinded placebo-controlled trial.[54] The test items or placebo were given in orange juice in two equal daily doses in order to provide the total study doses, and consumption of the study drinks was monitored by investigators in order to assure compliance. XOS used in the study was derived from wheat arabinoxylans and was characterized as 80% XOS (on a dry matter basis) with an average DP >10 and arabinose:xylose ratio >0.5 (indicating the test item may actually have been AXOS); as such, 6.64 g was used to provide the XOS (or AXOS) dosage of 5 g/day. Blood pressure, heart rate, and gastrointestinal well-being (visual analog scale questionnaire) were monitored before and at predetermined intervals during treatment, and a global digestive tolerance score (GDTS) was computed based on the questionnaire responses. One subject dropped out prior to treatment and was not included in analyses, and the remaining 59 subjects completed the study. No statistically significant differences in GDTS, stool consistency or frequency, general well-being, or effects on professional or social activities were observed in the XOS only group compared to controls. GDTS and stool frequency were significantly increased, and stool consistency was perceived as more liquid, compared to placebo in the XOS-inulin group following 2 weeks of treatment; however, the changes were transient becoming non-significant following the 4th week of treatment. The increased GDTS was due to flatulence and bloating with only flatulence showing significance for the entire treatment period and there were small, but statistically significant impairments in general well-being and profession activities. No effects on blood pressure or heart rate were observed in either group. Given that the transient AEs observed did not occur in the XOS only group, it is possible that they can be attributed to inulin alone; however, as the study did not include an inulin only group, we do not make a definitive conclusion in this respect.

In a single-blinded crossover design, 29 healthy females were randomly assigned to two groups and administered 1 g/day XOS or placebo for three consecutive weeks.[51] Each randomized group received the XOS and placebo in opposite order; the test period was preceded by a one-week run-in period, and there was a three-week washout period between crossover from XOS to placebo or vice versa. The test item was described as a 95% pure XOS, derived from hardwood xylan, of which 17% was comprised of xylobiose. All subjects were asked to record stool frequency, volume, and condition using a provided point scale questionnaire; any abdominal or non-abdominal symptoms; and medication use during the entire 10-week period from run-in through the end of the second crossover period. No diarrhea or other GI or non-GI adverse effects that could be attributed to ingestion of the test item were reported during the study. Some minor and fleeting abdominal symptoms were

associated with menstruation, but there was no correlation between these symptoms and intake XOS or placebo.

Forty healthy adult females ingested 0.4 g XOS daily for 4 weeks in an open-label clinical trial.[59] While no safety parameters were evaluated and no specific procedures were reported to monitor adverse events, the authors reported that one subject dropped out on Day 4 due to abdominal distension and one subject reported use of antidiarrheal medication during the study.

The tolerance of WBE was investigated in a randomized double-blinded placebo-controlled crossover trial.[58] Twenty healthy Caucasian adults were randomized to receive 15 g/day WBE for one week followed by an increase to 30 g/day WBE for an additional week (administered in three (morning, mid-day, and evening) equal doses in a non-carbonated soft drink) or placebo (same composition soft drink alone) for 2 weeks. Each treatment phase was followed by a 2-week washout period (other oligosaccharides included in the crossover design are not discussed here). WBE used in the study was manufactured by the notifier of GRN 343 and was reported to contain 80.9% AXOS (average DP = 5) of which 34.1% of the total ingredient dry mass was XOS. Therefore, the 15 and 30 g doses delivered approximately 5 and 10 g/day XOS, respectively. A gastrointestinal (GI) symptom questionnaire was administered weekly in order to assess occurrence, frequency, and severity of any GI adverse effects, and the Bristol Stool Form Scale was administered daily during the week prior to dose initiation and the last weeks of the treatment and washout periods. Subjects were asked to record other adverse effects (including medication use) daily as well as being questioned about these at each study visit; AEs were categorized according to the National Cancer Institute Common Terminology Criteria for Adverse Events v. 3.0. Fasting serum and plasma samples were collected for evaluation of hematological and clinical chemistry (including vitamin and mineral status) parameters. All subjects completed the study are were included in the safety evaluation. No statistically significant differences compared to placebo were observed in overall GI symptoms scores with either dose of WBE. With respect to the 18 individual GI symptoms scored, there was a statistically significant increase compared to placebo in frequency of abdominal stretching following ingestion of 15 g/day WBE and a statistically significant increase in severity of abdominal cramping following ingestion of 30 g/day WBE compared to placebo. No other statistically significant differences were observed. The increased severity of abdominal cramping was the result of increases in minimal to mild severity of cramping, as one subject receiving placebo versus four subjects receiving WBE reported minimal severity, and zero subjects receiving placebo versus one receiving WBE reported mild severity while one subject in each group reported severe cramping and the remaining subjects of both groups reported no

cramping. As no differences in flatulence were observed in this study in contrast to earlier studies using lower doses of WBE, the authors hypothesized the lack of effect may have resulted from induction of tolerance due to the relatively high basal fiber intake (26.4 g/day) of the study subjects. The Bristol Composite Measure was statistically significantly increased compared to placebo following ingestion of 30 g WBE; however, the increase was slight (0.48) and no differences were observed in defecation frequency, fecal output, or stool consistency. No statistically significant differences in non-GI AEs or hematological or clinical chemistry (included vitamin and mineral status) parameters were observed following consumption of either dose of WBE compared to placebo. The overall conclusion is that WBE extract was well-tolerated at daily doses up to 30 g (containing approximately 10 g XOS) for one week.

The effects of AXOS in bread were investigated by Walton et al. using a randomized double-blinded placebo-controlled crossover design in healthy adult subjects.[56] Forty-four subjects were administered wheat/rye bread containing enzymatically *in situ* produced AXOS with an average DP of 18 or placebo bread for 21 days. Each treatment or placebo period was separated from the next by a 21-day washout period in which subjects ate the same amount of a wheat bread as a baseline control. Total arabinoxylan content of the baseline control (wheat) and placebo (wheat/rye) breads were 2.0 and 4.2% (the latter formulated to match the arabinoxylan + AXOS content of the test item) and the average DPs were 157 and 174, respectively. Total daily ingestion of AXOS from the test bread was 2.2 g (insufficient information provided to calculate XOS content). Subjects were asked to record any GI symptoms, other AEs, emotional changes, medication use, and failures to consume the study breads in a diary on a daily basis. Three subjects dropped out during the first feeding period due to the quantity of bread (7 slices daily) they were required to consume, and an additional subject dropped out due to a change in travel plans; these four subjects were not included in analyses. An additional subject dropped out for personal reasons midway through the study, and his data collected to date were included in the analyses; therefore, 40 subjects were included in the analyses. In comparison to pre-treatment scores, there were no statistically significant differences in subjects ingesting AXOS bread while during placebo bread treatment, a statistically significant increase in number of stools was observed compare to pre-treatment. Thus, the AXOS bread was well-tolerated.

6.4.2 Toxicological Data and Information on Related Compounds

The results of a two-year oral toxicity/carcinogenicity study of D-xylose were reported on page 26, section IV.F.2 of GRN 458, which is incorporated herein by

reference.[12] D-Xylose (CAS RN 58-86-6) is the monosaccharide backbone unit of XOS, AXOS, and xylans and occurs naturally at low levels (<1%) in Prenexus XOS derived from sugarcane. It is listed in FDA's Substances Added to Food database (accessed July 24, 2018) as in use as a flavoring agent or adjuvant and nutritive sweetener, having been the subject of a GRAS conclusion by the Flavor and Extract Manufacturers Association, and is also listed as food additive of natural origin in Japan.[64] Kuroiwa et al. administered D-xylose (99% purity) to three groups of 55 SPF Fischer 344/DuCrj (F344) rats/sex/group in the diet at concentrations of 0, 2.5, and 5% for 104 weeks.[65] No differences in survival and no adverse effects or carcinogenicity that could be attributed to treatment were observed. Therefore, we are in agreement with the GRN 458 conclusion of a NOAEL of 5% D-xylose in the diet of F344 rats (equivalent to 2214 and 2513 mg/kg bw/day in males and females, respectively).

6.5 Allergenicity

Prenexus XOS derived from sugarcane does not contain or have added, and is manufactured in a facility free of, all eight major allergens (milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans) identified, and required to be disclosed in labeling, in the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). Additionally, Prenexus XOS derived from sugarcane does not contain gluten; barley, rye, rice, oats or other cereal grains; mustard; sesame seeds; sulfites; monosodium glutamate; artificial colors or flavors; or preservatives or any derivatives or products of the aforementioned. Prenexus has established and maintains allergen control protocols to manage the risk of contamination of these allergens into Prenexus XOS derived from sugarcane during production and manufacturing.

No reports of allergic reactions to XOS were found in our investigations.

6.6 History of Consumption

While xylan occurs naturally in plants used as food, XOS (a hydrolysis product of xylan) is not expected to be naturally present in food at any appreciable quantities. Nonetheless, consumption of XOS in the U.S. as a discrete dietary component is expected to have occurred since at least 2011 as a GRAS notice (GRN 343) for use of an ingredient containing approximately 40% XOS received FDA's "no questions" letter on November 22, 2010. A second GRAS notice (GRN 458) for a family of ingredients containing up to 95% XOS received FDA's "no questions" letter on August 23, 2013, which likely lead to an increase in XOS consumption in

the U.S. While no qualitative or quantitative data were located to characterize XOS consumption, both GRAS notices contained conservative estimates of maximum daily intakes, and in Part 3 of this notice, we used intended use data from these two GRAS notices in order to derive our estimates of background exposure to XOS (approximately 4.8 g/day (85 mg/kg bw/day) and 7.6 g/day (160 mg/kg bw/day) at the mean and lifetime 90th percentile, respectively). XOS has also been consumed in Japan as a food ingredient where, as of 2000, it was reported to be in use by about 60 food manufacturers and present in about 100 food products.[1]

Xylan is widely present in plant foods consumed by humans,[1-3] and is the most abundant of the hemicelluloses, which in turn are the second most abundant naturally occurring polysaccharide.[2, 4] Rasper reported that common cereal grains contain from 21.0% to 67.8% xylose-based hemicelluloses depending on the method of analysis and part of the grain assayed.[66] While the β-1,4-linked xylose backbone of different xylans can be decorated with various substituents, substituent to xylose ratios are typically low with long regions of unsubstituted xylose residues occurring (e.g., Figure 2 shows sugarcane xylans with unsubstituted regions of 61–79 xylose residues), and linear homoxylans also occur in plants.[4, 9] Given the only structural and compositional differences between XOS and linear homoxylan or unsubstituted regions of other xylans is the xylose chain length (i.e., DP) it is reasonable to think of human consumption of xylans as supportive of a more extensive history of exposure to XOS than what can be attributed to specific XOS exposure (discussed in the above paragraph) alone although we did not locate qualitative and quantitative data characterizing xylan consumption specifically. As the use of xylanases as raw material in baking, brewing, and cereal beverage and cereal grain processing is GRAS,[67, 68] and their use in bread dough has been reported (and is presumed in the other categories as well),[69, 70] it is further reasonable to presume that some XOS would be formed *in situ* resulting in exposure from consumption of such foods. However, we did not locate any quantitative or qualitative data regarding such exposures.

A general Internet search (July 25, 2018) revealed that sugarcane fiber (the specific source of xylan from which Prenexus XOS derived from sugarcane is derived) is an ingredient in various food products, illustrating that this ingredient is widely available in the U.S. Examples of products containing sugarcane fiber are listed in Table 8:

Table 8. U.S. Products Containing Sugarcane Fiber

Company	Website	Product Name
Ener-G	https://www.ener-g.com/	High Fiber Loaf
The Essential Baking Company	http://essentialbaking.com/	Gluten-Free Cinnamon Raisin
American Quality Foods	http://www.americanqualityfoods.com/	Graham Pie Crusts
Rudi's Organic Bakery	https://www.rudisbakery.com/	Hearty Fiber
Promise Gluten Free	https://www.promiseglutenfree.com/	Baked In-Store Sourdoughs

6.7 Reported Adverse Events

No FDA letters regarding concern for safety to companies that market products containing XOS were located. A search of MedWatch, FDA's adverse event reporting program, FDA's Recalls, Market Withdrawals, & Safety Alerts search engine, and FDA's Center for Food Safety and Applied Nutrition Adverse Event Reporting System (January 2004–September 2017) did not uncover any mention of XOS products. All databases were accessed on June 27, 2018.

6.8 Basis for the GRAS Conclusion

The scientific procedures establishing the safety of Prenexus XOS derived from sugarcane comprise the technical element of the GRAS standard. The common knowledge element is comprised of the general availability and general acceptance, throughout the scientific community of qualified experts, of the technical element. Together, the technical element and the common knowledge element form the basis for Prenexus's conclusion of GRAS status of Prenexus XOS derived from sugarcane for its intended use.

6.8.1 Technical Element

Prenexus XOS derived from sugarcane has been the subject of a thorough safety assessment as described above. The totality of evidence supporting the safety of Prenexus XOS derived from sugarcane is comprised of data and information that establish the safety of Prenexus XOS derived from sugarcane under the conditions of its intended use (the technical element) and data and information this is corroborative of safety. The scientific data, information, and methods forming the technical element of this conclusion are:

- The establishment of identity, demonstrating that the ingredient is well-characterized and sourced from edible material (i.e., sugarcane fiber);

- The method of manufacture and specifications, demonstrating the safe production and the high quality control standards of Prenexus XOS derived from sugarcane;
- The established non-digestibility of XOS and its metabolism by gut microbes to common SCFAs that can be absorbed and utilized by humans as an energy source;
- The GRAS status of uses of two similar ingredients including the following data and information:
    - In vitro and in vivo genetic toxicity studies demonstrating a lack of mutagenic and genotoxic potential of XOS under the tested conditions;
    - 90-day repeated-dose oral toxicity studies in rats establishing NOAELs up to 14.96 and 11.51 g/kg bw/day XOS in male and females, respectively, the highest dose tested;
- Randomized double-blinded placebo-controlled clinical trials establishing that XOS is well-tolerated in humans at doses up to 12 g/day with only minor GI adverse effects similar to those observed with other non-digestible and poorly digestible carbohydrates occurring at the highest dose levels (with transient diarrhea occurring in some subjects on only the first day of administration followed by adaption) and no AEs in comparison to placebo at lower, more typical doses.

Based on the oral toxicity studies in rats, the toxicity of XOS is quite low and well above physiological tolerance levels at which gastrointestinal tolerance would limit consumption. As XOS has only been tested in humans at doses up to 12 g daily, a true tolerance level has not been established; thus, we conclude that the physiological tolerance of XOS is $\geq$12 g/day.

Based on the intended use of Prenexus XOS derived from sugarcane in addition to background exposure levels to XOS due to uses specified in GRAS notices 458 and 343, the lifetime exposure in the total U.S. population at the 90th percentile of consumers was conservatively estimated in Part 3 of this GRAS notice using a 20% presence probability as approximately 3845 mg/day or 84.9 mg/kg bw/day of XOS (equivalent to approximately 4747 mg/day or 104.8 mg/kg bw/day Prenexus XOS derived from sugarcane). This is considerably lower than some amounts that have been well tolerated in humans in several clinical trials as well as a theoretical physiological limit due to laxation effects. Furthermore, a NOAEL as high as 10% of the diet of rats (equivalent to 14.95 and 11.51 g/kg bw/day in males and females) has been observed in a formal toxicological study.[45] Nonetheless, due to the

difficulty of dosing animals at extremely high doses to provide many fold increases in exposure over typical human exposure to food-like substances, other toxicological studies used lower doses, and it is not usually necessary to consider a typical margin of safety (MOS) for such substances that are food-like and act in the body according to well-known and established physiological processes. Moreover, it is well established that XOS is a non-digestible oligomeric carbohydrate that is not systemically absorbed at any appreciable amount. The end products of fermentation of XOS by gastrointestinal microorganisms are also known and are comprised of common dietary substances of nutritional benefit. Therefore, it is more appropriate in this case to discuss exposure to XOS in terms of human physiological tolerance (≥12 g/day), which supports a conclusion that the intended use of Prenexus XOS derived from sugarcane is reasonably certain to be safe. Even if consumption were to exceed 12 g/day, which, as discussed in Part 3, is highly unlikely, this would not present a safety concern as bowel discomfort, including and up to onset of transient osmotic diarrhea, would likely be quickly self-regulating by consumers.

The safety of XOS is corroborated by acute and short-term oral toxicity studies in rats and mice in which adverse effects were not observed, a chronic repeated-dose oral toxicity study in dogs in which a NOAEL was determined as 2500 mg/kg bw/day XOS, a 2-year carcinogenicity study on xylose (the ultimate hydrolysis product of XOS and xylan) in which adverse effects were not observed, the lack of serious adverse events reported in clinical trials using XOS, the history of human consumption of XOS following two previous GRAS notices, and the long history of human consumption of xylan as a component of plant-based foods.

6.8.2 Common Knowledge Element

The scientific data, information, and methods herein reported, that provide the basis of this GRAS conclusion by scientific procedures are published and available in the public domain. Part 7 of this GRAS notice contains the citations for the published studies. These publicly available data and information fulfill the requirement for general availability of the scientific data, information, and methods relied on to establish the technical element of the GRAS standard. The peer-review of the published studies, absence of Letters to the Editor or other dissenting opinions, and FDA "no questions" response letters to two previous GRAS notices for similar ingredients provide ample evidence of consensus among qualified experts that there is reasonable certainty that consumption of Prenexus XOS derived from sugarcane for its intended use is not harmful. The general availability and acceptance of these scientific data, information, and methods satisfy the common knowledge element of this GRAS conclusion.

6.9 Data and Information that are Inconsistent with the GRAS Conclusion

In a recently published chronic repeated-dose oral toxicity study in dogs, using the test item that was the ingredient subject of GRN 458, a NOAEL was determined as 2500 mg/kg bw/day XOS under a LOAEL of 5000 mg/kg bw/day XOS.[46] Under the idea that the highest NOAEL under the lowest LOAEL from toxicological studies in laboratory animals should be used in establishing an MOS for human exposure, this study would provide an MOS of approximately 29-fold. While this is lower than typically used when establishing the basis of safety of the use of a food ingredient, as discussed in Subpart 6.3.7 above, the LOAEL was determined on the basis of vomiting observed in dogs of the high-dose group, which resolved following the post-treatment recovery period. Because rats are unable to vomit, it cannot be definitively concluded that the cause of vomiting was an isolated effect that is only present in dogs, or the result of a particular methodology, although it is presumed that some form of adverse effect might have been observed in rats, which was not the case, even at much higher doses. As the authors did not report any information that would have allowed us to speculate as to a specific cause or to conclude that the cause was related to methodological problems, such as excessive dose volume, vehicle effects, and/or viscosity, we were unable to disagree with their conclusion of a LOAEL at the high-dose.

Nonetheless, humans have tolerated XOS very well in clinical trials at doses up to 12 g/day and in general, adverse effects associated with ingestion of XOS occur with low incidence, tend to be mild, rapidly adapted to, and limited to minor gastrointestinal effects of a physiological nature. Furthermore, vomiting has not been observed or reported in any clinical trials using XOS or a XOS-containing test item. Compared to other non- or poorly digested carbohydrates in the food supply, XOS appears to be generally better tolerated. Additionally, two GRAS notices for XOS-containing ingredients received FDA's "no questions" letters in 2010 and 2013, respectively, and have presumably been in the U.S. food supply since at least those times without, to the best of our knowledge, resulting in any post-market safety concerns. Thus, based on the current history of human consumption, it would appear, even if the LOEAL in dogs is a real toxicological effect, that the MOS determined from the NOAEL of this study in dogs is sufficient to assure a reasonable certainty of safety for the intended uses of Prenexus XOS derived from sugarcane. If on the other hand, this effect were either not a toxicologically relevant effect or not relevant to humans, an MOS of 136-fold could be calculated based on a NOAEL of 11.51 g/kg bw/day in female rats.

Because GRAS conclusions were able to be drawn for two other XOS-containing ingredients, and received FDA's "no questions" letters, without consideration of this

recently published study in dogs, and because of the presumed history of human use without post-market safety concerns and results of clinical trials without serious adverse effects, we considered this study as unnecessary to form the basis of a conclusion that the intended use of Prenexus XOS derived from sugarcane is safe, and therefore, we considered it to be corroborative in nature.

We are not aware of any additional data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

6.10 Information that is Exempt from Disclosure under FOIA

There are no data or information in this GRAS notice that are considered exempt from disclosure under FOIA as trade secret or commercial or financial information that is privileged or confidential.

Part 7: Supporting Data and Information

Initial literature searches for the safety assessment described in Part 6 of this GRAS notice were conducted from February 23 through June 23, 2017. In addition, literature cited in GRN 485 and GRN 343 was incorporated by reference, obtained, and reviewed during the preparation of this GRAS notice. Additional literature searches were conducted during the course of time spanning June 22, 2018 through September 26, 2018.

7.1 Data and Information that are *not* Generally Available

All of the information described in this GRAS notice is generally available.

7.2 References that *are* Generally Available

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MEMORANDUM OF MEETING (COR2018-2556)

Date: May 31, 2018

Time: 11:00 a.m. – 12:00 p.m.

Location: FDA, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, 5001 Campus Drive, College Park, MD 20740

Participants:

Visitors:

John R. Endres, Ph.D.	AIBMR Life Sciences, Inc.
Amy Clewell, Ph.D. (via WebEx)	AIBMR Life Sciences, Inc.
Anne Thiel, Ph.D. (via WebEx)	AIBMR Life Sciences, Inc.
David Keller, Ph.D.	Keller Consulting Group
Tim Brummels	Prenexus Health
Georges Bergen	DSM Nutritional Products

CFSAN/OFAS/DBGNR:

Rachel Morissette, Ph.D.	HFS-255
Jeremy Mihalov, M.S.	HFS-255
Supratim Choudhuri, Ph.D.	HFS-255

Subject: Pre-submission meeting for the intended use of xylooligosaccharide (XOS) in conventional foods

In an electronic mail message dated April 28, 2018, Dr. John Endres requested a meeting with FDA/DBGNR to discuss a GRAS conclusion for the intended use of XOS as a texturizer and source of dietary fiber in conventional foods. During the presentation, Dr. Endres stated that Prenexus Health intends to use XOS in similar foods and at use levels similar to those in GRN 000458.[1] Dr. Endres stated that the uses will be primarily substitutional; however, there will be some additional uses compared to those described in GRN 000458 that will be accounted for in their exposure assessment. Dr. Endres noted that the estimate of dietary exposure to XOS may include consideration of the market share of XOS with similar types of ingredients. FDA advised Dr. Endres to ensure that estimates of exposure that include a market share adjustment are practical given the intended use level per serving. Dr. Endres also provided information on proposed specifications, stating that they would be providing batch analyses data, and

[1] XOS for use as a bulking agent in beers and ales at a level of 0.5 grams per serving (g/serving); and, as an ingredient in breakfast bars, cereal bars, cheese, chewing gums, custards, flavored and soy milk, gelatin desserts and salads, medical foods, milk and milk products, isotonic beverages, milk desserts, ready-to-drink milk-based meal replacements, power bars, puddings, protein bars, processed fruits, juice drinks and punch, ready-to-eat cereals, sports drinks, and yogurt at levels ranging from 0.2 to 2.4 g/serving was the subject of GRN 000548, which received a "no questions" letter dated August 23, 2013.

safety studies that they intend to include as part of their safety narrative. These studies include a 26-week study in beagles, a 13-week study in rats, and human clinical studies. Dr. Endres stated that a complete and balanced literature search would be conducted since GRN 000458 was submitted, and safety information from GRN 000458 would be incorporated into the notice. DBGNR discussed the concept of incorporation into a notice and the need for the notifier to "take ownership" of the GRAS conclusion. Dr. Endres and Dr. Keller stated that though an Expert Panel was convened for this GRAS notice, the Expert Panel and its findings would not be included in the notice. Dr. Endres stated that no confidential information would be included in the notice.

In addition to the points discussed above, DBGNR attendees provided the following recommendations:

1. Include the date range for which the literature search was conducted.

2. If incorporating data and information into the notice, include the page number where the prior information can be found. Include a brief summary of the key findings from the study, emphasizing any effect that could be interpreted as potentially adverse. Provide an explanation of why the effect is not adverse. In doing so, you may incorporate the explanation provided in the previous notice, and also add your own interpretation. State clearly your independent conclusion about the outcome of the study.

3. Consider the totality and the weight of the evidence when constructing the safety narrative, including any adverse events with discussion why they are not a safety concern.

Rachel Morissette -S

Digitally signed by Rachel Morissette -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0014346356, cn=Rachel Morissette -S
Date: 2018.06.05 11:49:51 -04'00'

Rachel Morissette, Ph.D.

ATTACHED:

1. Meeting Request (email dated April 28, 2018)
2. Presentation

R/D:HFS-255:RMorissette:6/4/18, 6/5/18
Edit/Comment/Init:HFS-255:SChoudhuri:6/4/18
Edit/Comment/Init:HFS-255:JMihalov:6/4/18
Edit/Comment/Init:HFS-255:SWestBarnette:6/5/18
F/T:HFS-255:RMorissette:6/5/18

From: Perrier, Judith
To: Morissette, Rachel
Cc: West-Barnette, Shayla
Subject: FW: AIBMR: PRENEXUS FDA Pre-GRAS Notification meeting request
Date: Tuesday, May 01, 2018 10:09:30 AM

Hi Rachel,

As I mentioned on the phone, we received a presubmission meeting request from John Endres (see below). Thank you for agreeing to handle this request.

Regards,

Judy

From: johnaibmr@gmail.com [mailto:johnaibmr@gmail.com] **On Behalf Of** John Endres
Sent: Saturday, April 28, 2018 4:27 PM
To: Perrier, Judith <Judith.Perrier@fda.hhs.gov>; Carlson, Susan <Susan.Carlson@fda.hhs.gov>
Subject: AIBMR: PRENEXUS FDA Pre-GRAS Notification meeting request

Dear Judy, Susan:

We would like to schedule a GRAS pre-notification meeting per usual with you all in College Park, MD.

Would it be possible to schedule something for either:

1. Wednesday, May 30, 2018 to finish before 2 pm or
2. Thursday, May 31, 2018 to finish before 2 pm.

The ingredient in question is XOS (xylooligosaccharide) from sugar cane.
We will preparing a Powerpoint for the presentation ASAP.

We hope these dates could work for you.
Thanks very much in advance!

Best Regards,
John

John R. Endres, ND
Chief Scientific Officer
AIBMR Life Sciences, Inc.
Ph. (253) 286-2888
john@aibmr.com
www.aibmr.com
www.toxicoop.com

FDA GRAS Pre-notification Meeting,

Xylooligosaccharide (XOS)—Prenexus Health XOS from Sugarcane

AIBMR Life Sciences, Inc.

Prenexus Health

May 31, 2018

AIBMR
Life Sciences, Inc

Prenexus™
Health

ATTENDEES

John R. Endres, ND, Chief Scientific Officer
AIBMR Life Sciences, Inc.

David Keller, CEO, LLC
Keller Consulting Group,

Tim Brummels, CEO, President & Founder
Prenexus Health, Inc.

Georges Bergen, Sr. Regulatory Affairs Manager
DSM Nutritional Products, LLC

Prenexus Health

Who we are

A Natural Health Ingredient Company

Our focus

Digestive Health and Wellness

What we do

Production of Natural Prebiotic Ingredients
supported by science and clinical research

Our advantage

High Quality, High Value Nutrition from Nature that is
GMO-free, Organic, Sustainable

Our solution

Xylooligosaccarhide (XOS) Prebiotic, pure, selective - feeds the good bacteria,
effective - at a clinically effective low dose in functional foods, beverages and supplements.

Prenexus™ Health

Prenexus Health Xylooligosaccharide (XOS)

Purpose of Meeting/GRAS Panel

- Prenexus Health has independently concluded that its XOS is GRAS for its intended use as a prebiotic ingredient in food and now intends to submit a voluntary GRAS notice to FDA for evaluation.
- Prenexus Health would like to present the GRAS strategy and appreciates FDAs comments, suggestions, and any concerns

  - GRAS Panel members*:
    - Robert L. Martin., Ph.D.
    - John A. Thomas, Ph.D., FACT, FATS.
    - Madhusudan G. Soni, Ph.D, FACN, FATS

  - GRAS Panel Report prepared by:
    - Soni & Associates

* FDA GRAS Notification will not include mention of this Expert Panel

Identity, Manufacture, Specifications:

Prenexus Health Xylooligosaccharide (XOS) from Sugarcane

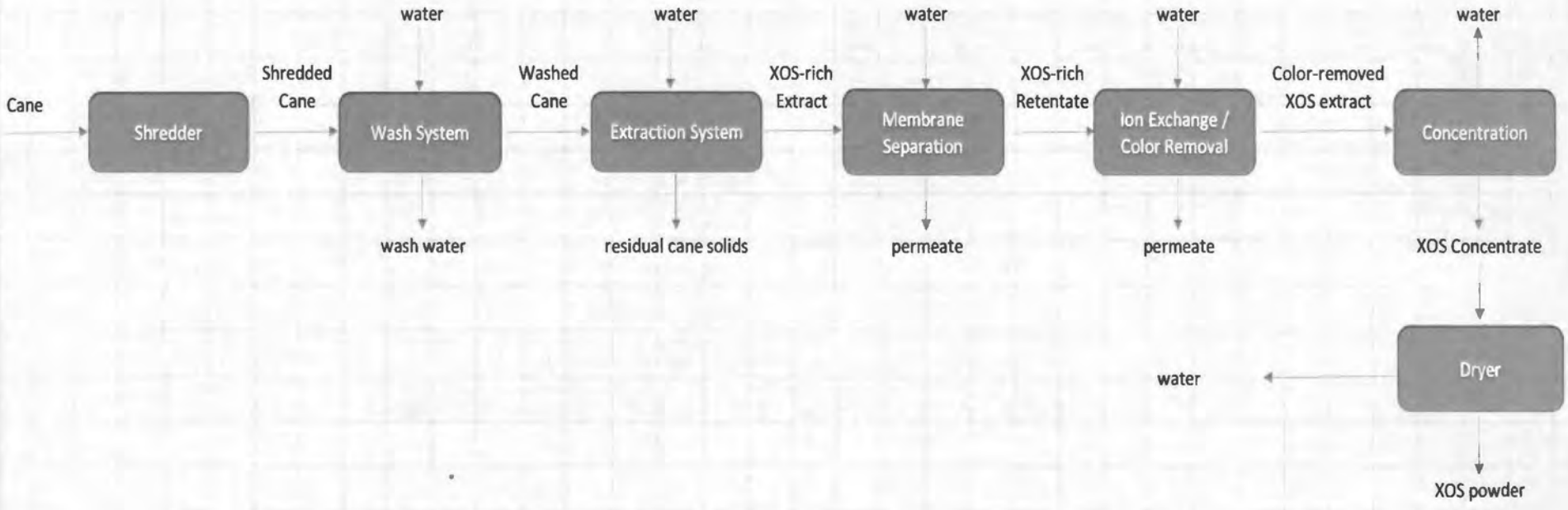
- XOS is a standardized preparation derived from sugarcane. It is non-digestible oligosaccharides comprised of 3 to 12 xylose moieties linked by β-(1-4) glycosidic bonds. The XOS preparation is a white to yellowish color powder with characteristic odor and sweet light taste.

General Descriptive Characteristics of XOS derived from Sugarcane

Parameter	Description (Prenexus, 2017)*
Botanical source	Sugarcane; *Saccharum officinarum* L.
Source synonyms	Saccharum
Product Appearance	Powder
Color	White to yellowish
Odor	Characteristic
Taste	Sweet
Storage	Store in a well closed, air tight container, protected from light and moisture, in a dry place at room temperature
Shelf life	Three years

- XOS are mixtures of oligosaccharides formed by D-xylopyranosyl residues (xylose) residues primarily linked through β-(1→4)-linkages (Figure 1). The structures of XOS vary in degree of polymerization (DP).
- *Prenexus Health XOS is* primarily composed of non-digestible xylose-based oligosaccharides (>75%) (non-digestible oligomers; n=3-12) with xylose backbones and carbohydrate monomers (12%) such as glucose, fructose, sucrose.

Manufacturing Flowchart*



** Only water is used in the extraction of XOS from sugarcane to produce Prenexus Health XOS*

Specifications

Physical, Chemical and Microbiological Specifications of *Prenexus Health XOS*

Parameter	Specifications	Assay method
Physical parameters		
Appearance	White powder	Visual, ICUMSA
Taste	Slight sweet	
Chemical parameters		
Total solids wt%	>93%	Halogen Moisture Analyzer
Total oligosacchrides (dry) wt%	>87%	HPLC
Xyoligosaccharides (XOS) wt%	> 75%	HPLC
Average DP* of XOS	3-12	HPLC
Carbohydrate monomers wt%	<12%	HPLC
Glucose/Fructose/Sucrose wt%	<12%	HPLC
Xylose wt%	<1%	HPLC
Polyphenols wt%	<2%	HPLC
Organic acids wt%	<1%	HPLC
Acetic acid wt%	<1%	HPLC
Ash	<1%	NREL Standard method
Dry matter (dm)	>96%	Halogen moisture analyzer
Heavy metals		
Lead	<0.5 ppm	AOAC 2011:19 & 993:14
Arsenic	<0.3 ppm	AOAC 2011:19 & 993:14
Cadmium	<0.5 ppm	AOAC 2011:19 & 993:14
Mercury	<0.5 ppm	AOAC 2011:19 & 993:14
Microbiological parameters		
Aerobic plate count	<25,000 cfu/g	USP C2021:7
Listeria spp.	Negative	AOAC RI 030502
Total Coliform	Negative	COLIPET:10
Salmonella	Negative	USP S2022:6
Pseudomonas	Negative	USP U2022:6
Staphylococcus	Negative	USP A2022:7
Yeast	<1000 cfu/g	USP M2021:7
Mold	<1000 cfu/g	USP M2021:7

*DP = Degree of polymerization

Specifications, cont.

- Product identity and quality is standardized by parameters such as:
  - Levels of total oligosaccharides
  - XOS
  - Carbohydrate monomers
  - Xylose
  - Polyphenols
  - Organic acids
- Final product:
  - >75% XOS
  - Carbohydrate monomers (glucose, fructose and sucrose up to 12%)
  - Xylose (<1%)
  - Polyphenols (<2%)
  - Organic acids (<1%)
- Sum of all analyzed components demonstrates that *Prenexus Health XOS* is fully characterized (~100%) for its constituents

Technical Effects

- Texturizer
- Ingredient added to food to increase dietary fiber in the diet (pending citizen's petition)

Intended Uses

- Baked goods and Baking mixes
- Beverages and beverage bases
- Breakfast cereals
- Frozen dairy desserts
- Gelatin and puddings
- Grain products and pastas
- Jams and jellies
- Milk products
- Processed fruits and fruit juices
- Processed vegetables and vegetable juices
- Snack foods

Exposure

- Similar foods as were in GRN 458
- Based on NHANES; 2009-2010*
- 100% presence probability for each category at an average addition of 1.3 g/serving.
- In GRN 458 the cumulative daily exposure was:
  - Mean: 5.1 g/day
    (equivalent to 85 mg/kg bw/day)
  - 90th percentile: 9.8 g/day
    (equivalent to 163 mg/kg bw/day)

- Intended uses of *Prenexus Health XOS* are largely expected to have a substitutive effect for the intended uses in GRN 458

*This will be updated by AIBMR using Creme software and the most recent 2013–2014NHANES data (2015–2016 NHANES data is expected to be released soon)

Exposure Discussion

- The 100% presence probability factor being used is considered to be an extremely conservative estimation of exposure for the following reasons:
  - It is nearly impossible that an individual will randomly or intentionally consume a product containing this powder every single time that he/she consumes a product from the intended use food categories daily over a lifetime.
  - It is assumed that the ingredient will likely be invisible to many consumers, which decreases the chance that only food products that contain the ingredient will be chosen by those consumers.
  - There will be cost and market share limitations of adding this specialty ingredient to foods in general.
- Actual exposure is likely to be considerably less
- A 20% Presence probability is still quite conservative and may be used

Self-limiting Levels of Use

- Excessive amounts of use levels of *Prenexus Health XOS* is technologically self-limiting because of organoleptic properties, texture, and formulation challenges that will limit use in foods/beverages.

Experience Based on Common Use in Food Prior to 1958

- The GRAS conclusion for Prenexus Health *XOS* is based on scientific procedures and thus experience based on common use in food prior to 1958 is not considered pivotal information.

Safety Narrative

- Based on the totality of available evidence
- Incorporates by *inclusion* the safety narrative and references of GRN 458 (and 343) for which the FDA had *no questions* with regard to the intended uses

Comparison of XOS with Xylose Based Polysaccharides and other Non-digestible Oligosaccharides Commonly Found in Foods

Carbohydrates	Chemical classification	Digestibility/ absorption	Fermentation	Food Uses; GRAS status
Xylose based mono and polysaccharides				
XOS	Oligosaccharides	Non-digestible	Fermented	GRAS- GRN 343; 458
Xylose	Monosaccharide	Mostly absorbed	Negligible	Commonly found in food
Xylan	Polysaccharide	Non-digestible	Fermented	Commonly present in plants; humans are exposed
AXOS	Oligosaccharide	Non-digestible	Fermented	GRAS- GRN 343
Similar non-digestible oligosaccharides				
FOS	Oligosaccharide	Non-digestible	Fermented	GRAS- GRN 623; 605; 576; 537; 392; 44
GOS	Oligosaccharide	Non-digestible	Fermented	GRAS- GRN 620; 569; 518, 495; 489; 484; 334; 286; 285; 236; 233
IMOS	Oligosaccharide	Non-digestible	Fermented	GRAS- GRN 246;

Safety Narrative, cont.

- 26-week study in beagles (0, 1250, 2500, 5000 mg/kg bw/day)
  - NOAEL 2500 mg/kg bw/day
- 13-week study in Sprague-Dawley rats:
  - NOAEL (the highest dose groups tested):
    - 11,510 mg/kg bw/day (males)
    - 14,950 mg/kg bw/day (females)
- Clinical Trials in which notifier of GRN 458 discussed were well tolerated:
  - For example:
    - Graduated dosing over 4 weeks from 3 g/day to 12g/day was well tolerated
    - Incidence of diarrhea was similar to the control group
- The safety narrative presented in GRN 458 will be discussed in this FDA GRAS Notification
- A thorough review of the literature published since GRN 458 with respect to safety will be conducted and will discuss the "good the bad and the ugly"

Basis for GRAS Conclusion

- Use in foods will necessarily be at relatively high levels due to the intended use as a prebiotic fiber
- The amount of test material that is possible to administer to animals is limited with respect to the typical goal of establishing a ≥ 100-fold MOS.
- A 15-fold MOS can be calculated by dividing the NOAEL in beagles of 2500 mg/kg bw/day (the middle dose group) from a 26-week study by the estimated daily intake at the 90th percentile (163 mg/kg bw/day) based and a 100% presence probability.
- A 70-fold MOS can be calculated by dividing the NOAEL of 11,510 mg/kg bw/day (the highest dose group in male rats) from a 90-day feeding study by the estimated daily intake at the 90th percentile (163 mg/kg bw/day) based and a 100% presence probability.
- The MOS will likely increase significantly when Creme Global probabilistic modeling software is used for the specific NHANES food categories and a 20% presence probability for the total population.
- Not mutagenic, not clastogenic.

Basis for GRAS Conclusion cont.

- Because of the food-like nature of the ingredient, being essentially composed of typical components of plant foods commonly found in the human diet for thousands of years, as well as the totality of evidence supporting the safety of the ingredient as described above, the MOS is considered reasonable and adequate for this ingredient and supports a conclusion that the intended use of Prenexus Health *XOS* is reasonably certain to be safe.

- We are not aware of any data or information that are, or may appear to be, inconsistent with a conclusion of GRAS status.

- There are no data or information in this presentation that is considered trade secret or commercial or financial information that is privileged or confidential.

Thank You!

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Prenexus™ Health

From: Tim Murbach, ND, DABT <tim@aibmr.com>
Sent: Wednesday, March 6, 2019 4:55 PM
To: Harry, Molly <Molly.Harry@fda.hhs.gov>
Subject: Re: GRN 000816 - Xylooligosaccharides Derived from Sugarcance

Dear Molly,

Pleases find our responses to your March 5, 2019 questions below in red and the requested amended Tables 4 and 5 attached. Please let me know should you have further questions.

1. In section 2 (page 13), you state that your XOS contains "small quantities" of organic acids (e.g., acetic acid). You have also provided the specifications for polyphenols and organic acids to be <3.0% w/w (Tables 1 and 2 (pages 14 & 15)). Please clarify if your final product is acidic, if so, why you have not specified the pH of the XOS final product.

   Response: pH is measured in solution. Prenexus XOS derived from sugarcane is a dry powder. A pH specification has no meaning in the context of a dry powder ingredient, and thus, has no bearing on the safety of a powered ingredient. For these reasons it was not considered necessary to set a specification for pH of the Prenexus XOS derived from sugarcane final product.

2. In section 3.1 (page 17), many of the food categories that you intend to add XOS to are also the same food categories listed in previous XOS GRNs (e.g., GRN 000458). Please confirm if the intended use of your XOS in food categories that are common with previous GRNs on XOS will be substitutional.

   Response: Yes, the intended uses of Prenexus XOS derived from sugarcane in food categories common with previous GRNs on XOS will be substitutive in terms of the XOS content of the respective ingredients. For our exposure analysis, we calculated these substitutive uses as background exposure as shown, in Tables 4 & 5, and discussed in section 3.2 on pages 18–22.

3. In section 3.2 (pages 18-22) you estimated the dietary exposure to be 3845 mg/d or 84.9 mg/kg bw/d. We would like you to provide the number of servings per day, and express the EDI (at the mean and 90th percentile levels) as per serving XOS consumption as well (mg/serving). Please provide updated Tables 4 and 5.

   Response: Our exposure estimates were calculated as g/p/d (or g/kg bw/d). These numbers do not vary based on serving size while daily intake of XOS stated as servings is only relevant if the serving size of the specific food consumed is known. As food serving sizes are quite variable, it is not possible to perform probabilistic aggregate exposure analyses based on servings per day. Therefore, we have back calculated this information in order to provide a response to this question.

   Use concentration inputs to Creme Global software need to be given as standardized concentrations (e.g., g XOS/100 g food). Food serving sizes vary widely. For example,

if 2.4 g XOS is to be added to a 40 g nutrition bar, the input to Creme Global for that nutrition bar could be 6 g XOS/100 g bar (or 6%).

Creme Global software uses NHANES consumption data in order to calculate estimates of aggregate exposure in the total population and/or subpopulations from the addition of XOS at input concentrations per food code in the selected foods. Based on input concentrations, the amount of XOS per serving size of any specific food may vary from food to food as different foods have different serving sizes and different input concentrations.

Based on the intended use, back calculations using input concentrations and NHANES consumption data indicate the amount of XOS from Prenexus XOS derived from sugarcane may range from 0.3 g to 2.4 g XOS in a serving of the various selected foods. The mean amount of XOS in a serving of food contained in the intended use is 2.2 g. The closeness of the mean to the maximum amount shows that XOS will be added to the majority of foods in which Prenexus XOS derived from sugarcane is intended for use at the maximum amount of 2.4 g per serving of food.

Based on a mean additional level of 2.2 g per serving of food the EDI can be back calculated "as per serving XOS consumption." For example, 3845 mg/p/d (or 84.9 mg/kg bw/d) can be expressed as follows:

> 3.845 g/2.2 g = 1.7 serving XOS/p/d (or 0.0386 servings XOS/kg bw/d) at the 90th percentile at the mean serving size added to foods.

Based on the minimum and maximum serving range of 0.3 g to 2.4 g XOS the 90th percentile EDI ranges from 1.6 to 12.8 servings of foods containing XOS/p/d.

Please find amended Tables 4 and 5 attached.

Kind Regards,

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Table 4. Total (Aggregate) Absolute Exposure to XOS by Proposed Use Food Consumers Using NHANES 2013–14 data (mg/day)

Data Set	Presence Probability (%)	N (% of total population)	Aggregate absolute consumption of XOS by food consumers (ages 2+) Daily Average (mg/day)					Lifetime 90th% Exposure Estimates (mg/day)	Addition level of XOS per serving of food (mg/serving at min, mean, and max)	Lifetime 90th% Exposure Estimates (servings/kg bw/day and min, mean, and max)
			Mean	Mean std err	90th%	90th% std err	90th% RSE Value			
Background uses[a]	100	6847 (97.6)	4814	78.0	9352	183.5	2.0	7647*	300, 2200, 2400	25.5, 3.5, 3.2
Background uses plus intended uses[b]	100	7046 (99.7)	12589	116.7	20738	241.6	1.2	18424*		61.4, 8.4, 7.7
Background uses plus intended uses[c]	100 (bkgd) 20 (uses)	6949 (98.7)	7206	87.0	13234	192.2	1.5	10468*		34.9, 4.8, 4.4
Background uses plus intended uses[d]	20	5883 (83.4)	2987	49.5	5882	120.0	2.0	3845*		12.8, 1.7, 1.6

[a]Creme run #308; [b]Creme run #392; [c]Creme run #394; [d]Creme run #395

*Creme Warning -2048 "Number of days per person should be consistent for a foods calculation"; data can still be used

Table 5. Total (Aggregate) Exposure to XOS by Proposed Use Food Consumers Relative to Body Weight Using NHANES 2013–14 data (mg/kg bw/day)

Data Set	Presence Probability (%)	N (% of total population)	Aggregate consumption of XOS by food consumers (ages 2+) relative to body weight Daily Average (mg/kg bw/day)					Lifetime 90th% Exposure Estimates (mg/kg bw/day)	Addition level of XOS per serving of food (mg/serving at min, mean, and max)	Lifetime 90th% Exposure Estimates (servings/kg bw/day and min, mean, and max)
			Mean	Mean std err	90th%	90th% std err	90th% RSE Value			
Background uses[a]	100	6847 (97.6)	84.6	1.5	184.3	4.3	2.3	160.2*	300, 2200, 2400	0.53, 0.07, 0.07
Background uses plus intended uses[b]	100	7046 (99.7)	214.4	3.0	415.0	11.3	2.7	395.1*		1.32, 0.18, 0.16
Background uses plus intended uses[c]	100 (bkgd) 20 (uses)	6949 (98.7)	126.8	2.1	264.9	7.5	2.8	232.7*		0.78, 0.11, 0.10
Background uses plus intended uses[d]	20	5883 (83.4)	51.0	1.0	107.1	3.8	3.5	84.9*		0.28, 0.04, 0.04

[a]Creme run #308; [b]Creme run #392; [c]Creme run #394; [d]Creme run #395

*Creme Warning -2048 "Number of days per person should be consistent for a foods calculation"; data can still be used.