合泰新光
SYNLIGHT BIO
四川合泰新光生物科技有限公司

5 May 2020

Office of Food Additive Safety HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Re: β-glucan from *Agrobacterium sp. ZX09* for Use in Select Food Categories

Dear Sir or Madam:

Accompanying this letter is a Notice, pursuant to the regulations of the United States Food and Drug Administration found at 21 CFR Part 170, setting forth the basis for the conclusion reached by the submitter, Sichuan Synlight Biotech, Ltd., that beta-glucan from *Agrobacterium sp.* ZX09 is Generally Recognized as Safe (GRAS) under the intended conditions of use described in the Notice. The Notice is contained in a binder. In addition, we have included a CD that contains a complete copy of the Notice. I hereby certify that the electronic files on the CD were scanned for viruses using Symantec Endpoint Protection prior to submission and the CD is virus-free.

Sincerely,

Du Lingling
Technical Director
Sichuan Synlight Biotech, Ltd
Email: wangmeng@salecan.com.cn

GRAS NOTICE FOR BETA-GLUCAN FROM *AGROBACTERIUM SP.* ZX09 FOR USE IN SELECT FOOD CATEGORIES

SUBMITTED TO:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

SUBMITTED BY:
Sichuan Synlight Biotech Co., Ltd.
Chengnan Industrial Park
Economic Development Area
Luojiang District, Deyang, City
Sichuan Province, China

DATE:
27 July 2020

GRAS Notice for Beta-Glucan from *Agrobacterium sp.* ZX09 for Use in Select Food Categories

TABLE OF CONTENTS

List of Appendices

List of Figures and Tables

GRAS Notice for β-Glucan from *Agrobacterium sp.* ZX09 for Use in Select Food Categories

Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through §170.285, Sichuan Synlight Biotech, Ltd. hereby informs the United States (U.S.) Food and Drug Administration (FDA) that beta-glucan derived from *Agrobacterium sp.* ZX09 (referred to as β-glucan herein), manufactured by Sichuan Synlight Biotech, Ltd., is not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act* based on Sichuan Synlight Biotech, Ltd.'s view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below. In addition, as a responsible official of Sichuan Synlight Biotech, Ltd., the undersigned hereby certifies that all data and information presented in this notice represents a complete and balanced submission that is representative of the generally available literature. Sichuan Synlight Biotech, Ltd. considered all unfavorable as well as favorable information that is publicly available and/or known to Sichuan Synlight Biotech, Ltd. and that is pertinent to the evaluation of the safety and GRAS status of β-glucan from *Agrobacterium* sp. ZX09 as a food ingredient for addition to select food products, as described herein.

Signed,

[redacted] 2020.07.22

Du Lingling Date

1.1 Name and Address of Notifier

Sichuan Synlight Biotech Co., Ltd.
Chengnan Industrial Park
Economic Development Area
Luojiang District, Deyang, City
Sichuan Province, China

1.2 Common Name of Notified Substance

β-glucan; Salecan®

1.3 Conditions of Use

β-glucan from *Agrobacterium sp.* ZX09 is intended to be added as a food ingredient to the food categories listed below in Table 1.3-1. β-glucan in the added foods will function as a stabilizer and thickener. Food codes representative of each proposed food use were chosen from the U.S. National Health and Nutrition Examination Survey (NHANES) 2015-2016 (CDC, 2018).

1.3 Conditions of Use

β-glucan from *Agrobacterium sp.* ZX09 is intended to be added as a food ingredient to the food categories listed below in Table 1.3-1. β-glucan in the added foods will function as a stabilizer and thickener. Food codes representative of each proposed food use were chosen from the U.S. National Health and Nutrition Examination Survey (NHANES) 2015-2016 (CDC, 2018).

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for β-glucan from *Agrobacterium sp.* ZX09 in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Food Uses[a]	β-glucan from *Agrobacterium sp.* ZX09 Use Levels (%)
Baked Goods and Baking Mixes	Biscuits	0.25–0.5
	Cookies	0.25–0.5
	Cornbread, Corn Muffins, or Tortillas	0.25–0.5
	Crackers	0.25–0.5
	Croissants	0.25–0.5
	Doughnuts (Donuts)	0.25–0.5
	Muffins	0.25–0.5
	Pastries	0.25–0.5
	Pies	0.25–0.5
Beverages and Beverage Bases	Energy Drinks	0.1–0.3
	Non-Milk-Based Meal Replacement Beverages and Protein Drinks	0.1–0.3
	Soft Drinks	0.1–0.3
	Sport or Electrolyte Drinks, and Fluid Replacement Drinks	0.1–0.3
Breakfast Cereals	Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	0.1–0.3
	Ready-to-Eat Breakfast Cereals	0.6
Dairy Product Analogs	Non-dairy-based Beverages	0.3–0.9
Frozen Dairy Desserts	Ice Cream	0.3–0.9
	Frozen Yogurt (Frozen Yoghurt)	0.3–0.9
Grain Products and Pastas	Cereal and Granola Bars	0.1– 0.6
	Energy Bars or Protein Bars	0.1– 0.6
	Macaroni and Noodle Products	0.3–0.9
Gravies and Sauces	Tomato-Based Sauces	0.1– 0.6
Jams and Jellies	Jams, Jellies, Preserves, and Marmalades	0.1– 0.6
Milk Products	Evaporated, Condensed, and/or Dry Milks	0.25–0.5
	Fermented Milks, Plain	0.1–0.25
	Flavored Milk and Milk Drinks	0.1–0.25
	Milk-Based Smoothies	0.1–0.3
	Milk-Based Meal Replacement and Protein Beverages	0.1–0.25
	Milk Shakes	0.1–0.25
	Plain or Flavored Yogurt (Yoghurt)	0.1–0.25
	Yogurt Drinks	0.3–0.9

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for β-glucan from *Agrobacterium sp.* ZX09 in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Food Uses[a]	β-glucan from *Agrobacterium sp.* ZX09 Use Levels (%)
Processed Fruits and Fruit Juices	Fruit Drinks and Ades, and Smoothies	0.1–0.3
	Fruit Juices	0.1–0.3
	Fruit Nectars	0.1–0.3
Processed Vegetables and Vegetable Juices	Vegetable Juices	0.1–0.3

CFR = Code of Federal Regulations; U.S. = United States.
[a] β-glucan from *Agrobacterium sp.* ZX09 is intended for use in unstandardized products when standards of identity, as established under 21 CFR §130 to §169, do not permit its addition.

β-glucan from *Agrobacterium sp.* ZX09 is not intended for use in infant formula or in products under the U.S. Department of Agriculture's jurisdiction. The intended use of that beta-glucan derived from *Agrobacterium sp.* ZX09 is substitutional for other β-glucans on the market.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a)(b) of the Code of Federal Regulations (CFR) (U.S. FDA, 2019), Sichuan Synlight Biotech, Ltd. has concluded that the intended uses of β-glucan as described herein are GRAS on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. FDA upon request, or will be available for review and copying at reasonable times at the offices of:

Sichuan Synlight Biotech Co., Ltd.
Chengnan Industrial Park
Economic Development Area
Luojiang District, Deyang, City
Sichuan Province, China

Should the FDA have any questions or additional information requests regarding this Notification, Sichuan Synlight Biotech, Ltd. will supply these data and information upon request.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Sichuan Synlight Biotech, Ltd.'s view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the Freedom of Information Act, 5 U.S.C. 552.

Part 2. § 170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

Sichuan Synlight Biotech, Ltd.'s β-glucan is a novel soluble extracellular β-glucan obtained as a result of a bacterial fermentation process by *Agrobacterium sp*. ZX09 also known as *Rhizobium pusense* ZX09. It is a high-molecular-mass polymer (about 2 x 10^6 Da) composed of a linear chain of glucosyl residues linked through a repeat unit of 7 β-(1,3) and 2 α-(1,3) glucosidic bonds (Zhang *et al.,* 2013).

Information about β-glucan is provided below. Information characterizing the identity of the production organism, *Agrobacterium sp*. ZX09, is presented in Section 2.2.

2.1.1 Chemical Name

{3)-β-D-Glup-(1→3)-[β-D-Glup-(1→3)-β-D-Glup-]3-(1→3)-α-D-Glup-(1→3)-α-D-Glup-(1→}n

2.1.2 Chemical Abstract Service (CAS) Number

1439905-58-4

2.1.3 Comparison to Other Beta-Glucans

The chemical and physical characteristics of β-glucan from *Agrobacterium sp.* ZX09 are compared to those of other β-glucans in Table 2.1.3-1.

Table 2.1.3-1 Chemical and Physical Characteristics of β-Glucans

Parameter	β-glucan from *Agrobacterium sp.* ZX09	GRN No. or CFR Regulation							
		GRN 239 (U.S. FDA, 2008)	21 CFR §172.809 (U.S. FDA, 2019)	GRN 437, 544 (U.S. FDA, 2013, 2015)	GRN 513, 698 (U.S. FDA, 2014, 2017)	GRN 413 (U.S. FDA, 2012a)	GRN 309 (U.S. FDA, 2010)	GRN 207, 344 (U.S. FDA, 2006, 2011)	GRN 412 (U.S. FDA, 2012b)
		Baker's yeast β-glucan	Bacterial Curdlan	Oat β-glucan	Algae containing paramylon	Mushroom β-glucan	Black Yeast β-glucan	Barley betafiber	Chitin-glucan
Source organism	*Rhizobium pusense*	*Saccharomyces cerevisiae*	*Alcaligenes faecalis* var. *myxogenes*	*Avena sativa*	*Euglena gracilis*	*Ganoderma lucidum*	*Aureobasidium pullulans* SM-2001	*Hordeum vulgare*	*Aspergillus niger*
Water solubility	Good	Insoluble	Insoluble	Common	Minimally	N/A	Highly soluble	Soluble	Insoluble
Appearance	Off-white or canary yellow powder	Canary yellow or tan powder	White or off-white	White	Beige to light grey/brown powder	fine light beige colored powder.	Reddish yellow powder	White to light tan powder	Light, yellowish brown powder
Odor/taste	Odorless/tasteless	Specific odor/taste	Odorless/ tasteless (or nearly)	Odorless/ tasteless	slight floral flavor and tea aroma	Mild/bland	Slight/ N/A	Odorless/ tasteless (or nearly)	Odorless/ N/A
pH of 1% water solution	Neutral	Neutral	Neutral (6.0 to 7.5%)	Neutral	N/A	N/A	Neutral (5 to 8, 20% solution)	N/A	N/A
Viscosity	High	Low	High	Middle	N/A	N/A	400 to 900 cp (18 s spindle, 3 rpm, 25°C)	High	N/A
Stability to heat and pH	Stable	Alkali-insoluble	Stable	Stable	Stable	N/A	N/A (no reported conditions to avoid)	Stable	Stable
Rheological property	Good	Good	Good	Good	N/A	N/A	N/A	N/A	N/A
CAS No.	1439905-58-4	N/A	54724-00-4	9041-22-9*, 55965-23-6**	9051-97-2	N/A	N/A	9041-22-9*, 55965-23-6**	N/A

Table 2.1.3-1 Chemical and Physical Characteristics of β-Glucans

Parameter	β-glucan from *Agrobacterium sp.* ZX09	GRN No. or CFR Regulation							
		GRN 239 (U.S. FDA, 2008)	**21 CFR §172.809 (U.S. FDA, 2019)**	**GRN 437, 544 (U.S. FDA, 2013, 2015)**	**GRN 513, 698 (U.S. FDA, 2014, 2017)**	**GRN 413 (U.S. FDA, 2012a)**	**GRN 309 (U.S. FDA, 2010)**	**GRN 207, 344 (U.S. FDA, 2006, 2011)**	**GRN 412 (U.S. FDA, 2012b)**
		Baker's yeast β-glucan	**Bacterial Curdlan**	**Oat β-glucan**	**Algae containing paramylon**	**Mushroom β-glucan**	**Black Yeast β-glucan**	**Barley betafiber**	**Chitin-glucan**
Linkage type	β-(1,3)-D-glucan and α-(1,3)-D-glucan	β-(1,3)/(1,6)-glucan (small amounts of β-1,6-glucan and chitin)	Exclusively β-1,3-linked glucose residues (Zhang and Edgar, 2014)	Linear (unbranched), mixed-linkage (1,3),(1,4)-β-D-glucans	Linear (unbranched) β-(1,3)–glucan polymer	β-(1,3),(1,6)-D-glucan	β-(1,3)/(1,6)-glucan (small amounts of β-(1,4)-glucan)	Linear (unbranched), mixed-linkage β-(1,3),(1,4)-D-glucans	Mainly β-(1,3) (~70%), and β-(1,4) (~7%) linkages with various other linkages, each present at <4%
Structure	The basic structure consists of 7 β-(1,3)-D-glucose monomers and 2 α-(1,3)-D-glucose monomers.	≥70% β-(1,3),(1,6)-D-glucan, Poly-β-(1,6)-D glucopyranosyl -β-(1,3)-D-glucopyranose.	Linear (unbranched) homopolymer of D-glucose. Average molecular weight of 100 kDa.	β-(1,3),(1,4)-D-glucan, approximately 70% (1,4) linkages and 30% (1,3) linkages.	Linear (unbranched) β-(1,3)–glucan polymer. Average molecular weight of 500 kDa.	≥50% β-(1,3),(1,6)-D-glucan, Poly-β-(1,6)-D-glucopyranosyl β-(1,3)-D-glucopyranose.	Mainly β-(1,3),(1,6)-D-glucan.	Blocks of 3 or 4 (1,4)-linked β-glucosyl units connected by (1,3) linkages. Blocks of 5 to 11 (1,4)-linked glucosyl units also present in small amounts.	Composed mainly of chitin (poly-N-acetyl-D-glucosamine) and β-(1,3)-D-glucan.

CAS = Chemical Abstracts Service; CFR = Code of Federal Regulations; GRN = Generally Recognized as Safe (GRAS) notice; N/A = not applicable.
* CAS number allocated to β-glucan that applies to β-glucan of any origin (barley, oat, mushroom, yeast, *etc.*).
** CAS number for the mixed-linkage (1-3),(1-4).-3-D-glucan.

2.2 Source Organism

β-glucan is produced *via* fermentation using *Agrobacterium sp.* ZX09 (CCTCC No. M2010020), also known as *R. pusense* ZX09. There is some debate in the literature regarding the classification of genus Agrobacterium/Rhizobium. *R. pusense* was reportedly first isolated from the rhizosphere of chickpea (Aujoulet *et al.*, 2015). Additional information related to this organism and strain in the published scientific literature was limited.

Although no reports of pathogenicity directly attributed to *Agrobacterium sp.* ZX09 (*R. pusense*) have been identified in the literature, *Agrobacterium radiobacter*, also named *Rhizobium radiobacter*, has been isolated in human beings with diverse underlying diseases and caused healthcare-associated outbreaks. Phylogenetic analysis by Aujoulet *et al.* (2015) suggests many of these opportunistic bacterial infections attributed to *R. radiobacter* could instead be attributed to *R. pusense*. Sichuan Synlight Biotech notes that during the β-1,3-glucan isolation process the fermentation organism is killed and is not actively present in β-glucan from *Agrobacterium sp.* ZX09, and thus does not pose a potential pathological safety concern to consumers.

The safety of the production organism is supported by its history of use in the production of another beta-glucan (*i.e.*, curdlan). In accordance with 21 CFR §172.809 (U.S. FDA, 2019), curdlan produced by *Alcaligenes faecalis* var. myxogenes is a food additive permitted for direct addition to food for human consumption when used in accordance with good manufacturing practice as a formulation aid, processing aid, stabilizer and thickener, and texturizer in foods for which standards of identity established under Section 401 of the Act do not preclude such use (U.S. FDA, 2019c). Subsequently, the taxonomy of this non-pathogenic curdlan-producing bacterium has been reclassified as *Agrobacterium* species (Zhan *et al.,* 2012)

Although the organism is not present in the finished ingredient, the safety of the organism was evaluated using the decision tree approach outlined by Pariza and Johnson (2001) and found to be acceptable for use. This analysis is shown in Appendix A.

Sichuan Synlight Biotech Ltd. evaluated the safety of *Agrobacterium sp*. ZX09 in a series of toxicology studies. These studies are discussed in greater detail in Section 6.1.4.

2.3 Manufacturing

2.3.1 Raw Material Specifications

The raw materials and processing aids used in the production of β-glucan from *Agrobacterium sp.* ZX09 are presented in Table 2.3.1-1. Specifications are provided in Appendix B.

Table 2.3.1-1 Raw Materials and Processing Aids

Compound	CFR Citation (U.S. FDA, 2019)
Fermentation Medium	
Calcium chloride	§184.1193 Calcium chloride
Canola oil	§184.1555 Rapeseed oil
Ferrous sulfate heptahydrate	§184.1315 Ferrous sulfate
Magnesium sulfate	§184.1443 Magnesium sulfate
Magnesium sulfate monohydrate	
Sodium chloride	§182.1 Substances that are generally recognized as safe

Table 2.3.1-1 Raw Materials and Processing Aids

Compound	CFR Citation (U.S. FDA, 2019)
Sodium dihydrogen phosphate	§181.29 Stabilizers
Potassium nitrate	§172.160 Potassium nitrate
Raw Materials	
Cane sugar	§184.1854 Sucrose
Processing Aids	
Diatomaceous earth	§182.60 Substances migrating to food from paper and paperboard products
Ethanol	§184.1293 Ethyl alcohol
Sodium Hydroxide	§184.1763 Sodium hydroxide

CFR = Code of Federal Regulations.

2.3.2 Manufacturing Process

The β-glucan ingredient is produced *via* fermentation using *Agrobacterium sp.* ZX09, also known as *Rhizobium pusense* (CCTCC No. M2010020), by Sichuan Synlight Biotech., Ltd. The preserved strain is inoculated on agar slant culture medium. After cultivation, the strain is diluted with sterile physiological saline solution and grown in solid medium to obtain a single colony. The colony is inoculated into the primary-stage seed culture medium. After shaking, the primary-stage seed liquid is obtained and inoculated into the secondary seed culture medium for culture expansion. The seeding liquid is inoculated into a sterilized fermentation medium for fermentation and food-grade cane sugar is added. The fermentation broth is pumped into a tank and ethanol is added. The precipitate is dried, milled, and sieved, yielding crude β-glucan. The crude β-glucan is then dissolved in purified water. Diatomaceous earth and sodium hydroxide are added into the crude β-glucan and the solution is filtered. The feed liquid is pumped into a tank and cooled. Ethanol is added to form a precipitate. After drying, the precipitate is milled and sieved to obtain the finished product. A schematic overview of the manufacturing process of β-glucan is provided below in Figure 2.3.2-1.

Figure 2.3.2-1 Schematic Overview of the Manufacturing Process for β-glucan from *Agrobacterium sp.* ZX09

Strain Activation

↓

Preparation of a Single Colony

↓

Preparation of Seeding Liquid

↓

Fermentation

↓

Primary Alcohol Precipitation

↓

Drying, Milling, and Sieving

↓

Crude β-1,3-glucan

↓

Dissolving

↓

Filtration

↓

Secondary Alcohol Precipitation

↓

Drying, Milling, and Sieving

↓

β-1,3-glucan

2.4 Product Specifications and Batch Analyses

2.4.1 Chemical Specifications

The chemical specifications for β-glucan from *Agrobacterium sp.* ZX09 are presented in Table 2.4.1-1. Details of the in-house analytical methods are provided in Appendix C.

Table 2.4.1-1 Chemical Specifications for β-Glucan from *Agrobacterium sp.* ZX09

Parameter	Specification	Analytical Method
Sensory Index		
Appearance[a]	Off-white or faint yellow powder	Organoleptic
Odor	Odorless	Organoleptic
Taste	Tasteless	Organoleptic

Table 2.4.1-1 Chemical Specifications for β-Glucan from *Agrobacterium sp.* ZX09

Parameter	Specification	Analytical Method
Physical and Chemical Properties		
Assay, w%	≥90.0%	UV spectrophotometry (in-house method)
Ethanol	≤0.5%	Gas chromatography (in-house method)
Nitrate[b]	≤50 ppm	HPLC (in-house method)
Moisture	≤5.0%	GB 5009.3 (Gravimetric)
Ash	≤5.0%	GB 5009.4 (Gravimetric)
Protein[c]	≤3.0%	GB 5009.5 (Kjeldahl method)
Heavy Metal		
Arsenic	≤0.5 ppm	GB 5009.11
Lead	≤0.5 ppm	GB 5009.12
Cadmium	≤0.2 ppm	GB 5009.15
Mercury	≤0.02 ppm	GB 5009.17

HPLC = high-performance liquid chromatography; ppm = parts per million; UV = ultraviolet.
[a] In the production of Salecan, the β-glucan with moisture can turn yellow in the process of drying. In addition, Salecan also contain some inorganic salts that come from fermentation medium, such as Fe^{3+}, which can also make it exhibit colors to some extent.
[b] The nitrate comes from the fermentation medium for production of β-glucan. Although the initial specification for nitrate was set to ≤100 ppm in accordance with China national standard *GB 2762 Maximum levels of contaminants in foods*, repeated analysis indicates the nitrate content is typically <20 ppm. Thus, the specification has been lowered to ≤50 ppm.
[c] Protein is a soluble protein released during the bacteria lysis in the fermentation process. The protein content in other fermented product, such as curdlan and xanthan gum, is usually far more than 3%.

2.4.2 Microbiological Specifications

The microbiological specifications for β-glucan from *Agrobacterium sp.* ZX09 are presented in Table 2.4.2-1.

Table 2.4.2-1 Microbiological Specifications for β-glucan from *Agrobacterium sp.* ZX09

Specification Parameter	Specification	Method
Total bacteria count	1,000 CFU/g	GB 4789.2
Coliforms	≤3 MPN/g	GB 4789.3
Salmonella spp.	Negative	GB 4789.4
Staphylococcus aureus	Negative	GB 4789.10
Mold and yeast	≤100 CFU/g	GB 4789.15

CFU = colony-forming units; MPN = most probable number.

2.4.3 Batch Analyses

Analysis of 3 non-consecutive lots of β-glucan from *Agrobacterium sp.* ZX09 demonstrates that the manufacturing process as described in Section 3.2 produces a consistent product that meets specifications. A summary of the chemical analysis for the 3 lots of β-glucan from *Agrobacterium sp.* ZX09 is presented in Table 2.4.3-1. Compliance with microbial specifications is shown in Table 2.4.3-2. Certificates of analysis are provided in Appendix D.

Table 2.4.3-1 Summary of the Chemical Product Analysis for 3 Batches of β-glucan from *Agrobacterium sp.* ZX09

Specification Parameter	Specification	Batch Number		
		20180115	20180111	20180518
Sensory Index				
Appearance	Off-white or faint yellow powder	Pass	Pass	Pass
Odor	Odorless	Pass	Pass	Pass
Physical and Chemical Index				
Assay, w%	≥90.0%	91.0%	90.5%	92.1%
Ethanol	≤0.5%	0.25%	0.21%	0.31%
Nitrate	≤50 ppm	24 ppm	23 ppm	43 pp
Moisture	≤5.0%	2.8%	3.5%	3.3%
Ash	≤5.0%	4.3%	4.1%	4.1%
Protein	≤3.0%	1.1%	0.8%	1.3%
Heavy Metals				
Arsenic	≤0.5 ppm	BLD	BLD	BLD
Lead	≤0.5 ppm	BLD	BLD	BLD
Cadmium	≤0.2 ppm	BLD	BLD	BLD
Mercury	≤0.02 ppm	BLD	BLD	BLD

BLD = below limit of detection; ppm = parts per million.
Limits of detection: arsenic = 0.010; lead = 0.05; cadmium = 0.03; mercury = 0.010.

Table 2.4.3-2 Summary of the Microbiological Product Analysis for 3 Lots of β-glucan from *Agrobacterium sp.* ZX09

Specification Parameter	Specification	Batch Number		
		20180115	2018011	20180518
Total bacteria count[a]	1,000 CFU/g	<10	<10	<10
Coliforms	≤3 MPN/g	<3.0	<3.0	<3.0
Salmonella spp.	Negative	Negative	Negative	Negative
Staphylococcus aureus	Negative	Negative	Negative	Negative
Mold and yeast	≤100 CFU/g	<10	<10	<10

CFU = colony-forming units; MPN = most probable number.
[a] The specification for total bacterial count is based on Chinese National Standards and is based on the limit of total bacterial count of all foods that β-glucan from *Agrobacterium sp.* ZX09 is added to. Analysis shows the actual level is far below this limit.

2.5 Stability

Sensory and chemical stability of β-glucan from *Agrobacterium sp.* ZX09 were evaluated under both real-time (25°C, 60% relative humidity) (Table 2.5-1) and accelerated storage conditions (40°C, 75% relative humidity) (Table 2.5-2). Together, these data demonstrate that β-glucan from *Agrobacterium sp.* ZX09 is stable for a period of 2 years at room temperature.

Table 2.5-1 Real Time Stability of β-glucan from *Agrobacterium sp.* ZX09

Batch #	Parameter	Standard	Time, month						
			0	**3**	**6**	**9**	**12**	**18**	**24**
131206	Appearance	Off-white or faint yellow powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder
	Assay (on the dried basis), %	≥90.0%	92.4	92.7	92.3	92.1	92.4	91.8	92.6
	Loss on drying, %	≤4.0%	1.7	1.7	1.6	1.7	1.9	2.5	2.2
131211	Appearance	Off-white or faint yellow powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder
	Assay (on the dried basis), %	≥90.0%	92.7	92.1	92.1	92.3	92.1	92.1	92.5
	Loss on drying, %	≤4.0%	1.7	1.7	1.7	1.7	1.9	2.6	2.4
131216	Appearance	Off-white or faint yellow powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder
	Assay (on the dried basis), %	≥90.0%	92.2	92.1	92.0	91.6	91.8	91.8	92.1
	Loss on drying, %	≤4.0%	1.6	1.7	1.7	1.8	2.0	2.6	2.5

Table 2.5-2 Stability of β-glucan from *Agrobacterium sp.* ZX09 Under Accelerated Storage Conditions

Batch #	Parameter	Standard	Time, month				
			0	**1**	**2**	**3**	**6**
131206	Appearance	Off-white or faint yellow powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder
	Assay (on the dried basis), %	≥90.0%	92.4	92.9	92.0	91.7	91.7
	Loss on drying, %	≤4.0%	1.7	1.6	1.6	1.7	2.2
121311	Appearance	Off-white or faint yellow powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder
	Assay (on the dried basis), %	≥90.0%	92.7	92.4	92.3	91.9	91.7

Table 2.5-2 Stability of β-glucan from *Agrobacterium sp.* ZX09 Under Accelerated Storage Conditions

Batch #	Parameter	Standard	Time, month				
			0	1	2	3	6
131206	Appearance	Off-white or faint yellow powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder	Off-white powder
	Assay (on the dried basis), %	≥90.0%	92.2	92.9	91.9	92.4	92.0
	Loss on drying, %	≤4.0%	1.6	1.6	1.8	2.3	2.8

Part 3. §170.235 Dietary Exposure

3.1 Background Dietary Intake of β-glucans

As components of cereals (*e.g.*, barley, oats) β-glucans have a long history of safe use. In GRN 344, Cargill estimated the combined average intake of barley betafiber by consumers from all uses of barley betafiber (*i.e.,* general food use and meat and poultry) would be 12.4 g/person/day (8.7 g β-glucan/person/day) (U.S. FDA, 2011). The 90th percentile intake was estimated to be 23.5 g/person/day (16.5 g β-glucan/person/day). Cargill stated that barley betafiber would be added to food at levels of 4.3 g/serving, resulting in approximately 3 g of β-glucan/serving. In GRN 544, Tate & Lyle estimated intake of β-glucan from the proposed use of its PromOat® product to be 4.35 and 8.25 β-glucan/person/day for the mean and 90th percentile, respectively) (U.S. FDA, 2015).

Bacterial curdlan (*Alcaligenes faecalis* var. myxogenes, CAS No. 54724-00-4, 21 CFR §172.809 – U.S. FDA, 2019) is used or intended for use in accordance with good manufacturing practice as a formulation aid, processing aid, stabilizer and thickener, and texturizer in foods for which standards of identity established under Section 401 of the Act do not preclude such use (U.S. FDA, 2019c). An estimate of curdlan intake is not readily available.

3.2 Regulatory Status of β-glucan from *Agrobacterium sp.* ZX09

The application for approval of β-glucan from *Agrobacterium sp.* ZX09 as a Novel Food Material in China is in progress. The application has passed the technical evaluation held by the Expert Evaluation Committee of National Health Commission of China (NHC) last year, and the result has been published on the NHC website for 3 months of public review. The application is currently undergoing administrative examination and approval, and it is expected to be approved in the first half of 2020.

3.3 Estimated Intake of β-glucan from *Agrobacterium sp.* ZX09 from Intended Food Uses in the U.S.

3.3.1 Methods

Estimates for the intake of β-glucan from *Agrobacterium sp.* ZX09 were based on the proposed food uses and maximum use levels for β-glucan (Table 1.3-1) in conjunction with food consumption data included in the U.S. National Center for Health Statistics' NHANES 2015-2016. Calculations for the mean and 90th percentile *per capita* and consumer-only intakes were performed for all proposed food uses of β-glucan and the percentage of consumers were determined. Similar calculations were used to estimate the intake of β-glucan resulting from each individual proposed food use, including the calculations of percent consumers. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

- Infants and young children, up to and including 2 years;
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;

- Male adults, ages 20 and up; and
- Total population (all age and gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of β-glucan from *Agrobacterium sp.* ZX09 by the U.S. population[1]. Estimates for the daily intake of β-glucan represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2015-2016; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of β-glucan averaged over all individuals surveyed, regardless of whether they consumed food products in which β-glucan is proposed for use, and therefore includes individuals with "zero" intakes (*i.e*., those who reported no intake of food products containing β-glucan during the 2 survey days). "Consumer-only" intake refers to the estimated intake of β-glucan by those individuals who reported consuming food products in which the use of β-glucan is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which β-glucan is proposed for use on either Day 1 or Day 2 of the survey.

3.3.2 Intake Estimates for β-glucan from *Agrobacterium sp.* ZX09

Table 3.3.2-1 summarizes the estimated total intake of β-glucan from *Agrobacterium sp.* ZX09 (g/person/day) from all proposed food uses in the U.S. population group. Table 3.3.2-2 presents this data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of consumers was high among all age groups evaluated in the current intake assessment; 76.7% of the population groups consisted of consumers of food products in which β-glucan from *Agrobacterium sp.* ZX09 is currently proposed for use. Children and female teenagers had the greatest proportion of consumers at 99.9%. The consumer-only estimates are more relevant to risk assessments, as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of β-glucan from Agrobacterium sp. ZX09 were determined to be 2.2 and 4.5 g/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of Salecan® on an absolute basis, at 2.7 and 5.5 g/person/day, respectively, while infants had the lowest mean and 90th percentile consumer-only intakes of 0.6 and 1.7 g/person/day, respectively (Table 3.3.2-1).

Table 3.3.2-1 Summary of the Estimated Daily Intake of β-glucan from *Agrobacterium sp.* ZX09 from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to <1	0.2	0.6	31.7	104	0.6	1.7
Young Children	1 to 2	1.5	3.3	98.9	366	1.6	3.3
Children	3 to 11	2.1	3.5	99.9	1,175	2.1	3.5
Female Teenagers	12 to 19	2.0	3.9	99.9	472	2.0	3.9

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

Table 3.3.2-1 Summary of the Estimated Daily Intake of β-glucan from *Agrobacterium sp.* ZX09 from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Male Teenagers	12 to 19	2.4	4.5	98.9	489	2.5	4.5
Female Adults	20 and up	1.9	3.8	97.7	2,166	1.9	3.8
Male Adults	20 and up	2.6	5.4	96.9	1,942	2.7	5.5
Total Population	All ages	2.2	4.4	97.0	6,714	2.2	4.5

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of β-glucan from *Agrobacterium sp.* ZX09 were determined to be 38 and 80 mg/kg body weight/day, respectively. Among the individual population groups, young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 124 and 263 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 27 and 54 mg/kg body weight/day, respectively (Table 3.3.2-2).

Table 3.3.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of β-glucan from *Agrobacterium sp.* ZX09 from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to <1	21	60	31.7	104	66	200
Young Children	1 to 2	123	262	98.9	361	124	263
Children	3 to 11	81	151	99.9	1,170	81	151
Female Teenagers	12 to 19	34	68	99.9	464	34	68
Male Teenagers	12 to 19	37	69	98.9	488	38	70
Female Adults	20 and up	26	53	97.6	2,152	27	54
Male Adults	20 and up	30	62	96.8	1,917	31	64
Total Population	All ages	37	79	97.0	6,656	38	80

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

3.3.3 Summary and Conclusions

Consumption data and information pertaining to the individual proposed food uses of β-glucan from *Agrobacterium sp.* ZX09 were used to estimate the *per capita* and consumer-only intakes of β-glucan from *Agrobacterium sp.* ZX09 for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates that may be considered suitably conservative. For example, it has been assumed in both exposure assessments that all food products within a food category contain β-glucan from *Agrobacterium sp.* ZX09 at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that β-glucan from *Agrobacterium sp.* ZX09 will have 100% market penetration in all identified food categories.

In summary, on consumer-only basis, the resulting mean and 90th percentile intakes of β-glucan from *Agrobacterium sp.* ZX09 by the total U.S. population from all proposed food uses, were estimated to be

2.2 g/person/day (38 mg/kg body weight/day) and 4.5 g/person/day (80 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile consumer-only intakes of β-glucan from *Agrobacterium sp.* ZX09 were determined to be 2.7 g/person/day (31 mg/kg body weight/day) and 5.5 g/person/day (64 mg/kg body weight/day), respectively, was identified among male adults.

When intakes were expressed on a body weight basis, young children had the highest mean and 90th percentile consumer-only intakes of 124 and 263 mg/kg body weight/day, respectively. Children were also identified with high mean and 90th percentile consumer-only intakes of β-glucan from *Agrobacterium sp.* ZX09 (*i.e.*, 81 and 151 mg/kg body weight/day, respectively). Although younger populations were identified as the groups having higher exposures to β-glucan from *Agrobacterium sp.* ZX09 on a body weight basis, it should be noted that products containing β-glucan from *Agrobacterium sp.* ZX09 will not be specifically targeted towards infants and children, and estimates described herein assume *all* products, including those consumed by younger individuals, would contain the ingredient at the maximum intended use levels. In actuality, these products would, in the worst case, only be consumed incidentally and intakes described in the older populations (*i.e.*, not more than 38 and 70 mg/kg body weight/day at the mean and 90th percentile, respectively) are expected to be more accurate estimates of dietary exposure among the intended population.

The intended use of that beta-glucan derived from *Agrobacterium sp.* ZX09 is substitutional for other β-glucans on the market. Thus, overall consumption is not expected to increase.

Part 4. §170.240 Self-Limiting Levels of Use

The use levels of β-glucan from *Agrobacterium sp.* ZX09 may be self-limiting at high concentrations due to undesirable effects on certain characteristics of the food such as flavor and texture. Specific thresholds for these effects have not been determined.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. §170.250 Narrative and Safety Information

To obtain information pertaining to the safety of β-glucan from *Agrobacterium sp.* ZX09, comprehensive and detailed searches of the published scientific literature were conducted by Intertek Scientific & Regulatory Consultancy through February 2019. AdisInsight: Trials, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile® served as the primary sources of published literature pertinent to the safety of β-glucan from *Agrobacterium sp.* ZX09.

6.1 Toxicological Studies

6.1.1 Acute Studies

The acute oral toxicity of β-glucan from *Agrobacterium sp.* ZX09 was evaluated (test guideline not specified) in 4-week-old male and female ICR mice (5/sex/group) (Xiu *et al*., 2011). Animals were orally administered a single dose of 3,000 mg/kg body weight (nominally 45 mg in a 15-g mouse) *via* gavage delivered in a 250-µL saline vehicle. A vehicle control group was established where animals were orally administered a single dose of the vehicle *via* gavage. All animals were observed for signs of toxicity and behavioral changes at 1 and 3 hours post-administration and once daily for 14 days. Mortality and moribundity were observed once daily and the median lethal dose (LD_{50}) values were calculated. Body weights were recorded pre-treatment, on Day 7, and upon termination on Day 15. Surviving mice were terminated on Day 15 and all organs and tissues were subject to macroscopic examination. Based on a lack of compound-related, toxicologically relevant adverse effects, the LD_{50} value for β-glucan from *Agrobacterium sp.* ZX09 was reported to be >3,000 mg/kg body weight in ICR mice (Xiu *et al.,* 2011).

The acute oral toxicity of β-glucan from *Agrobacterium sp.* ZX09 was also evaluated (test guideline not specified) in male and female Sprague-Dawley (specific-pathogen free [SPF]) rats (10/sex/group; 180 to 220 g body weight) (Sichuan University, 2015 [unpublished]). Animals were given diets providing 3,840 mg/kg body weight/day of granulated β-glucan from *Agrobacterium sp.* ZX09 for 7-consecutive days, followed by a 14-day observation period. The feed consumption was calculated based on 8% animal weight and the dose was selected based on the recommended adult dose of 3,000 mg/day. Based on a lack of compound-related, toxicologically relevant adverse effects, an LD_{50} value of >3,840 mg/kg body weight was implied in rats.

6.1.2 Subchronic and Chronic Studies

A 90-day dietary toxicity study was conducted in ICR mice to evaluate the repeat-dose toxicity of β-glucan from *Agrobacterium sp.* ZX09 (Xiu *et al.,* 2011). Mice were divided into 4 groups (10/sex/group) and were given diets providing 0, 1, 2.5, or 5% (w/w) (approximately 2,896, 7,239, or 14,478 mg/kg body weight/day) for 13 weeks. The mice were caged individually and given water and diets *ad libitum*. The mice were monitored daily for mortality and clinical signs. Body weights and individual food and water consumptions were recorded weekly. Feed conversion efficiency was calculated using the method described by Janaki and Sashidhar (2000). Two days before the end of the treatment period the mice were housed in metabolic cages and the 24-hour fecal samples were collected, dried, and analyzed for moisture content (%). Prior to exsanguination but after administration of ether (the anesthetic), body temperatures were measured and 3 µL of blood was obtained by tail bleed to determine the total blood glucose. Blood samples were collected by cardiac puncture and analyzed for hemoglobin concentration, red blood cell count, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, white blood cell count (WBC), and the differential WBC (neutrophils, eosinophils, basophils, lymphocytes, and monocytes). Serums from the blood samples collected in separator tubes were analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, triglyceride, total cholesterol, total protein, total bilirubin, creatinine, blood urea nitrogen, calcium, inorganic phosphorous, potassium, sodium, and chloride. After exsanguination, organs and tissues (spleen, heart, liver, lung, cholecystic, pancreas, stomach, colon, kidney, and urinary bladder) of the mice from the treated groups were examined macroscopically and compared to the control group. Histopathological examinations of the liver, kidney, spleen, and heart were performed for all animals.

All animals survived, were healthy, and did not show any unusual behavior throughout the study period. A statistically significant increase in fecal bulk was reported in β-glucan-fed mice in both sexes compared to the control group; however, the authors reported that these significant differences did not demonstrate a dose-response relationship and that the increase in fecal bulk could have been due to the highly water-soluble, hydroscopic, and viscous nature of this glucan. A statistically significant decrease in inorganic phosphorous was reported in high-dose males compared to the control group; however, the authors reported that this effect did not correlate with concurrent changes in other clinical parameters and that the effect was within historical control values of the lab and was therefore not considered toxicologically relevant and attributed to random occurrence. A statistically significant decrease in fasting blood glucose was reported in the mid- and high-dose females compared to the control group; however, the authors discussed how this effect is generally considered a desirable therapeutic effect and how of β-glucan from *Agrobacterium sp.* ZX09 may have a role in functional foods for diabetics. The authors reported that there were no other significant effects regarding any other measured parameter.

Based on the results of this study, the authors concluded that doses of β-glucan from *Agrobacterium sp.* ZX09 up to 14,478 mg/kg body weight/day (the highest dose tested) did not result in any compound-related, toxicologically relevant adverse effects. The authors therefore reported a no-observed-adverse-effect level (NOAEL) of 14,478 mg/kg body weight/day for mice in a 90-day feeding study (Xiu *et al*., 2011).

A second 90-day dietary toxicity study was conducted in Sprague-Dawley rats (SPF) to evaluate the repeat-dose toxicity of β-glucan from *Agrobacterium sp.* ZX09 (test guideline not specified) (Sichuan University, 2015 [unpublished]). Rats were randomly divided into 4 groups (10/sex/group) and given diets providing 960 (low-dose), 1,920 (middle-dose), or 3,840 (high-dose) mg/kg body weight/day of granulated β-glucan from *Agrobacterium sp.* X09 for 90 consecutive days. The control group was given a basal diet with ordinary feed. The feed consumption was calculated based on 8% animal weight. General condition and clinical signs were recorded daily, animals and surplus feed were weighed once and twice a week, respectively, for the first month, to calculate feed consumption and weekly feed utilization rates. Monthly utilization rates were calculated from weekly body weight and surplus feed weights taken during the second month. Blood was collected from the eye sockets on Day 45 for routine analysis. All groups were terminated on Day 90 and blood samples were collected from the femoral artery to evaluate hematological and biochemical parameters. Gross pathological changes of the liver, kidney, spleen, and testes or ovaries were evaluated, and these tissues were hematoxylin- and eosin-stained and evaluated for microscopic pathological changes.

All animals survived until the end of the study period and no significant changes were observed in behavior, feed and water consumption, urination and defecation, clinical signs of toxicity, organ weights, or any hematologic parameters on Day 45. Significantly decreased body weight gains in treated male rats were observed in Month 2, but there were no significant differences in body weight gain between other test groups and the control group and it was reported that β-glucan from *Agrobacterium sp.* ZX09 had no significant negative impacts on animal growth and development. Significantly decreased food utilization rates in female rats in the low- and middle-dose group were observed in Week 3 but there were no significant differences in weekly or monthly feed consumption, utilization rates, or total food utilization between other test groups and the control group and the authors reported that of β-glucan from *Agrobacterium sp.* ZX09 had no harmful effects on animal body weight and feed utilization rates. Significantly higher kidney and spleen organ coefficients (*i.e.,* organ weight/animal weight) were observed in females from the low-dose group but there were no significant differences in the organ weights and coefficients of the major organs between the other test groups and the control group. Significantly increased granulocyte rates and middle cells rates[2] were reported in females in the low- and middle-dose groups at the end of the study whereas the lymphocyte rates were significantly decreased in the same groups. Significantly increased WBCs were reported in the male rats in the high-dose group; however, the authors also reported that all noted changes in hematological parameters were within historical control values of the laboratory and were not considered biologically relevant. There were no other significant differences in the measured hematological parameters between other test groups and the control groups. The ALT and AST levels were significantly reduced in the male and female rats of the low- and middle-dose groups compared to the control groups; however, the authors reported that all noted changes in biochemical parameters were within historical control values of the laboratory and were not considered biologically relevant. There were no other significant differences in the measured biochemical parameters between other test groups and the control groups. Histological examinations were only performed on the livers, kidneys, stomachs, jejunums, spleens, and testes or ovaries of the high-dose and control groups because no pathological changes were observed in the gross examinations. Slight hepatic granuloma and acute episodes of chronic pyelitis and calcium deposition in kidneys were reported in individual animals; however, the abnormal alterations were not significant between treated and control groups and the authors reported that these effects were therefore considered spontaneous pathological alterations (Sichuan University, 2015 [unpublished]).

Based on the results of this study, a no-observed-effect level of 3,840 mg/kg (the highest dose tested) was implied due to a lack of compound-related, toxicologically relevant adverse effects in regard to the measured clinical, histological, biochemical, and hematological parameters (Sichuan University, 2015 [unpublished]).

6.1.3 Genotoxicity

6.1.3.1 In Vitro Tests

A reverse bacterial mutation assay (test guideline not specified) of β-glucan from *Agrobacterium sp.* ZX09 was conducted in *Salmonella typhimurium* TA97, TA98, TA100, and TA102 using the plate incorporation method (Sichuan University, 2015 [unpublished]). β-glucan from *Agrobacterium sp.* ZX09 was tested twice in triplicate at concentrations of 1.6, 8, 40, 200, or 1,000 μg/plate in the presence and absence of metabolic activation. A solvent control group, consisting of distilled water (100 μL/plate), was also evaluated, as were the following positive control groups: 2,4,7-Trinitro-9-fluorenone (0.2 μg/plate) for TA97 and TA98 without metabolic activation, sodium azide (1.5 μg/plate) for TA100 without metabolic activation, and mitomycin C (0.5 μg/plate) for TA102 without metabolic activation, 2-aminofluorene (10 μg/plate) for TA97, TA98, and TA100 with metabolic activation, and 1,8-dihydroxyanthraquinone (10 μg/plate) for TA102 with metabolic activation. The number of revertant colonies was detected by standard plate incorporation assay.

The number of revertant colonies did not significantly change at concentrations of β-glucan from *Agrobacterium sp.* ZX09 up to 1,000 μg/plate in the presence or absence of metabolic activation compared to the solvent control group. Based on the results of this study, the authors concluded that β-glucan from

[2] The classification of leukocytes in rat blood was carried out by 3 classification software, namely, lymphocytes, neutrophils and "middle cells". The "middle cells", also called "intermediate cells", refer to the leukocytes other than neutrophils and lymphocytes, which include eosinophils, basophils and monocytes. Because the quantity and proportion is very small, they are commonly grouped in 1 category. The normal value of middle cells rate generally range from 3 to 6%. In this experiment, the proportion of these 3 types of leukocytes has certain difference between groups, but the change is in the normal range of the laboratory, and therefore not considered to be of toxicological significance.

Agrobacterium sp. ZX09 was not mutagenic to tested strains under the conditions of this study (Sichuan University, 2015 [unpublished]).

6.1.3.2 In Vivo Studies

A mammalian erythrocyte micronucleus test was conducted (test guideline not specified) in male and female Kunming mice (5/sex/group; 25 to 30 g in weight) (Sichuan University, 2015 [unpublished]). Animals were given diets providing 960, 1,920, or 3,840 mg/kg body weight/day of granulated β-glucan from *Agrobacterium sp.* ZX09 for 5 consecutive days, where feed consumption was calculated based on 8% animal weight. A negative control group was given ordinary feed for 5 days and a positive control group was given 40 mg cyclophosphamide (CTX)/kg body weight *via* gavage (2 mL/100 g body weight) 6 and 32 hours before sacrifice. The micronucleus polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) of the bone marrow were observed and measured.

Significantly increased micronucleus induction rates were reported in the positive control group compared to the negative control group and there was no significant difference in the micronucleus induction rate of the β-glucan-treated and negative control groups. The proportions of PCEs in the total erythrocyte counts were above 20% of the control values and there was no significant difference in the PCE/NCE between β-glucan-treated and negative control groups. Based on the results of this study, the authors concluded that doses up to 3,840 mg/kg body weight/day β-glucan from *Agrobacterium sp.* ZX09 had no impact on proliferation of rat bone marrow cells and did not increase the micronucleus induction rate in male and female Kunming mouse bone marrow cells (Sichuan University, 2015 [unpublished]).

A sperm abnormality test was conducted (test guideline not specified) in male Kunming mice (5/group; 25 to 35 g in weight) (Sichuan University, 2015 [unpublished]). Animals were given diets providing 960, 1,920, or 3,840 mg/kg body weight/day of granulated β-glucan from *Agrobacterium sp.* ZX09 for 35 consecutive days, where feed consumption was calculated based on 8% animal weight. A negative control group was given ordinary feed and a positive control group was given 40 mg CTX/kg body weight *via* gavage (2 mL/100 g body weight) for 5 days. Animals were sacrificed after 35 days of the dosing regimen and bilateral epididymis were taken for pathological assessment of the number of deformed sperm.

Significantly increased sperm abnormality rates were reported in the positive control group compared to the negative control group and there was no significant difference in the rate of abnormal sperm in the β-glucan-treated and negative control groups. Based on these results, the authors concluded that doses up to 3,840 mg/kg body weight β-glucan from *Agrobacterium sp.* ZX09 did not cause a higher sperm abnormality rate in male Kunming rats (Sichuan University, 2015 [unpublished]).

6.1.4 Studies on the Production Organism

The safety of *Agrobacterium sp*. ZX09 was evaluated in a series of toxicology studies. The acute oral LD_{50} of the bacterial suspension of *Agrobacterium sp*. ZX09 to determined greater than 10,000 mg/kg body weight in rats and mice. No mutagenicity or genotoxicity was seen in a bacterial reverse mutation test, a mammalian erythrocyte micronucleus test, and an *in vitro* chromosomal aberration test on mammalian cells) indicate that the sample has no genetic toxicity (Sichuan Synlight Biotech, 2018 [unpublished]).

Finally, no toxicologically significant adverse events were seen on hematology, clinical chemistry, or urinalysis parameters in male or female Sprague Dawley rats (10 animals/sex/group) administered suspensions of Agrobacterium sp. ZX09 at 2.5, 5.0, or 10 mL/kg body weight/day for 90 days. Although feed consumption and body weight were significantly decreased in high dose females, food utilization was not significantly affected. Upon histopathological examination, hyperplasia of gastrointestinal and mesenteric lymph node was observed in female animals of high dose females. These changes were considered to be compensatory response to the foreign living bacterium (Sichuan Synlight Biotech Ltd. 2018, [unpublished]).

6.1.5 Other Studies

The protective effects of β-glucan from *Agrobacterium sp.* ZX09 against alcoholic liver injury, carbon tetrachloride induced liver injury, and concanavalin A-induced acute liver injury have been evaluated (Chen *et al.*, 2012, 2014; Sun *et al.*, 2017). In addition, β-glucan from *Agrobacterium sp.* ZX09 was shown to control postprandial blood glucose (Xiu *et al*., 2010), serve as hydrophilic laxative for constipation (Zhou *et al*., 2013), decrease fat absorption and improve glucose tolerance and (Zhang *et al.,* 2013) in rodents. Additional studies in animal models demonstrated prebiotic effects (Zhou *et al.,* 2014a), anti-fatigue effects (Xu *et al.* 2018), and anti-inflammatory effects with colitis induced by dextran sulphate sodium (Zhou *et al.*, 2014b). Finally, β-glucan from *Agrobacterium* sp. ZX09 was shown to improve growth performance and to exhibit beneficial effects on mucosal barrier function and microbial populations in weaned piglets (Luo *et al*., 2019). Although these studies were not intended to evaluate the safety of β-glucan from *Agrobacterium sp.* ZX09, the absence of adverse effects in any of the treated animals provides supporting evidence of safety.

6.2 Allergenicity

The potential allergenicity of β-glucan was researched in the available literature, but no relevant information was located. Sources of β-glucan concentrates, including *Agrobacterium* (as a source of curdlan), have been safely consumed for decades. β-glucan is not listed among the U.S. FDA's list of the 8 major food allergens (U.S. FDA, 2018). The lack of available information and absence from the FDA's list suggests that allergic reactions to β-glucan itself are not of concern.

6.3 Immunomodulatory Effects

Numerous articles have reported the immunostimulatory effects of β-glucans. Activation of cells of the mucosal immune system *via* β-glucan receptors depends on its branched structure. A β-1,3-glucan without any side chains (branches) does not activate macrophages. Products with only 1 single glucose molecule in the 'side chain', as in most mushroom beta-1,3/ 1,6-glucans (*e.g.*, lentinan), have lower macrophage activating activity than yeast cell-wall beta-1,3/1,6-glucan (Raa, 2015). As an unbranched molecule, that β-glucan from *Agrobacterium sp.* ZX09 would be anticipated to have little or no immunomodulating activity compared with other commonly consumed β-glucans.

6.4 GRAS Panel Evaluation

Sichuan Synlight Biotech, Ltd. has concluded that β-glucan from *Agrobacterium sp.* ZX09 is GRAS for use in select categories as described in Section 1.3, on the basis of scientific procedures. This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of β-glucan from *Agrobacterium sp.* ZX09, as discussed herein, and on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel consisted of the following qualified scientific experts: Dr. Stanley M. Tarka, Jr. (Adjunct Associate Professor, The Pennsylvania State University College of Medicine), John A. Thomas (Indiana University School of Medicine Indianapolis), and David H. Bechtel (President, Bechtel Consulting Inc.).

The GRAS Panel, convened by Sichuan Synlight Biotech, Ltd., independently and critically evaluated all data and information presented herein, and also concluded that β-glucan from *Agrobacterium sp.* ZX09 is GRAS for use in conventional food products as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the GRAS Panel, and evaluation of such data as it pertains to the proposed GRAS uses of β-glucan from *Agrobacterium sp.* ZX09 is presented in Appendix E.

6.5 Conclusion

Based on the above data and information presented herein, Sichuan Synlight Biotech, Ltd. has concluded that β-glucan from *Agrobacterium sp.* ZX09 is GRAS, on the basis of scientific procedures, for use in food and beverage products as described in Section 1.0. General recognition of Sichuan Synlight Biotech, Ltd.'s GRAS conclusion is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of β-glucan from *Agrobacterium sp.* ZX09 in food, who similarly concluded that the proposed uses of β-glucan are GRAS on the basis of scientific procedures.

β-glucan from *Agrobacterium sp.* ZX09 therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

Part 7. §170.255 List of Supporting Data and Information

Aujoulat F, Marchandin H, Zorgniotti I, Masnou A, Jumas-Bilak E (2015). *Rhizobium pusense* is the main human pathogen in the genus *Agrobacterium/Rhizobium*. Clin Microbiol Infect 21(5):472.e1-472.e5. DOI:10.1016/j.cmi.2014.12.005.

CDC (2018). *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Last updated: July 2018].

Chen P, Wang Z, Zeng L, Wang S, Dong W, Jia A, et al. (2012). Protective effects of Salecan against carbon tetrachloride-induced acute liver injury in mice. J Appl Toxicol 32(10):796-803. DOI:10.1002/jat.1694.

Chen P, Wang Z, Zeng L, Yang X, Wang S, Dong W, et al. (2014). A novel soluble beta-glucan Salecan protects against acute alcohol-induced hepatotoxicity in mice. Biosci Biotechnol Biochem 75(10):1990-1993. DOI:10.1271/bbb.110412.

Dazult Ltd. (2018). *DaDiet - The Dietary Intake Evaluation Tool [Software]*. (Version 17.04). Straffan, Ireland: Dazult Ltd. Available at: http://dadiet.daanalysis.com.

Janaki B, Sashidhar RB (2000). Subchronic (90-day) toxicity study in rats fed gum kondagogu (*Cochlospermumgossypium*). Food Chem Toxicol 38(6):523-534. DOI:10.1016/s0278-6915(00)00037-5.

Luo J, Chen D, Mao X, He J, Yu B, Cheng L, Zeng D (2019) Purified β-glucans of different molecular weights enhance growth performance of LPS-challenged piglets via improved gut barrier function and microbiota. Animals 9(9):602 [13pp]. DOI:10.3390/ani9090602.

Pariza MW, Johnson EA (2001). Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Regul Toxicol Pharmacol 33(2):173-186. DOI:10.1006/rtph.2001.1466.

Raa J (2015) Immune modulation by non-digestible and nonabsorbable beta-1,3/1,6-glucan Microbial Ecology in Health and Disease. 26:1, 27824.

Sichuan Synlight Biotech (2018) [unpublished]. *Toxicological Assessment on the Bacterial Suspension of Rhizobium pusense (Agrobactium ZX 09). [Confidential].* Sichuan Synlight Biotech

Sichuan University (2015) [unpublished]. *Inspection Report [Sample Description: β-1,3-Glucan (Salecan)]. [Confidential].* (Report No. BJ201411601; Application No.: BJ2014116, July 8, 2015). Prepared by Sichuan Province, China: Sichuan University, Center for Analysis and Assay, West China School of Public Health for Sichuan Synlight Biotech Ltd.

Sun Q, Xu X, Yang X, Weng D, Wang J, Zhang J (2017). Salecan protected against concanavalin A-induced acute liver injury by modulating T cell immune responses and NMR-based metabolic profiles. Toxicol Appl Pharmacol 317:63-72. DOI:10.1016/j.taap.2017.01.007.

U.S. FDA (2006). *Agency Response Letter GRAS Notice No. GRN 207 [Barley Fiber, Wayzata (MN): Cargill Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=207 [Dec. 19, 2006 - FDA response - no questions].

U.S. FDA (2008). *Agency Response Letter GRAS Notice No. GRN 239 [Bakers Yeast beta-Glucan, Eagan (MN): Biothera, Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=239 [July 22, 2008 - FDA response - no questions].

U.S. FDA (2010). *Agency Response Letter GRAS Notice No. GRN 309 [beta-Glucan Derived from Aureobasidium pullulan, Busan, South Korea: Glucan Corporation, Limited]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=309 [June 14, 2010 - FDA response - no questions].

U.S. FDA (2011). *Agency Response Letter GRAS Notice No. GRN 344 [Barley Fiber, Wayzata (MN): Cargill Incorporated*]. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=344 [May 23, 2011 - FDA response - no questions].

U.S. FDA (2012a). *Agency Response Letter GRAS Notice No. GRN 413 [Beta Glucans Derived from Ganoderma lucidum mycelium, Irvina (CA): Super Beta Glucan, Inc.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=413 [Aug. 10, 2012 - FDA response - no questions].

U.S. FDA (2012b). *Agency Response Letter GRAS Notice No. GRN 412 [Chitin-glucan from Aspergillus niger, Herstal, Belgium: KitoZyme sa]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=412 [June 18, 2012 - FDA response - no questions].

U.S. FDA (2013). *Agency Response Letter GRAS Notice No. GRN 437 [Beta-Glucans from Oat Bran, Exeter (CA): Garuda International, Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=437 [Jan. 27, 2013 - FDA response - no questions].

U.S. FDA (2014). *Agency Response Letter GRAS Notice No. GRN 513 [Dried Biomass of Euglena gracilis Containing beta-1,3-Glucan from Euglena gracilis, Indianapolis (IN): Algaeon, Inc.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=513 [Jul. 9, 2014 - At the notifier's request, FDA ceased to evaluate the notice].

U.S. FDA (2015). *Agency Response Letter GRAS Notice No. GRN 544 [β-Glucans from Oat Bran, Hoffman Estates (IL): Tate and Lyle]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=544 [May 5, 2015 - FDA response - no questions].

U.S. FDA (2017). *Agency Response Letter GRAS Notice No. GRN 698 [Paramylon Isolate from Euglena gracilis, Des Moines (IA): Kemin Foods, L.C.].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=698 [Jul. 19, 2017 - FDA response - no questions].

U.S. FDA (2018). *Food Allergies: What You Need to Know. Food Facts*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at. Available online at: https://www.fda.gov/food/buy-store-serve-safe-food/what-you-need-know-about-food-allergies. [Last updated 09/26/2018].

U.S. FDA (2019). U.*S. Code of Federal Regulations (CFR). Title 21—Food and Drugs*. (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/2019/title21.

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
170—Food additives	170.3	Subpart A—General provisions. Definitions
172—Food Additives Permitted for Direct Addition to Food for Human Consumption	172.160	Potassium nitrate
	172.809	Curdlan
181—Prior-sanctioned Food Ingredients	181.29	Stabilizers
182—Substances Generally Recognized as Safe	182.1	Substances that are generally recognized as safe
	182.60	Substances migrating to food from paper and paperboard products
184—Direct Food Substances Affirmed as Generally Recognized as Safe	184.1193	Calcium chloride
	184.1293	Ethyl alcohol
	184.1315	Ferrous sulfate
	184.1443	Magnesium sulfate
	184.1555	Rapeseed oil
	184.1763	Sodium hydroxide
	184.1854	Sucrose

Xiu A, Kong Y, Zhou M, Hu B, Wang S, Zhang J (2010). The chemical and digestive properties of soluble glucan from *Agrobacterium* sp. ZX09. Carbohydr Polym 82(3):623-628. DOI:10.1016/j.carbpol.2010.05.027.

Xiu A, Zhan Y, Zhou M, Zhu B, Wang S, Jia A, et al. (2011). Results of a 90-day safety assessment study in mice fed a glucan produced by Agrobacterium sp. ZX09. Food Chem Toxicol 49(9):2377-2384. DOI:10.1016/j.fct.2011.06.050.

Xu X, Ding Y, Yang Y, Gao Y, Sun Q, Liu J, et al. (2018). β-glucan Salecan improves exercise performance and displays anti-fatigue effects through regulating energy metabolism and oxidative stress in mice. Nutrients 10(7):858 [13pp]. DOI:10.3390/nu10070858.

Zhan X-B, Lin C-C, Zhang H-T (2012). Recent advances in curdlan biosynthesis, biotechnological production, and applications. Appl Microbiol Biotechnol 93(2):525-531. DOI:10.1007/s00253-011-3740-2.

Zhang R, Edgar KJ (2014). Properties, chemistry, and applications of the bioactive polysaccharide curdlan. Biomacromolecules 15(4):1079-1096. DOI:10.1021/bm500038g.

Zhang Y, Xia L, Pang W, Wang T, Chen P, Zhu B, et al. (2013). A novel soluble β-1,3-D-glucan Salecan reduces adiposity and improves glucose tolerance in high-fat diet-fed mice. Br J Nutr 109(2):254-262. DOI:10.1017/S0007114512000980.

Zhou M, Jia P, Chen J, Xiu A, Zhao Y, Zhan Y, et al. (2013). Laxative effects of Salecan on normal and two models of experimental constipated mice. BMC Gastroenterol 13:52 [6pp]. DOI:10.1186/1471-230x-13-52.

Zhou M, Pu C, Xia L, Yu X, Zhu B, Cheng R, et al. (2014a). Salecan diet increases short chain fatty acids and enriches beneficial microbiota in the mouse cecum. Carbohydr Polym 102:772-779. DOI:10.1016/j.carbpol.2013.10.091.

Zhou M, Wang Z, Chen J, Zhan Y, Wang T, Xia L, et al. (2014b). Supplementation of the diet with Salecan attenuates the symptoms of colitis induced by dextran sulphate sodium in mice. Br J Nutr 111(10):1822-1829. DOI:10.1017/s000711451300442x.

Appendix A
Pariza and Johnson Decision Tree

DECISION TREE ANALYSIS (based on Pariza and Johnson, 2001)

This analysis is based on the Decision Tree of Pariza and Johnson (2001)

1. Is the production strain genetically modified? NO
If yes, go to 2. If no, go to 6.

2. Is the production strain modified using rDNA techniques?
If yes, go to 3. If no, go to 5.

3. Issues relating to the introduced DNA are addressed in 3a–3e.

3a. Do the expressed enzyme product(s) which are encoded by the introduced DNA have a history of safe use in food?
If yes, go to 3c. If no, go to 3b

3b. Is the NOAEL for the test article in appropriate short-term oral studies sufficiently high to ensure safety?
If yes, go to 3c. If no, go to 12.

3c. Is the test article free of transferable antibiotic resistance gene DNA?
If yes, go to 3e. If no, go to 3d.

3d. Does the resistance gene(s) code for resistance to a drug substance used in treatment of disease agents in man or animal? If yes, go to 12. If no, go to 3e.

3e. Is all other introduced DNA well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce food-grade products?
If yes, go to 4. If no, go to 12.

4. Is the introduced DNA randomly integrated into the chromosome?
If yes, go to 5. If no, go to 6.

5. Is the production strain sufficiently well characterized so that one may reasonably conclude that unintended pleiotropic effects which may result in the synthesis of toxins or other unsafe metabolites will not arise due to the genetic modification method that was employed?
If yes, go to 6. If no, go to 7.

6. Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure? YES
If yes, the test article is ACCEPTED. If no, go to 7.

7. Is the organism nonpathogenic?
If yes, go to 8. If no, go to 12.

8. Is the test article free of antibiotics?
If yes, go to 9. If no, go to 12.

9. Is the test article free of oral toxins known to be produced by other members of the same species?
If yes, go to 11. If no, go to 10.

10. Are the amounts of such toxins in the test article below levels of concern?
If yes, go to 11. If no, go to 12.

11. Is the NOAEL for the test article in appropriate oral studies sufficiently high to ensure safety?
If yes, the test article is ACCEPTED.

12. An undesirable trait or substance may be present and the test article is not acceptable for food use. If the genetic potential for producing the undesirable trait or substance can be permanently inactivated or deleted, the test article may be passed through the decision tree again.

Appendix B
Raw Material Specifications

SPECIFICATION

Name: Diatomaceous Earth

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Appearance	White or pinkish white powder
Physical & Chemical Index	
As, mg/kg	≤5
Lead, mg/kg	≤4
Loss on drying, w/%	≤3.0
Loss on ignition (on dried basis), w/%	≤0.5
Non silicide (on dried basis),w/%	≤25.0
pH (100g/L)	8.0~11.0

SPECIFICATION

Name: Calcium Chloride

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Colour	White, off-white or slightly yellow
Appearance	Bulk, flake or grain
Physical & Chemical Index	
Content (calculated on $CaCl_2$), w/%	≥93.0
Content (calculated on $CaCl_2 \cdot 2H_2O$), w/%	99.0%~107.0%
Free alkali content, w/%	
$CaCl_2$	≤0.25
$CaCl_2 \cdot 2H_2O$	≤0.15
Alkali salt, w/%	≤5.0
Heavy metal, w/%	≤0.002
Pb, mg/kg	≤0.0005
As, mg/kg	≤0.0003
F, mg/kg	≤0.004

SPECIFICATION

Name: Cane Sugar

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Colour	Dry, loose, pure white, brilliant, without obvious impurities
Appearance	Crystal grain
Physical & Chemical Index	
Content, %	≥99.5
Reducing sugar, %	≤0.15
Conductivity Ash	≤0.13
Loss on drying, w/%	≤0.10
Color value, IU	≤240
Turbidity, MAU	≤220
Insoluble impurities, mg/kg	≤60
SO_2, mg/kg	≤30

SPECIFICATION

Name: Canola Oil

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Colour (Lovibond comparator 25.4 mm)	≤yellow 35, red 7.0
Odour/taste	Specific odour/taste
Transparency	--
Physical & Chemical Index	
Moisture and volatiles, %	≤0.20
Insoluble impurities, %	≤0.05
Acid value (NaOH), mg/g	≤3.0
Superoxide value, mmol/kg	≤6.0
Heat test (280°C)	Traces of precipitation Lovibond comparator:yellow value does not change, red value increase<0.5
Saponified matter content, %	--
Freeze test (store 5.5 h at 0℃)	--
Solvent residue, mg/kg	N.D.

SPECIFICATION

Name: Ethanol

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Appearance	Colorless, transparent liquid
Physical & Chemical Index	
Assay, w/%	≥94.9
Acidity (as acetic acid), g/L	≤0.05
Alkalinity (as ammonia), w/%	≤0.003
Residue on evaporation, mg/100mL	≤2
Lead, mg/kg	≤0.5
Ketones and other alcohols	
Total, w/%	≤0.5
Methanol, w/%	≤0.02
Any other impurities	≤0.1
Fusel oil test	Pass
Readily carbonizable substances test	Pass
Readily oxidizable substances test	Pass

SPECIFICATION

Name: Ferrous Sulfate Heptahydrate

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Colour	Gray or bluish green
Appearance	Crystal grain
Physical & Chemical Index	
Content, %	99.5%~104.5%
Pb, mg/kg	≤2
Hg, mg/kg	≤1
As, w/%	≤3
Acid-insoluble solid, w/%	-

Sichuan Synlight Biotech., Ltd.
Add: Rm. 703&704, B6 Building, No. 88 South Keyuan Rd., TLSP, Chengdu, 610041
Tel: 86-28-852163941

SPECIFICATION

Name: Magnesium Sulfate

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Colour	Colourless or white
Appearance	Grain or powder
Physical & Chemical Index	
Content (after ignition), %	≥99.0%
Heavy Metal	≤10
Pb, mg/kg	≤2
Se, mg/kg	≤30
pH (50 g/L solution)	5.5~7.5
Chloride, mg/kg	≤0.03
As, mg/kg	≤3
Fe, mg/kg	≤20
Loss on iginition, w/%	
Magnesium sulfate anhydrous	≤2
Magnesium sulfate monohydrate	13.0~16.0
Magnesium sulfate trihydrate	29.0~33.0
Magnesium sulfate heptahydrate	40.0~52.0
Magnesium sulfate (after dry)	22.0~32.0

SPECIFICATION

Name: Manganese Sulfate Monohydrate

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Colour	Pale pink
Appearance	Grain or powder
Physical & Chemical Index	
Content, %	98.0%~102.0%
As, mg/kg	≤3
Pb, mg/kg	≤4
Se, mg/kg	≤30
Loss on ignition, w/%	10.0~13.0

SPECIFICATION

Name: Sodium Chloride

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Granularity	Big grain: 2 mm~4 mm Medium grain: 0.3 mm~2.8 mm Small grain: 0.15 mm~0.85 mm
Physical & Chemical Index	
Whiteness	≥67
Content, g/100g	≥97.2
Sulfate radical, g/100g	≤1.00
Moisture, g/100g	≤0.80
Insoluble solid, g/100g	≤0.10

SPECIFICATION

Name: Sodium Dihydrogen Phosphate

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Colour	White
Appearance	Crystal powder or grain
Physical & Chemical Index	
Content, %	98.0%~103.0%
Insoluble solid, w/%	≤0.2%
As, mg/kg	≤3
Heave metal, mg/kg	≤10
Pb, w/%	≤4
Fluoride	≤50
pH (10 g/L solution)	4.1~4.7
Loss on drying	
NaH_2PO_4	≤2.0
$NaH_2PO_4 \cdot H_2O$	10.0~15.0
$NaH_2PO_4 \cdot 2H_2O$	10.0~15.0

SPECIFICATION

Name: Sodium Hydroxide

Grade: Food Grade

DETERMINATION	SPECIFICATION
Sensory Index	
Appearance	White or off white solid
Physical & Chemical Index	
Assay, w/%	98.0~100.5
Carbonate (as Na_2CO_3), w/%	≤2.0
As, mg/kg	≤3.0
Lead, mg/kg	≤5.0
Mercury, mg/kg	≤0.1
Insoluble substance	Pass
Organic impurities	Pass

Add: Rm. 703&704, B6 Building, No. 88 South Keyuan Rd., TLSP, Chengdu, 610041
Tel: 86-28-852163941

Appendix C
Analytical Methods

Content Determination of β-1, 3-Glucan

1. Instruments

1.1 Ultraviolet spectrophotometer

1.2 Test tubes with glass stopper

2. Reagents

2.1 Concentrated sulfuric acid: AR, 95.5%

2.2 6% Phenol solution: Prepared with 90% phenol solution (dilute it when it will be used)

2.3 Anhydrous glucose, AR (dry at 105℃ for 2.5 hours to constant weight)

3. Preparation of standard reserving solution for glucose

100.0 mg glucose is weighed precisely and dissolved in distilled water to make 1000 mL, the concentration of standard reserving solution will be 0.1 mg/mL.

3.1 Establishment of glucose standard curve

Accurately measure 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mL standard reserving solution to eight 50 mL test tubes; add 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3 mL distilled water respectively; add 6% phenol solution 1.0 mL; quickly add 5.0 mL concentrated sulfuric acid, shake well and stopper the test tubes. The prepared standard solutions will contain 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07 mg glucose respectively.

3.2 Preparation of sample solution

Precisely weigh 35.0 mg β-1,3-glucan sample in a 1000 mL volumetric flask, add appropriate amount of distilled water and dissolve the sample by ultrasound and shaking (about 4 hours), then dilute with distilled water to volume, and mix. Accurately measure 1.0 mL sample solution to a 50 mL test tube, add 1.0 mL 6% phenol solution and 5.0 mL concentrated sulfuric acid, shake well and stopper the test tubes.

4. Determination

Place the prepared standard reserving solutions and sample solution at room temperature for 60 min. Determine the absorbance at 480 nm of above solutions by UV spectrophotometer. Calculate content of β-1,3-glucan according to the standard curve.

5. Calculation

According to the absorbance of sample solution, the mass of β-1,3-glucan can be detected with the graph. Then the content of β-1,3-glucan can be calculated by using the following formula

$$X=C\times1000/m\times0.9\times100\%\ldots\ldots(1)$$

X—content of β-1,3-glucan, %;

m—sampling weight of β-1,3-glucan (anhydride), mg;

C—mass of β-1,3-glucan detected with standard curve, mg;

0.9—conversion coefficient of molecular weight

6. Acceptable Error

The test result hereby refers to the arithmetic mean of multiple determination results. At the same test condition, the absolute difference of determination results should not exceed 5% of the arithmetic mean.

Add: Rm. 703&704, B6 Building, No. 88 South Keyuan Rd., TLSP, Chengdu, 610041
Tel: 86-28-852163941

Content Determination of Ethanol

1. Instruments

a) Gas chromatograph: Shimadzu GC-2014C, with headspace sampler

b) Chromatographic column: Shimadzu InertCap 624 capillary column, 0.32 mm×30 m, 3.0 μm

c) Column temperature: the initial temperature is 80℃ and last 10 min. Then increase to 180 ℃ at the rate of 30℃/min, and last 10 min.

d) Detector: FID, 250℃; Injection temperature: 250℃; Carrier gas: nitrogen, 2.0 mL/min; Split ratio: 5:1

e) Sample size: 1 μL

f) Balance temperature of headspace bottle: 90℃; Balance time: 30 min

g) Electronic balance: Shimadzu AUW220D

2. Reagent and solution

a) Ethanol, AR

b) DMSO for HPLC

3. Solution preparation

3.1 Ethanol reference solution

Weigh ethanol and dilute with DMSO to prepare 100 mg/L solution as reference solution. Accurately transfer 5 mL to 20 mL headspace bottle, and seal.

3.2 Test solution

Accurately weigh 100.0 mg ethanol and 5.0 mL DMSO to 20 mL headspace bottle, and seal.

4. Determination

Detect the blank solution, reference solution and test solution respectively under the chosen chrmatographic condition, and register the chart. The concentration will be calculated using external reference method of peak area. The specific calculation formula is as follow:

$$A \times Cr/(Ar \times C) \times 100\%$$

In this formula:

A-The ethanol peak area of test solution;

Ar-The ethanol peak area of reference solution;

C-The ethanol concentration of sample solution, mg/mL

Cr-The ethanol concentration of reference solution, mg/mL

4. Margin of error

The final result should be subject to the arithmetic average value of parallel determination results. The absolute difference between the two independent determination results under repeatability conditions should not exceed 5% of the arithmetic average value.

Sichuan Synlight Biotech., Ltd.
Add: Rm. 703&704, B6 Building, No. 88 South Keyuan Rd., TLSP, Chengdu, 610041
Tel: 86-28-852163941

Content Determination of Nitrate

1. Instruments

a) HPLC, with UV detector

b) Chromatographic column: Acclaimt Trinity P1, 3.0×100 mm, 3 μm

2. Reagent and solution

a) Ammonium acetate, AR, 95.5%

b) Acetonitrile for HPLC

c) 20 mmol/L ammonium acetate solution: accurately weigh 1.54166 g ammonium acetate in a beaker and dilute with 1,000 mL distilled water. Adjust pH value to 5.0 with acetic acid.

d) Mobile phase: 20 mmol/L ammonium acetate solution-acetonitrile (45:55)

e) Diluent: water-acetonitrile (30:70)

f) Detection wavelength: 210 nm

g) Flow rate: 0.7 mL/min

h) Column temperature: 40℃

i) Reference substance: potassium nitrate

3. Determination

Accurately weigh 500.0 mg of β-1,3-glucan in a 50 mL volumetric flask. Add diluent and ultrasonicate for 10 min. Dilute with diluent to volume. Mix and filter slowly. Accurately measure 50 μL of the solution and inject into the chromatogram instrument, and register the chart. Weigh potassium nitrate and dilute with diluent to prepare 1 mg/L solution as reference solution. Detect the reference solution with same HPLC method. The concentration will be calculated using external reference method of peak area. The specific calculation formula is as follow:

$$A \times Cr/(Ar \times C) \times 100\%$$

In this formula:

A-The principal peak area of test solution;

Ar-The principal peak area of reference solution;

C-The concentration of sample solution, mg/mL

Cr-The concentration of reference solution, mg/mL

4. Margin of error

The final result should be subject to the arithmetic average value of parallel determination results. The absolute difference between the two independent determination results under repeatability conditions should not exceed 1% of the arithmetic average value.

Appendix D
Certificate of Analysis

CERTIFICATE OF ANALYSIS

Product name	**β-1,3-Glucan**	**Manufacturing date**	2018.01.15
Batch number	20180115	**Expiry date**	2020.01
Inspection criteria	Enterprise quality standard	**Batch quantity**	1000 kg
Package	Two-layer plastice bag (food grade) with fibre drum outside, 25kg per drum.		
Storage condition	Store in cool and dry place. Avoid from strong light and heat.		

ITEMS	SPECIFICATION	RESULTS
Sensory Index		
Appearance	Off-white or canary powder	Pass
Odor	Odorless	Pass
Physical & Chemical Index		
β-1,3-Glucan ,%	≥90.0%	91.0%
Ethanol	≤0.5%	0.25%
Nitrate	≤100 ppm	24 ppm
Moisture	≤4.0%	2.8%
Ash	≤5.0%	4.3%
Protein	≤3.0%	1.1%
Heavy Metals		
Arsenic	≤0.5 ppm	Pass
Lead	≤0.5 ppm	Pass
Cadmium	≤0.2 ppm	Pass
Mercury	≤0.02 ppm	Pass
Microbiology		
Toal bacteria count	≤1000 CFU/g	Pass
Coliforms	≤3 MPN/g	Pass
Salmonella	Negative	Pass
Staphylococcus aureus	Negative	Pass
Mould and Yeast	≤100 CFU/g	Pass
Conclusion: According to the enterprise quality standard, the product is conform to quality requirement.		

Principal: Du Lingling **Reviewer**:Sun shulin **Technician**: Tian Jie

四川合泰新光生物科技有限公司
质量检验专用章

CERTIFICATE OF ANALYSIS

Product name	**β-1,3-Glucan**	**Manufacturing date**	2018.01.11
Batch number	20180111	**Expiry date**	2020.01
Inspection criteria	Enterprise quality standard	**Batch quantity**	909 kg
Package	Two-layer plastice bag (food grade) with fibre drum outside, 25kg per drum.		
Storage condition	Store in cool and dry place. Avoid from strong light and heat.		

ITEMS	SPECIFICATION	RESULTS
Sensory Index		
Appearance	Off-white or canary powder	Pass
Odor	Odorless	Pass
Physical & Chemical Index		
β-1,3-Glucan ,%	≥90.0%	90.5%
Ethanol	≤0.5%	0.21%
Nitrate	≤100 ppm	23 ppm
Moisture	≤4.0%	3.5%
Ash	≤5.0%	4.1%
Protein	≤3.0%	0.8%
Heavy Metals		
Arsenic	≤0.5 ppm	Pass
Lead	≤0.5 ppm	Pass
Cadmium	≤0.2 ppm	Pass
Mercury	≤0.02 ppm	Pass
Microbiology		
Toal bacteria count	≤1000 CFU/g	Pass
Coliforms	≤3 MPN/g	Pass
Salmonella	Negative	Pass
Staphylococcus aureus	Negative	Pass
Mould and Yeast	≤100 CFU/g	Pass
Conclusion: According to the enterprise quality standard, the product is conform to quality requirement.		

Principal: Du Lingling **Reviewer**: Sun shulin **Technician**: Tian Jie

四川合泰新光生物科技有限公司 质量检验专用章

CERTIFICATE OF ANALYSIS

Product name	**β-1,3-Glucan**	**Manufacturing date**	2018.05.18
Batch number	20180518	**Expiry date**	2020.05
Inspection criteria	Enterprise quality standard	**Batch quantity**	1001 kg
Package	Two-layer plastice bag (food grade) with fibre drum outside, 25kg per drum.		
Storage condition	Store in cool and dry place. Avoid from strong light and heat.		

ITEMS	SPECIFICATION	RESULTS
Sensory Index		
Appearance	Off-white or canary powder	Pass
Odor	Odorless	Pass
Physical & Chemical Index		
β-1,3-Glucan ,%	≥90.0%	92.1%
Ethanol	≤0.5%	0.31%
Nitrate	≤100 ppm	43 ppm
Moisture	≤4.0%	3.3%
Ash	≤5.0%	4.1%
Protein	≤3.0%	1.3%
Heavy Metals		
Arsenic	≤0.5 ppm	Pass
Lead	≤0.5 ppm	Pass
Cadmium	≤0.2 ppm	Pass
Mercury	≤0.02 ppm	Pass
Microbiology		
Toal bacteria count	≤1000 CFU/g	Pass
Coliforms	≤3 MPN/g	Pass
Salmonella	Negative	Pass
Staphylococcus aureus	Negative	Pass
Mould and Yeast	≤100 CFU/g	Pass
Conclusion: According to the enterprise quality standard, the product is conform to quality requirement.		

Principal: Du Lingling **Reviewer**: Sun shulin **Technician**: Tian Jie

四川合泰新光生物科技有限公司 质量检验专用章

Appendix E
GRAS Panel Statement

GRAS Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of β-glucan from *Agrobacterium sp.* ZX09 for Use in Select Food Categories

01 November 2019

INTRODUCTION

At the request of Sichuan Synlight Biotech Co., Ltd., a GRAS Panel (the Panel) of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information related to the safety of the company's β-glucan ingredient, and to determine whether, under the conditions of intended use of β-glucan from *Agrobacterium sp.* ZX09 as an adhesive, stabilizer, and thickener in select food categories would be Generally Recognized as Safe (GRAS), based on scientific procedures.

The Panel consisted of the below-signed qualified scientific experts: Stanley Tarka, Jr., Ph.D., F.A.T.S., (Adjunct Associate Professor, The Pennsylvania State University College of Medicine and The Tarka Group, Inc.), John A. Thomas, Ph.D., F.A.T.S., (Adjunct Professor, Department of Pharmacology & Toxicology, Indiana University School of Medicine), and David Bechtel, Ph.D., D.A.B.T. (Bechtel Consulting). The Panel was selected and convened in accordance with the United States (U.S.) Food and Drug Administration (FDA)'s guidance for industry on *Best Practices for Convening a GRAS Panel* (U.S. FDA, 2017). Sichuan Synlight Biotech Co., Ltd ensured that all reasonable efforts were made to identify and select a balanced GRAS Panel with expertise in food safety and toxicology. Efforts were placed on identifying conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the Panel; no such conflicts of interest or "appearance issues" were identified. The Panel received a reasonable honorarium as compensation for their time; the honoraria provided to the Panel were not contingent upon the outcome of their deliberations.

The Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources based on searches of the published scientific literature conducted through February of 2019 in a dossier titled, "*Documentation Supporting the Evaluation of Beta-Glucan from Agrobacterium SP. ZX09 as Generally Recognized as Safe (GRAS) for Use in Select Food Categories"*(17 October 2019). In addition, the Panel evaluated other information deemed appropriate or necessary, including both published and unpublished data and information provided by Sichuan Synlight Biotech Co., Ltd. The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and a comprehensive literature assessment on the safety of β-glucan from *Agrobacterium sp.* ZX09.

Following its independent and collective critical evaluation of the data and information, the Panel convened *via* teleconference on 01 November 2019. The Panel reviewed their findings and, following discussion, unanimously concluded that the intended use in specified foods as described herein of β-glucan as an adhesive, stabilizer, and thickener from *Agrobacterium sp.* ZX09, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice (cGMP), is safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

COMPOSITION, MANUFACTURING, AND SPECIFICATIONS

Sichuan Synlight Biotech Co., Ltd's of β-1,3-glucan is produced *via* fermentation by a non-genetically modified and non-pathogenic organism, *Agrobacterium sp*. ZX09. It is a high-molecular-mass polymer (about 2 x 10^6 Da) and composed of a linear chain of glucosyl residues linked through a repeat unit of seven β-(1,3) and two α-(1,3) glucosidic bonds (Zhang *et al.,* 2013). It is produced in accordance with cGMP using food-grade raw materials and approved processing aids. Analysis of 3 non-consecutive lots demonstrate that this manufacturing process produces a final β-glucan ingredient that reproducibly meet appropriate food-grade specifications. Stability analysis under both accelerated and real-time storage conditions demonstrate that this β-glucan from *Agrobacterium sp.* ZX09 is stable for a period of 2 years at room temperature.

INTENDED USE AND ESTIMATED EXPOSURE

Sichuan Synlight Biotech Co., Ltd's β-glucan is intended for use as an adhesive, stabilizer, and thickener in select food categories. Specifically, it is intended for use in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, dairy product analogs, frozen dairy desserts, grain products and pastas, gravies and sauces, jams and jellies, milk products, processed fruits and fruit juices, and processed vegetables and vegetable juices at use levels summarized in Table 1.

Table 1 Intended Uses and Use Levels for β-glucan from *Agrobacterium sp.* ZX09 in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019a)	Food-Uses[a]	β-glucan from *Agrobacterium sp.* ZX09 Use Levels (%)
Baked Goods and Baking Mixes	Biscuits	0.25–0.5
	Cookies	0.25–0.5
	Cornbread, Corn Muffins, or Tortillas	0.25–0.5
	Crackers	0.25–0.5
	Croissants	0.25–0.5
	Doughnuts (Donuts)	0.25–0.5
	Muffins	0.25–0.5
	Pastries	0.25–0.5
	Pies	0.25–0.5
Beverages and Beverage Bases	Energy Drinks	0.1–0.3
	Non-Milk-Based Meal Replacement Beverages and Protein Drinks	0.1–0.3
	Soft Drinks	0.1–0.3
	Sport or Electrolyte Drinks, and Fluid Replacement Drinks	0.1–0.3
Breakfast Cereals	Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	0.1–0.3
	Ready-to-Eat Breakfast Cereals	0.6
Dairy Product Analogs	Non-dairy-based Beverages	0.3–0.9
Frozen Dairy Desserts	Ice Cream	0.3–0.9
	Frozen Yogurt (Frozen Yoghurt)	0.3–0.9

Table 1 Intended Uses and Use Levels for β-glucan from *Agrobacterium sp.* ZX09 in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019a)	Food-Uses[a]	β-glucan from *Agrobacterium sp.* ZX09 Use Levels (%)
Grain Products and Pastas	Cereal and Granola Bars	0.1– 0.6
	Energy Bars or Protein Bars	0.1– 0.6
	Macaroni and Noodle Products	0.3–0.9
Gravies and Sauces	Tomato-Based Sauces	0.1– 0.6
Jams and Jellies	Jams, Jellies, Preserves, and Marmalades	0.1– 0.6
Milk Products	Evaporated, Condensed, and/or Dry Milks	0.25–0.5
	Fermented Milks, Plain	0.1–0.25
	Flavored Milk and Milk Drinks	0.1–0.25
	Milk-Based Smoothies	0.1–0.3
	Milk-Based Meal Replacement and Protein Beverages	0.1–0.25
	Milk Shakes	0.1–0.25
	Plain or Flavored Yogurt (Yoghurt)	0.1–0.25
	Yogurt Drinks	0.3–0.9
Processed Fruits and Fruit Juices	Fruit Drinks and Ades, and Smoothies	0.1–0.3
	Fruit Juices	0.1–0.3
	Fruit Nectars	0.1–0.3
Processed Vegetables and Vegetable Juices	Vegetable Juices	0.1–0.3

CFR = Code of Federal Regulations; FDA = Food and Drug Administration; U.S. = United States.
[a] β-glucan from Agrobacterium sp. ZX09 is intended for use in unstandardized products when standards of identity, as established under 21 CFR §130 to 169, do not permit its addition.
[a] U.S. FDA (2019a)

Sichuan Synlight Biotech Co., Ltd's estimated the *per capita* and consumer-only intakes of β-glucan from *Agrobacterium sp.* ZX09 for specific demographic groups and for the total U.S. population using consumption data from the *National Health and Nutrition Examination Survey (NHANES): 2015-2016* (CDC, 2018) and information pertaining to the individual proposed food-uses of β-glucan from *Agrobacterium sp.* ZX09 summarized in Table 1. On a consumer-only basis, the resulting mean and 90th percentile intakes of β-glucan from *Agrobacterium sp.* ZX09 by the total U.S. population from all proposed food-uses, were estimated to be 2.2 g/person/day (38 mg/kg body weight/day) and 4.5 g/person/day (80 mg/kg body weight/day), respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of β-glucan from *Agrobacterium sp.* ZX09 on an absolute basis, at 2.7 and 5.5 g/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 1.4 and 3.2 g/person/day, respectively.

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of β-glucan from *Agrobacterium sp.* ZX09 were determined to be 38 and 80 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 116 and 253 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 27 and 54 mg/kg body weight/day, respectively.

DATA PERTAINING TO SAFETY

β-Glucans are naturally occurring polysaccharides with poly-branched β-1,3-(D)-glucans or β-1,6-(D)-glucose side chains. Originally found in the cell walls of fungi and cereal plants, β-glucans are also integral cell wall constituents in a variety of bacteria and yeasts. As such, they have a long history of safe consumption in the diet, with several types of β-Glucans approved for use in food *via* listing in the Code of Federal Regulations and GRAS Notices (GRNs) to the FDA (U.S. FDA, 2006, 2008, 2010, 2011, 2012a,b, 2013, 2015, 2019b).

The safety of β-glucan from *Agrobacterium sp.* ZX09 under the conditions of intended use in foods as described herein is based on scientific procedures. A search of the published scientific literature was conducted through February 2019 using the search program Proquest to identify published studies relevant to the safety -glucan from *Agrobacterium sp.* ZX09. The search was conducted on databases including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, and Toxfile®.

The production organism, *Agrobacterium sp.* ZX09, is a non-GMO and nonpathogenic strain closely related to another organism with a history of safe use in the production of curdlan, another β-glucan approved for use as a direct food additive in the U.S. (U.S. FDA, 2019b). An evaluation of the safety of *Agrobacterium sp.* ZX09 using the decision tree approach outlined by Pariza and Johnson (2001) indicates that it is acceptable for use in the production of food.

Sichuan Synlight Biotech Ltd. also evaluated the safety of *Agrobacterium* sp. ZX09 in a series of toxicology studies. The acute oral median lethal dose (LD_{50}) of the bacterial suspension of *Agrobacterium sp*. ZX09 to determined greater than 10,000 mg/kg body weight in rats and mice. No mutagenicity or genotoxicity was seen in a bacterial reverse mutation test, a mammalian erythrocyte micronucleus test and an *in vitro* chromosomal aberration test on mammalian cells) indicating that *Agrobacterium sp.* ZX09 was not genotoxic or mutagenic. No toxicologically significant adverse events were seen hematology, clinical chemistry, or urinalysis parameters in male or female Sprague Dawley rats (10 animals/sex/group) who had been administered suspensions of Agrobacterium sp. ZX09 at 2.5, 5.0, or 10 mL/kg body weight/day for 90 days (Sichuan Synlight Biotech, 2018 [unpublished]).

The safety of β-glucan from *Agrobacterium sp.* ZX09 was also evaluated in a series of toxicology studies. The acute oral LD_{50} value for β-glucan from *Agrobacterium sp.* ZX09 was reported to be >3,000 mg/kg in rats and mice. In the pivotal safety study, administration of β-glucan from *Agrobacterium sp.* ZX09 to mice at levels up to 14,478 mg/kg body weight/day for 90 days did not result in any compound-related, toxicologically relevant adverse effects (Xiu *et al.*, 2011). Thus, there exists a margin of safety of 180-fold over the estimate 90^{th} percentile intake of 4.5 g/person/day (80 mg/kg body weight/day). In a second supportive 90-day study, no compound-related, toxicologically relevant adverse effects were observed in clinical, histological, biochemical, and hematological parameters in rats administered doses of β-glucan from *Agrobacterium sp.* ZX09 up to 3,840 mg/kg body weight/day. No evidence of genotoxicity or mutagenicity was seen in a bacterial reverse mutation test, a mammalian erythrocyte micronucleus test, or a sperm abnormality test (Sichuan University, 2015 [unpublished]).

The potential allergenicity of β-glucan was researched in the available literature, and no relevant information was located. Sources of β-glucan concentrates, including *Agrobacterium* (as a source of curdlan), have been safely consumed for decades. β-glucan is not listed among FDA's list of the 8 major food allergens (U.S. FDA, 2018, 2019b). The lack of available information and absence from FDA's list suggests that allergic reactions to β-glucan are not of concern.

CONCLUSION

We, the undersigned independent qualified members of the Generally Recognized as Safe (GRAS) Panel, have, independently and collectively, critically evaluated the data and information summarized above that is pertinent to the safety of the proposed use of β-glucan obtained from *Agrobacterium sp.* ZX09 in specified conventional foods. We unanimously conclude that the proposed use of β-glucan obtained from *Agrobacterium sp.* ZX09, produced in a manner that is consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food grade specifications as presented in the supporting dossier *"Documentation Supporting the Evaluation of Beta-Glucan from Agrobacterium SP. ZX09 as Generally Recognized as Safe (GRAS) for Use in Select Food Categories"* is safe.

We further unanimously conclude that the proposed use of β-glucan obtained from *Agrobacterium sp.* ZX09, produced in a manner that is consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food grade specifications as presented in the supporting dossier is Generally Recognized as Safe (GRAS) based on scientific procedures under the conditions of intended use in foods specified herein.

It is our professional opinion that other qualified experts would also concur with this conclusion.

12 November 2019

Stanley M. Tarka, Jr., PhD, ATS
Adjunct Associate Professor, The Pennsylvania State University College of Medicine, Hershey, PA
The Tarka Group, Inc., Carlisle, PA

Date

11/11/19

John A. Thomas, Ph.D.
Adjunct Professor
Department of Pharmacology & Toxicology
Indiana University School of Medicine Indianapolis, IN

Date

11/13/19

David H. Bechtel, Ph.D., DABT
President,
Bechtel Consulting, Inc.
Monroe, NJ

Date

REFERENCES

CDC (2018). *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Last updated: July 2018].

Pariza MW, Johnson EA (2001). Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Regul Toxicol Pharmacol 33(2):173-186. DOI:10.1006/rtph.2001.1466.

Sichuan Synlight Biotech (2018) [unpublished]. Toxicological assessment on the bacterial suspension of *Rhizobium pusense* (*Agrobactium ZX 09*).

Sichuan University (2015) [unpublished]. *Inspection Report [Sample Description: β-1,3-Glucan (Salecan)]* (Report No. BJ201411601; Application No.: BJ2014116, July 8, 2015). Prepared by Sichuan Province, China: Sichuan University, Center for Analysis and Assay, West China School of Public Health for Sichuan Synlight Biotech Ltd.

U.S. FDA (2006). *Agency Response Letter GRAS Notice No. GRN 000207 [Subject: Barley Fiber, Wayzata (MN): Cargill Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=207 [Dec. 19, 2006].

U.S. FDA (2008). *Agency Response Letter GRAS Notice No. GRN 000239 [Bakers yeast beta-glucan, Eagan (MN): Biothera, Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=239 [July 22, 2008].

U.S. FDA (2010). *Agency Response Letter GRAS Notice No. GRN 000309 [Beta-glucan derived from Aureobasidium pullulan, South Korea: Glucan Corporation, Limited Marine Bio Center 221, Silla University]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=309 [June 14, 2010].

U.S. FDA (2011). *Agency Response Letter GRAS Notice No. GRN 000344 [Subject: Barley Fiber, Wayzata (MN): Cargill Incorporated*]. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=344 [May 23, 2011].

U.S. FDA (2012a). *Agency Response Letter GRAS Notice No. GRN 000413 [Beta glucans derived from Ganoderma lucidum mycelium, Irvina (CA): Super Beta Glucan, Inc.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=413 [Aug. 10, 2012].

U.S. FDA (2012a). *Agency Response Letter GRAS Notice No. GRN 000412 [Chitin-glucan from Aspergillus niger, Herstal, Belgium: KitoZyme sa]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=412 [June 18, 2012].

U.S. FDA (2013). *Agency Response Letter GRAS Notice No. GRN 000437 [Beta-glucans from oat bran, Exeter (CA): Garuda International, Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=437 [Jan. 27, 2013].

U.S. FDA (2015). *Agency Response Letter GRAS Notice No. GRN 000544 [β-glucans from oat bran, Hoffman Estates (IL): Tate and Lyle]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=544 [May 5, 2015].

U.S. FDA (2017). *Best Practices for Convening a GRAS Panel: Guidance for Industry: Draft Guidance*. (Docket Number: FDA-2017-D-0085, November 2017). College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Center for Veterinary Medicine (CVM). Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-best-practices-convening-gras-panel.

U.S. FDA (2018). *Food Allergies: What You Need to Know. Food Facts*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at. Available online at: https://www.fda.gov/food/buy-store-serve-safe-food/what-you-need-know-about-food-allergies. [Last updated 09/26/2018].

U.S. FDA (2019a). Part 170—Secondary direct food additives permitted in food for human consumption. Section §170.3—Definitions. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs*. (U.S. Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.accessdata.fda.gov/SCRIPTS/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=170.3.

U.S. FDA (2019b). Part 172—Food additives permitted for direct addition to food for human consumption. Section §172.809—Curdlan. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs*. (U.S. Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.accessdata.fda.gov/SCRIPTS/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=172.809.

Xiu A, Zhan Y, Zhou M, Zhu B, Wang S, Jia A, *et al.* (2011). Results of a 90-day safety assessment study in mice fed a glucan produced by Agrobacterium sp. ZX09. Food Chem Toxicol 49(9):2377-2384. DOI:10.1016/j.fct.2011.06.050.

Zhang Y, Xia L, Pang W, Wang T, Chen P, Zhu B, *et al.* (2013). A novel soluble β-1,3-D-glucan Salecan reduces adiposity and improves glucose tolerance in high-fat diet-fed mice. Br J Nutr 109(2):254-262. DOI:10.1017/S0007114512000980.

合泰新光
SYNLIGHT BIO
四川合泰新光生物科技有限公司

Authorization Letter

I, the undersigned Ms Du Lingling, representing Sichuan Synlight Biotech Ltd., hereby appoint Intertek Health Sciences Inc., to represent us with regard to our GRAS Notice (GRN 000961) and related matters in United States of America.

Signature:

Date: 2021.3.18

Seal:

Sichuan Synlight Biotech Ltd
地址：四川省成都市高新区科园南路 88 号天府生命科技园 B6 栋 703&704
电话：+86-28-8521 9331　　传真：+86-28-8521 6567
网址：www.salecan.com.cn

Kampmeyer, Christopher

From:	wangmeng@salecan.com.cn
Sent:	Friday, March 19, 2021 12:29 AM
To:	Kampmeyer, Christopher
Subject:	Re: RE: [EXTERNAL] RE: Beta-Glucan FDA GRAS Notice - GRN 000961
Attachments:	Authorization Letter.PDF
Follow Up Flag:	Follow up
Flag Status:	Flagged

CAUTION: This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Dear Mr. Kampmeyer

Please find the authorization letter in attachment.

Regards
Lingling

wangmeng@salecan.com.cn

From: Kampmeyer, Christopher

Subject: RE: [EXTERNAL] RE: Beta-Glucan FDA GRAS Notice - GRN 000961

Dear Mr. Lingling,

Your agent from Intertek has requested to communicate with us about your GRAS Notice (GRN 000961). Could you please provide us, in writing, your authorization for Intertek to discuss the GRAS Notice on your behalf?

Thank you,
Chris

Chris Kampmeyer, M.S.
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
christopher.kampmeyer@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

From: Ryan Simon Intertek <ryan.simon@intertek.com>
Sent: Thursday, March 11, 2021 5:59 PM
To: Kampmeyer, Christopher <Christopher.Kampmeyer@fda.hhs.gov>
Cc: wangmeng@salecan.com.cn
Subject: [EXTERNAL] RE: Beta-Glucan FDA GRAS Notice - GRN 000961

CAUTION: This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Dear Dr. Kampmeyer:

As Sichuan Synlight Biotech Co., Ltd's authorized agent, Intertek Health Sciences Inc. is reaching out to you to assist the company with addressing some questions the U.S. FDA has pertaining to the Synlight's GRAS Notice for *beta*-glucan in GRN 961. If you could let me know the best way to move forward so that we can provide the agency with the information they need in a timely fashion, it would be much appreciated.

Kind Regards,
Ryan

Ryan Simon
Sr. Director, Safety & Regulatory
Food & Nutrition Group
Health, Environmental & Regulatory Services (HERS)
Direct +1 905 286-4188
Mobile +1 905 301-2766
Office +1 905 542-2900
Skype ryan.simon.intertek
http://www.intertek.com/scientific-regulatory-consultancy/