SUPER BETA GLUCAN

August 7, 2020

Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Campus Drive
College Park, MD 20740

Subject: GRAS Notification for the intended use of Antrodia mushroom β-glucans as a Food Ingredient

Dear Sir/Madam:

In accordance with 21 CFR part 170, subpart E, Quorum Innovations, LLC, Florida, USA, hereby submits the enclosed notice of a claim that the food ingredient Antrodia mushroom β-glucans, derived from *Antrodia cinnamomea,* as described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be Generally Recognized As Safe (GRAS), based on scientific procedures.

As required, please find enclosed three copies of the GRAS notification. If you have any questions or require additional information, please feel free to contact me by phone at: 949-264-2888 or by email at <sherwin@superbetaglucan.com>.

Sincerely,

Sherwin Chen
Vice-President

Enclosure: Three copies of GRAS notification

5 Holland #109, Irvine, CA 92618
Tel: 949-264-2888 Fax: 626-203-0655
www.superbetaglucan.com

EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF

ANTRODIA MUSHROOM β-GLUCANS

AS A FOOD INGREDIENT

Prepared for:

Super Beta Glucan

5 Holland, Unit 109

Irvine, CA 92618

USA

Prepared by:

Soni & Associates Inc.

749 46th Square

Vero Beach, FL 32968

Panel Members

John A. Thomas, Ph.D., F.A.C.T., F.A.T.S.

Robert L. Martin., Ph.D.

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.

July, 2020

EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ANTRODIA MUSHROOM β-GLUCAN AS A FOOD INGREDIENT

TABLE OF CONTENTS

Part I- SIGNED STATEMENT AND CERTIFICATION

1.1. Basis of Conclusion

This GRAS conclusion for the use of Antrodia mushroom β-glucans, derived from *Antrodia cinnamomea,* as a food ingredient, has been reached in accordance with the requirements as defined in 21 CFR 170.220.

It should be noted that in 2012, Super Beta Glucan (SBG) submitted a GRAS notice (GRN 413) to FDA on Beta glucans derived from *Ganoderma lucidum* mycelium that received an "FDA has no questions" letter[1]. As discussed below, the subject of this present GRAS notification is similar to GRN 413, except that the β-glucan is derived from another species of mushroom, i.e., *Antrodia cinnamomea.*

1.2. Name and address of organization:

Super Beta Glucan
5 Holland, Unit 109
Irvine, CA 92618

Phone: 949-264-2888
Fax 626-203-0655
E-mail: sherwin@superbetaglucan.com

1.3. Name of substance:

The name of the substance of this GRAS assessment is Antrodia Beta Glucan, ABG, and Antrocan.

1.4. Intended conditions of use:

Antrodia mushroom β-glucans derived from *Antrodia cinnamomea* is intended to be used as a food ingredient in selected food categories such as baked goods and baking mixes, beverages and beverage bases, cereal and cereal products, dairy product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, and soft candy at levels up to 150 mg Antrodia mushroom β-glucans *per* serving (reference amounts customarily consumed, 21 CFR 101.12). The intended use of Antrodia mushroom β-glucans is in the same food products and at the identical levels mentioned in the GRN 413[1] (β-glucans derived from *G. lucidum* mycelium). The proposed food categories are also identical to another GRAS notification on β-glucans, i.e., GRN 309[2] (Beta-glucan derived from *Aureobasidium pullulans*) and are similar to GRN 239[3] (Bakers yeast beta-glucan). The intended use of Antrodia mushroom β-glucans in the above-mentioned food categories is estimated to result for "users only" at the mean and 90th

[1] Accessible at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm299311.pdf
[2] Accessible at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm269476.pdf
[3] Accessible at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm267500.pdf

percentile intakes of 291.3 and 583.4 mg/person/day, respectively. Antrodia mushroom β-glucans is not intended to be marketed for infant and toddler foods.

1.5. Statutory Basis for GRAS conclusion:

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

1.6. Exemption from Premarket approval requirements:

Super Beta Glucan (SBG) has concluded that Antrodia mushroom β-glucans is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on our conclusion that Antrodia mushroom β-glucans, meeting the specifications cited herein, and when used as a food ingredient, is GRAS and is therefore exempt from the premarket approval requirements.

It is also our opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion. Therefore, we have also concluded that Antrodia mushroom β-glucans, when used as described in this dossier, is GRAS based on scientific procedures.

1.7. Availability of data and information:

The data and information that are the basis for this GRAS conclusion will be made available to FDA upon request by contacting Dr. Chen at the below address. The data and information will be made available to FDA in a form in accordance with that requested under 21 CFR 170.225(c)(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

Sherwin Chen
Vice-President
Super Beta Glucan
5 Holland, Suite 109
Irvine, CA 92618

Phone: +1 949-264-2888
Fax: +1 626 203 0655
E-mail: sherwin@superbetaglucan.com

1.8. Data exempt from Disclosure:

Part I through Part VII of this GRAS assessment does not contain any privileged or confidential information such as trade secrets and/or commercial or financial information and can be made publicly available.

1.9. Certification:

SBG certifies that, to the best of its knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by SBG, including any favorable or unfavorable information, and pertinent to the

evaluation of the safety and GRAS status of the use of Antrodia mushroom β-glucan. SBG accepts responsibility for the GRAS conclusion that has been made for Antrodia mushroom β-glucan as described in this dossier.

1.10. Name, position/title of responsible person who signs dossier and signature:

Sherwin Chen
Vice-President
Super Beta Glucan
5 Holland, Suite 109
Irvine, CA 92618

Signature: 

1.11. FSIS/USDA – Use in Meat and/or Poultry:

SBG does not intend to add Antrodia mushroom β-glucans to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

Part II- IDENTITY AND TECHNICAL INFORMATION

2.1. Description

The subject of this GRAS assessment, Antrodia mushroom β-glucans, derived from *Antrodia cinnamomea,* is a standardized powder. The preparation is a fine light beige powder with characteristic mild odor and bland taste. It is extracted from *A. cinnamomea* culture. General descriptive characteristics of Antrodia mushroom β-glucans are summarized in Table 1. The active constituents of the extract are β-glucans.

Table 1. General Descriptive Characteristics of Antrodia mushroom β-glucans

Parameter	Description (SBG, 2019)*
Source microorganism	*Antrodia cinnamomea*
Active constituents	β-Glucan
Synonyms	Mushroom β-glucan
Appearance	Dried fine powder
Color	Light beige
Odor	Characteristic mild
Taste	Bland
Storage	Ambient, Dry
Shelf life	3 years

*Based on information provided by SBG (2019)

2.2. Microbiological Identification of Source Microorganism

The hierarchical classification of the source material, *Antrodia cinnamomea* is presented in Table 2. *A. cinnamomea*, a species of the genus Antrodia (Polyporaceae), is a parasitic fungus that lives in the inner cavity of the tree *Cinnamomum kanehirai*, which is endemic to Taiwan. *A. cinnamomea* or *A. camphorata* is a distinctive mushroom of Taiwan. The *A. cinnamomea* (ATCC® 200183™) used in the production of Antrodia mushroom β-glucan was identified by Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan. Mycelium from the *A. cinnamomea* strain was subcultured and maintained in YM agar medium.

Table 2. Taxonomical Classification of *Antrodia cinnamomea*

Rank	Scientific Name and Common Name
Kingdom	Fungi
Division	Basidiomycota
Class	Agaricomycetes
Order	Polyporales
Family	Fomitopsidaceae
Genus	*Antrodia*
Species	*Antrodia cinnamomea*
Strain	*A. cinnamomea* ATCC # 200183

2.3. Identity of Notified Substance

A. Chemical name:

Antrodia mushroom β-glucans. The product is composed mainly of β-glucans. β-D-Glucan; (1-3), (1-4)-β-D-Glucan; and/or β-Glucosylglucan.

B. Common/Trade Name:

The subject of this notification will be marketed as Antrodia Mushroom Beta Glucan, Antrodia Beta Glucan, ABG, or Antrocan.

C. Chemical Abstract Registry Number:

Not available. There is a CAS Registry Number 9041-22-9 allocated to β-glucan that applies to β-glucan of any origin (e.g., barley, oat, mushroom, yeast, etc.).

D. Chemical Formula:

(1-3),(1-6)-β-D-glucan; Poly-(l-6)-β-D-glucopyranosyl-(l,3)-β-D-glucopyranose, and consists of a β(1,3)-linked glucose backbone with short β(1,6)-linked branches.

E. Structure:

The main components of mushroom β-glucans along with locations and orientations of different β-glucan linkages is shown in Figure 1.

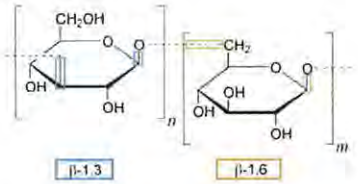

Figure 1. Orientation and Location of Different β-Glucan Linkages.

F. Molecular Weight

The molecular weight of mushroom β-glucans ranges from 9.6 kDa to 298 kDa

2.4. Specifications of Notified Substance

Food grade specifications of Antrodia mushroom β-glucans are presented in Table 3. It is a fine light beige powder soluble in water. Analyses of 5 independently produced, and representative non-consecutive batches (Appendix I) of Antrodia mushroom β-glucans demonstrate that the manufacturing process and final product are both highly reproducible and that the process is capable of producing material that consistently meets the specifications. The product is primarily composed of a minimum 65% β-glucans with a total carbohydrate content of over 90%. Antrodia mushroom β-glucans also contains approximately 1% fat, 1% protein, 3% ash and 5% moisture. As shown in Table 3, the sum of all analyzed components demonstrates that Antrodia mushroom β-glucans is fully characterized (approximately 100%) for its constituents. Among the carbohydrates (over 90%), β-glucans comprises 65%, the remaining carbohydrates and are primarily monosaccharides and disaccharides, as these molecules are water soluble. The use of a ceramic membrane in the manufacturing process removes most of the larger carbohydrate molecules and other large molecular weight compounds. Extensive analyses of different batches for potential external contaminants of Antrodia mushroom β-glucans such as heavy metals and microbes, generally associated with such food products, revealed that these contaminants were not detected within the limits of detection for the method used. In those

instances, it was assumed that the contaminant could be present at the LOD. At these low levels, it was concluded that the contaminant, if present, are unlikely to cause any adverse effects.

Table 3. Physical, Chemical and Microbiological Specifications of Antrodia Mushroom β-glucans

Parameter	Specifications	Assay method
Physical parameters		
Appearance	Fine light beige powder	Visual
Odor	Mild	Olfactory
Taste	Bland	Taste
Chemical parameters		
Total Carbohydrate (%)	> 90	By Difference (Calculation)
β-Glucans (%)	Minimum 65	Internal Methods
Fat (%)	<1.0	AOAC 996.06
Saturated Fat (%)	<1.0	AOAC 996.06
Trans Fat (%)	N.D.	AOAC 996.06
Protein (%)	<1.0	AOAC 922.15
Moisture (%)	<5.0	AOAC 925.45A/V.O
Ash (%)	<3.0	AOAC 900.02
Heavy metals		
Lead	<0.5 ppm	ICP-MS
Arsenic	<0.5 ppm	Cold Vapor
Cadmium	<0.5 ppm	ICP-MS
Mercury	<0.05 ppm	ICP-OES
Microbiological parameters		
Aerobic Plate Count (CFU/g)	<15,000	FDA BAM
Yeast and Mold (CFU/g)	<150 combined	FDA BAM/CMMEF APHA
Total Coliforms (MPN/G)	<10	AOAC 966.24
Staphylococcus aureus	Negative	AOAC 2003.07/2003.08
Escherichia coli	Negative	FDA BAM/AOAC 991.14
Salmonella sp.	Negative	FDA BAM

2.4. Manufacturing Process

The production process for Antrodia mushroom β-glucans is illustrated in Figure 1. Antrodia mushroom β-glucans is manufactured according to current good manufacturing practices (GMPs). The manufacturing process is initiated by preparing a culture medium containing glucose, galactose, sucrose, mannose and yeast extract. Following autoclaving procedure at 121°C for 20 mins, the mycelia of *A. cinnamomea* were introduced into the sterile medium and cultured using an incubator at 24-27°C with relative humidity at 60-70% for 6-8 weeks to allow full growth of the *A. cinnamomea* mushroom culture. Subsequently, Antrodia mushroom β-glucans in mycelium was extracted using a high-speed homogenizer (12500 rpm for 8 min) and ultrasonic vibration (30 kHz/25 min). The resulting solution was then filtered and separated using a ceramic membrane to strip most of the residual small carbohydrate molecules (Molecular Weight < 3 kDa). The concentrated Antrodia mushroom β-glucans were then pooled, dried and grounded into powder form.

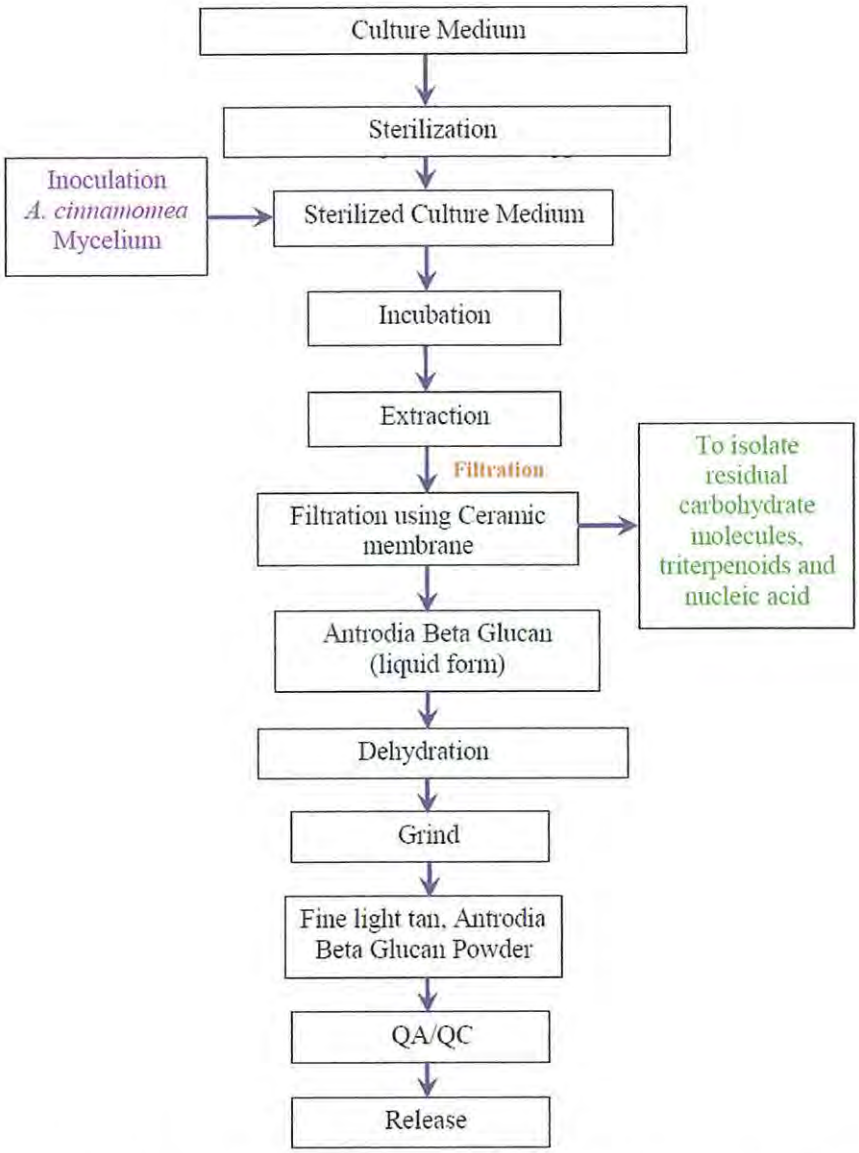

Figure 1. Manufacturing Process of Antrodia Mushroom β-glucans

2.4. Chemistry and Biological Activity

Beta-glucans are defined chemically as linear molecules of beta-1,3-and beta-1,4-linked D-glucopyranose units that are associated with cell wall structural components in both the bran and endosperm. These molecules, comprised of D-glucose polymers, are primarily produced in fungi, yeast and plants (grains) but not in mammalian cells (Driscoll et al., 2009). β-Glucans exists as a chain of glucose molecules linked together by β-glycosidic bonds. The D-glucose rings with six sides can be connected to one another, in a variety of positions on the D-glucose ring structure. Some β-glucans compounds are continual repeats of D-glucose attached at a specific position. Depending on the source, the primary chemical structure of β-glucans polymers differs. However, mainly consists of a linear glucose polymer with β(1,3)-, β(1,4)- or β(1,6)-linkages. β-glucans from oat and barley are primarily linear with large regions of β(1,4)-linkages

separating shorter stretches of β(1,3)-structures, whereas β-glucans from yeast have a β(1,3)-backbone with β(1,6)-linked β(1,3)-branches (Yan et al., 2005). β-Glucans from mushroom are similar to yeast except that they are comprised of short β(1,6)-branches coming off of a β(1,3)-backbone, thereby lacking the extra β(1,3)-branch extending from the β(1,6)-branch point (Borchers et al., 1999; 2004). These polymers have diverse structural variability including molecular weight, linkage pattern, degree of branching, triple helical conformation, and water solubility (Driscoll et al., 2009).

Zhao and Cheung (2011) attempted to elucidate the structures of β-glucans from different sources such as inulin (dahlia tuber), cereal (barley), bacteria (Curdlan), seaweed (laminarin) and mushroom. These investigations revealed that all of these β-glucans contained almost all glucose moieties as their sugar component with only trace amounts of mannose (<2%) being found in laminarin. The glycosidic linkage analysis on the β-glucans conducted using a methylation study revealed β-glucans from barley to be a linear chain polysaccharide with mixed 1,3- and 1,4-β-linkages in the ratio of 1:3, while β-glucans from both Curdlan and laminarin had a β-(1,3) linked linear chain. Curdlan was unbranched and laminarin was highly branched. Compared to other sources, β-glucans from mushrooms had a highly branched main chain with mixed glycosidic 1,3-, 1,4-, and 1,6-β-linkages. In another study, Kim et al. (2011) reported that β-glucans obtained from mushrooms contained 514 g/kg of (1,3)-β-glucans with (1,6)-β-linked side chains and its chemical structure was confirmed by ^{13}CNMR and FTIR spectroscopy. In an earlier study, Zhang et al. (2007) reported that the most common chemical structure of β-glucans from mushrooms is a β-1,3 backbone with different degrees of β-1,6- and/or β-1,4-branching.

The biological and physiochemical properties of β-glucans differ, depending on the source of extraction. Additionally, the degrees of purification, as well as the extraction method, also influences the physiological activity of β-glucans. Based on physiological properties, β-glucans are generally divided into soluble and insoluble β-glucans. In general, insoluble fibers decrease intestinal transit time as well as increase fecal bulk and the excretion of bile acids, while soluble fibers slow glucose absorption and increase the total transit time by delaying gastric emptying. Generally, β-glucans are derived from the cell walls of yeast, fungi, and cereals. The contents of β-glucans strongly depend on the environmental conditions. Among cereals, the high content of β-glucans are found in barley (20%), oat (8%), sorghum (6%), rye (3%), maize (1.7 g) and triticale (1.2 g). Other sources of β-glucans include yeasts, such as *Saccharomyces cerevisiae*, mushrooms, such as Maitake and Shiitake, and seaweeds, such as *Laminaria* sp. (Bashir and Choi, 2017).

Part III- DIETARY EXPOSURE

3.1. Proposed Use Levels and Food Categories

SBG intends to use Antrodia mushroom β-glucans derived from *A. cinnamomea* as a food ingredient in baked goods and baking mixes, beverages and beverage bases, cereal and cereal products, dairy product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, and soft candy at levels up to 150 mg mushroom β-glucans *per* serving (reference amounts customarily consumed, 21 CFR 101.12). Although some foods with standards of identity are included in the list of foods, the uses of Antrodia mushroom β-glucans are intended for foods without a standard of identity.

Antrodia mushroom β-glucan is intended for use in the same foods and at identical levels of addition to those previously described in the GRAS notification on β-glucans derived from *Ganoderma lucidum* mycelium (GRN 413) by SBG. Foods that are intended for infants and toddlers, such as infant formulas or foods formulated for babies or toddlers, and meat and poultry products that come under USDA jurisdiction are excluded from the list of intended food uses of Antrodia mushroom β-glucans.

3.2. Estimated Daily Intake

The intended use of Antrodia mushroom β-glucans in the same foods and at the same levels as those in GRN 413 (FDA, 2012; SBG, 2012) is not expected to noticeably affect the intake of β-glucans in the overall diet of the public from introduction into the market by another supplier who will have to compete in essentially the same markets and foods. Based on a statistical analysis of potential dietary intake presented in GRN 413 notice, it was estimated that the mean and 90th percentile all users intakes for the total population would be 291.3 and 583.4 mg/person/day or 6.3 and 14.5 mg/kg bw/day, respectively. For GRN 413, the dietary analysis described in GRN 309 was utilized. The intake analysis described in GRN 309 and in GRN 413 was not questioned by FDA in the response letter of June 14, 2010 and August 10, 2012, respectively.

In summary, the proposed use of Antrodia mushroom β-glucans as a food ingredient in baked goods and baking mixes, beverages and beverage bases, cereal and cereal products, dairy product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, and soft candy at levels up to 150 mg mushroom β-glucans *per* serving (RACC) is estimated to results in mean and 90th percentile all users intakes of 291.3 and 583.4 mg/person/day or 6.3 and 14.5 mg/kg bw/day, respectively.

3.3. Exposure to β-Glucans from Food

The available information shows that β-glucans, naturally present in cereal bran such as oat and barley, are commonly produced as agricultural by-products. β-Glucans are natural bioactive compounds and can be taken orally, as a food supplement, or as part of a daily diet, and are considered safe to use. Given the health benefits of products containing β-glucans, the FDA permitted a health claim on a food product label for reduction of the cholesterol level when cereal β-glucans (0.75 g per serving) is included in such foods (FDA, 1997). Similarly, the European Food Safety Authority (EFSA) authorized a health claim related to the maintenance of normal blood cholesterol concentrations for soluble cereal fibers, particularly β-glucans from oat and barley (EFSA, 2009). β-Glucans derived from different food sources are known to differ

slightly in chemical structure. However, as discussed later, biologically these molecules are expected to behave in the same manner.

In 1997, the FDA approved the health claim on the association of soluble fiber from rolled oats and reduced risk of heart disease (21 CFR 101.81) (62 FR 3584, January 23, 1997). For this claim, FDA (1997) recognized that β-glucan (soluble fiber) was the primary component of whole oat products in influencing serum lipid levels. The agency stated that β-glucan plays a significant role in the relationship between whole grain oats and the risk of coronary heart disease (CHD). This conclusion was based on two major findings:

- A dose response between the level of β-glucan soluble fiber consumed and the level of reduction in blood total- and LDL-cholesterol, and
- β-Glucan intakes of 3 g or more per day were effective in lowering serum lipids.

Since this initial health claim approval, manufacturers have attempted to market β-glucan containing products to consumers.

As per this regulation, substantiation of health claims for soluble fiber from certain foods and risk of coronary heart disease requires the daily intake of 3 g or more per day of β-glucan soluble fiber from either whole oats or barley, or a combination of whole oats and barley (FDA, 2005). Hence, it can be estimated that a diet aiming to reduce the risk of coronary heart disease provides at least 3 g β-glucan/day. As oats and barley have a β-glucan content of on average 5 and 7%, respectively (Peterson et al., 1995; Oscarsson et al., 1996; Izydorczyk and Dexter, 2008), it can be estimated that a serving of 50 g whole grain oat or barley provides 2.5 and 3.5 g β-glucan, respectively. Additionally, as per 21 CFR 172.898, the FDA has approved the use of bakers yeast glycan (synonymously called as glucans) for direct addition as a multi-purpose food additive to various food products including salad dressings, frozen desserts, sour cream and cheese spread analogs, and flavored snack dips. These approved uses of glycan also suggest a safe intake of β-glucans from food by humans. Additionally, consumers have been exposed to β-glucans through the consumption of baker's yeast, mushrooms and other foods that contain β-glucans.

Available information on background intake of oat β-glucan is summarized in a GRAS notice on barley β-glucan (FDA, 2011). This information indicates that oat-derived β-glucan concentrates, including oatrim with a β-glucan content of up to 15%, have been consumed safely for over 10 years. This ingredient was developed in the late 1980s as a fat replacer and has been extensively used by different manufacturers.

In a study on quantification and analysis of β-1,3-glucans in foods, Ko and Lin (2004) tested the levels of β-glucans in six food categories including legumes, cereals, tubers, vegetables, fruits, and mushrooms, and from 17 total items. The results of this quantitation revealed significant levels of β-1,3-glucans naturally present in a number of foods, such as edible mushrooms, specifically the Shiitake (*Lenbnus edodes*), Maitake (*Grifola frondosa*), Wood Cauliflower (*Sparassis crispa Fr*), and snow mushroom (*Tremella fucifomis*) varieties. The Snow mushroom (dry weight) had the highest levels of β-1,3-glucans (2.5%), and was also rich in both water (0.67%) and alkaline soluble (1.87%) forms. Additionally, these investigators also reported that several non-fungus-derived food sources, such as celery, chi-chian leaves, carrot, and radish, contain nearly 20% β-1,3-D-glucans in their total carbohydrate fraction, and soybeans were reported to contain up to 0.8% β-1,3-D-glucans (dry weight). The findings from this study suggest that humans are exposed to mushroom β-glucans from food items.

In recent years, *A. cinnamomea*, the source material of Antrodia mushroom β-glucans, has attracted great attention around the world as an extremely precious edible and therapeutic mushroom (Zhang et al., 2017). Commonly known as the fungus of fortune, *A. cinnamomea* in Taiwan is known as "niu zhang zhi" or "zhang-yi." Growing only inside the rotting heartwood of the indigenous Bull Camphor Tree (*Cinnamomum kanekirai*) endemic to Taiwan, this mushroom has been used as a natural therapeutic ingredient. The slow growth and host-specific requirements makes this mushroom one of the highly priced unique food items in the world.

In summary, the available information suggest that humans are regularly exposed to β-glucans from diet. β-Glucans from different dietary sources are known to differ slightly in chemical structure, but biologically these molecules are expected to behave in the same manner. Additionally, the source material of Antrodia mushroom β-glucans, *A. cinnamomea* is an edible mushroom.

Part IV- SELF LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with the intended use of Antrodia mushroom β-glucans. It should be noted that Super Beta Glucan does not intend to add Antrodia mushroom β-glucans at any level beyond what is listed in the GRAS document.

Part V- EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

The statutory basis for the conclusion of the GRAS status of Antrodia mushroom β-glucans in this document is not based on common use in food before 1958. The GRAS assessment is based on scientific procedures. Notwithstanding this, the source material of the extract - Antrodia mushroom - has been present in food prior to 1958, as described in this dossier.

Part VI- NARRATIVE

6.1. Data Pertaining to Safety

In a series of well-designed toxicity studies, conducted as per current accepted guidelines, SBG investigated the effects of Antrodia mushroom β-glucans in animals and *in vitro* experimental systems. The findings from all these investigations are published in the journal *Regulatory Toxicology and Pharmacology* (Chen et al., 2018). In the following section, relevant toxicological and other studies on Antrodia mushroom β-glucans and similar substances are summarized in the order of their importance and in support of the conclusions drawn in this assessment. This information is provided in the following sequence: published pivotal studies, secondary published studies, and regulatory agency assessments. Efforts have been made to present both the data supporting the safety as well as any data on the adverse effects of Antrodia mushroom and similar preparations.

The safety determination of Antrodia mushroom β-glucans is based on the totality of available evidence, particularly from experimental studies of Antrodia mushroom β-glucans and those conducted on β-glucans from other sources in animals, as well as from human trials. The experimental studies of Antrodia mushroom β-glucans were designed to evaluate its safety as a dietary supplement. Subsequent to the recent FDA evaluations of the GRAS notices on yeast β-glucans and mushroom β-glucan that also contained all relevant data from other sources of β-glucans, very few safety-related studies on β-glucans have appeared in the published literature. These studies do not raise any new safety concerns. A summary of the recent publications that appeared following the agency's review of the recent GRAS notices, along with some relevant findings are described below.

6.1.1. Pivotal Studies of Antrodia Mushroom β-Glucans

6.1.1.1. Subchronic Toxicity Study of Antrodia Mushroom β-Glucans

Chen et al. (2018) investigated adverse effects of a standardized Antrodia mushroom β-glucan preparation in a repeat-dose subchronic toxicity study in 96 CD® (SD) IGS strain rats. The study was conducted in according to a protocol based on the Organization for Economic Co-operation and Development (OECD) Guidelines for Testing Chemicals, Health Effects Test Guidelines, for Repeated Dose 90-Day Oral Toxicity Study in Rodents, Section 408, as per Good Laboratory Practices for Non-clinical Laboratory Studies (FDA, 21 CFR, Part 58), and OECD principles on Good Laboratory Practices. In this study, rats divided into four groups (12/sex/group; approximately 6 weeks old) were administered (gavage) once daily with Antrodia mushroom β-glucans dissolved in sterile water at dose levels of 0 (control- Group I), 500 (low dose- Group II), 1000 (mid dose- Group III), or 2000 (high dose- Group IV) mg/kg bw/day for 90 consecutive days. Throughout the study period, the animals were observed for mortality/morbidity (twice daily), detailed clinical observations (daily), body weight and feed consumption (weekly), and ophthalmologic examinations at the grouping day and before euthanasia. Body weights were recorded before the first dosing, weekly thereafter, prior to the termination of the study, and on the day of necropsy. Mean body weight and mean body weight gains were recorded. Feed consumption was measured at weekly intervals. After 90 days of treatment, hematology, serum chemistry, and urinalysis measurements were performed for surviving animals after 13 weeks of treatment. At termination, necropsy was performed and tissue weights were recorded at termination. Over 40 tissues and organs were fixed in 10%

buffered neutral formalin. Histopathological examination was carried on the full set of tissues collected from the high dose and control groups.

There were no mortalities reported during the treatment period that were related to Antrodia mushroom β-glucan preparation (Chen et al., 2018). One female rat, in the control group, was found dead on Day 78 due to individual spontaneous lesion not related to the β-glucan preparation. There were no treatment-related abnormal clinical signs in any of the groups during the entire study period. Ophthalmological examinations did not reveal any abnormalities in any group prior to dosing and prior to necropsy. The mean body weights and body weight gains of treatment groups were comparable to control group animals throughout the treatment period, except for the statistically significant higher mean body weight gain in the high-dose (Group IV) treated male rats at the week 11 measurement. This finding was not considered to be related to Antrodia mushroom β-glucan preparation administration. Similarly, there were no biologically significant differences in feed consumption in males and females in the vehicle control and treatment groups during the course of study. At week 10, in the high-dose group male rats a statistically significant decrease in feed intake was noted. At week one, in all female treatment group, significantly higher feed intake was noted. These significant changes were not considered related to Antrodia mushroom β-glucan preparation administration (Chen et al., 2018).

The results of urinalysis parameters (volume, pH, specific gravity, and urobilinogen) in male and female rats following administration of Antrodia mushroom β-glucan preparation did not reveal any significant changes, except for a statistically significant lower urine pH value in Group II (low-dose group). No treatment related biologically significant adverse effects in hematology parameters were noted except a statistically significant increase in blood levels of eosinophil in the high-dose treated group. This change in male rats lacked correlating changes in other red cell parameters, remained within physiological range, was of small magnitude, and/or was not noted in a dose-related manner. Hence, this change was considered as incidental variation and not treatment-related adverse effect. There were no other statistically significant differences when the respective control and treatment groups were compared (Chen et al., 2018).

There were no treatment-related biologically significant adverse effects of the Antrodia mushroom β-glucan preparation on serum chemistry parameters in male and female rats. However, in male rats, a significant increase in serum sodium levels in mid- and high-dose groups; serum albumin in the mid-dose group; and serum chloride in low-dose group was noted. The serum sodium levels in male control, low, medium and high dose groups was as follows: 145.23±1.38; 146.54±1.25; 147.23±1.56*; 148.35±1.57* mmol/L, respectively; historical control data range- 140.95 - 148.99 mmol/L. Similarly, in female rats, a statistically significant decrease in serum levels of protein in the low-dose group; increase in alkaline phosphatase in the low-dose group; and increase in serum sodium levels in the mid- and high-dose groups was noted. The serum sodium levels in female control, low, medium and high dose groups was as follows: 143.47±1.54; 144.28±1.55; 145.23±1.40*; 145.26±1.46* mmol/L, respectively; historial control data range- 132.51 - 151.47 mmol/L. The changes noted in sodium appears to be dose-related; however these changes were not toxicologically relevant as the increase was of low in magnitude, within the historical control range (normal physiological range), and may be due to the sodium content of the test article. These changes noted in serum sodium were also reported in a previous study by Chen et al. (2011) with another mushroom β-glucan preparation that was derived from *G. lucidum*. The above described changes in serum sodium, alkaline phosphatase and proteins

were well within the normal laboratory control or physiological range and hence considered as incidental changes or biological variations and not as treatment-related effects (Chen et al., 2018).

As regards organ weights, there were no statistically significant differences when the respective control and treatment groups were compared. No treatment related macroscopic findings were noted in any of the groups at the scheduled necropsy following administration of the β-glucan preparation to rats. At terminal euthanasia, only one female from the low-dose group showed focal mass in subcutaneous tissue (mammary gland). According to severity and incidence based on histopathological evaluation of this lesion (fibroadenoma), the finding was considered as spontaneous abnormality and not related to the β-glucan preparation. There were no treatment-related histopathological findings. The histopathological observations in the high-dose group were considered to be spontaneous due to incidence, significance, and severity. These changes were observed across all groups and no dose-related response was noted. All findings observed were consistent with normal background lesions in clinically normal rats of the age and strain used in this study, and were considered spontaneous and/or incidental in nature and unrelated to the treatment (Chen et al., 2018).

In summary, the findings of this subchronic (90-day) toxicity study suggest that oral administration of the Antrodia mushroom β-glucan preparation at levels up to 2000 mg/kg bw/day does not cause adverse effects in male and female rats. Based on the results of this study, the no-observed adverse effect level (NOAEL) of the Antrodia mushroom β-glucan preparation can be established as 2000 mg/kg bw/day, the highest dose tested (Chen et al., 2018). The findings from this study suggest that the resulting all user maximum intake of 14.5 mg/kg bw/day from the proposed uses of Antrodia mushroom β-glucan is over 135-fold lower as compared to the NOAEL. The findings from this study support the safe use of Antrodia mushroom β-glucan preparation by humans.

6.1.1.2. Genotoxicity Studies

6.1.1.2.1. Ames Assay

A study on the potential mutagenic effects of Antrodia mushroom β-glucans was conducted using the Bacterial Reverse Mutation Assay, also known as the Ames test (Chen et al., 2018). For this assay, *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were used, and the plate incorporation method in the presence or absence of a S9 metabolic activation system was applied. Based on an initial dose range findings study that revealed significant growth that interfered the counting of revertant colony at doses of 5.0, 2.5 and 1.25 mg of Antrodia mushroom β-glucan preparation/plate, following concentrations were tested: 0.039, 0.078, 0.157 and 0.313 mg/plate. The chemicals used as positive controls for assays without or with metabolic activation included 2-nitrofluorene, sodium azide, mitomycin C, 9-aminoacridine, 2-aminoanthracene, and benzo(a)pyrene.

There was no significant increase in the number of revertant colonies. Antrodia mushroom β-glucan preparation did not present any genotoxic effect at all concentrations tested with or without the presence of S9. The number of revertant colonies in the negative control groups of each strain was within the range of historic control data. The revertant colonies in the positive control group were more than two times (TA98, TA100, and TA102) and three times (TA1535 and TA1537) the negative control groups. There was no significant increase in the number of revertant colonies at all concentrations of test article in any of the strains whether with

or without S9 mixture, suggesting that Antrodia mushroom β-glucan preparation was not genotoxic (Chen et al., 2018).

6.1.1.2.2. Chromosome aberrations

The genotoxic potential of Antrodia mushroom β-glucans to induce chromosome aberrations in Chinese hamster ovary cells (CHO-K1) was evaluated (Chen et al., 2018). The test was performed following GLP guidelines and in accordance with the OECD guideline for testing of chemicals #473-*In vitro* Mammalian Chromosome Aberration Test. Five doses (0.313, 0.625, 1.25, 2.5, and 5 mg/ml) of Antrodia mushroom β-glucan preparation were tested for cytotoxicity using the MTT assay. CHO-K1 cells with epithelial-like morphology and modal chromosome number 20 ± 2 were used. The positive controls in the different treatments included mitomycin C (3 and 18 hours), benzo(a)pyrene (3 hours). The non-cytotoxic dosages of 1.25, 2.5, and 5 mg/ml of mushroom β-glucans with or without S9 in short-term (3 hours) and without S9 in long term (18 hours) were selected for the chromosomal aberration test.

In the three-hour exposure group, cell viability in the absence of S9 metabolic activation at the following concentrations 5, 2.5, 1.25, 0.625, and 0.313mg/ml were 97.06±0.89, 99.06±2.41, 97.64±2.21, 99.62±0.92, and 94.27±1.07%, respectively. In the short-term treatment in the presence of S9 metabolic mixture, the cell viabilities at 5, 2.5, 1.25, 0.625, and 0.313 mg/ml were 106.15±6.55, 107.60±2.26, 103.20±2.56, 132.29±10.01, and 128.55±7.63%, respectively. In the 18-h (long-term) treatment in the absence of S9 metabolic mixture, the cell viabilities were 87.72±2.13, 91.13±0.81, 94.21±0.88, 95.99±0.86, and 100.69±5.53%, respectively. As the cell viability was greater than 50%, the extract was not considered as cytotoxic. Antrodia mushroom β-glucan preparation treatment of CHO-K1 cells did not display genotoxicity. The results of this investigation suggest that, Antrodia mushroom β-glucan preparation is non-mutagenic and do not cause significant structural and numerical aberrations under the experimental conditions (Chen et al., 2018).

6.1.1.2.3. *In vivo* Micronucleus Assay

The mammalian peripheral blood micronucleus test was conducted in accordance with the OECD guideline for the testing of chemicals #474: mammalian erythrocyte micronucleus test (Chen et al., 2018). Antrodia mushroom β-glucans was administered orally to CD-1® (ICR) mice (SPF grade, about 7 weeks old) (5/sex/group; the control group 6/sex) at dose levels of 0, 80, 500 1000 and 2000 mg/kg bw. Cyclophosphamide (80 mg/kg bw) was chosen as the positive control and administered intraperitoneally. Following the treatment, peripheral blood samples (2 μl) from the tail vein of mice were collected at 24, 48, and 72 hours. The blood was smeared on acridine orange-coated slides and the staining was performed at room temperature for 2-3 hours. The positive control group was only sampled at 48 hours after dosing.

There were no abnormal clinical symptoms noted in the animals in any group during the study, and there were no mortalities observed during the study. No significant differences in mean body weights were noted between the groups. The PCE% of the positive control group at 48 hours after dosing in females and males was 1.38±0.37% and 1.40±0.62%, respectively. A decrease in the PCE % of the positive control group, at 48 hours after dosing, indicated inhibition of erythropoiesis by cyclophosphamide. However, the PCE% in all the treatment groups showed no significant decrease as compared to the negative control group indicating Antrodia mushroom β-glucan preparation did not inhibit erythropoiesis (Chen et al., 2018).

The micronucleus frequency in thousand PCE of the negative control group at 48 and 72 hours after dosing was 0.17±0.26, and 0.17±0.41 ‰PCE in females, and 0.67±0.52, and 0.50±0.45 in males, respectively. The micronucleus frequency in thousand PCE of the positive control group at 48 hours after dosing was 20.40±5.55 in females, and 21.00±7.62 in males. After Poisson distribution analysis, there was no significant difference between the three treatment groups and the negative control group, indicating Antrodia mushroom β-glucan preparation exhibited no genotoxicity (Chen et al., 2018).

6.1.2. Secondary Published Studies with Other products

6.1.2.1. Toxicity Studies of Source Mushroom

In a subchronic toxicity study, Chang et al. (2013) investigated oral toxicity of *A. cinnamomea* extracts in male and female BALB/c mice. *A. cinnamomea* and distilled water were mixed thoroughly and filtered (0.22 μm pore size) to provide a solution that was used in this study. For this study, BALB/c male and female mice (10/sex/group) divided into control (Group 1) and groups 2 (low), 3 (medium) and 4 (high) were and orally administered with 0, 16.67, 833.3 and 1666.67 mg/kg bw/day of *A. cinnamomea* daily for 90 consecutive days. All animals survived to the end of the study, and there were no significant differences in body weight among the control and treatment groups. No significant differences were found in hematological and serum biochemical parameters among the control and treatment groups. No abnormalities of internal organs as evaluated by histopathologic examinations were observed in the treated groups. The investigators concluded that oral administration of *A. cinnamomea* to male and female mice at levels up to 1666.67 mg/kg bw/day for 90 days does not produce any changes in blood cell counts and serum chemistry.

In another subchronic toxicity study, Chen et al. (2011a) investigated the toxicity of *A. cinnamomea* (freeze-dried mycelia) from submerged culture in male and female Sprague-Dawley (SD) rats. In this study, 80 rats were divided into four groups (10/sex/group). *A. cinnamomea* was administered by oral gavage to rats at dose levels of 0, 1500, 2200 and 3000 mg/kg bw/day for 90 consecutive days. All standard parameters commonly evaluated in toxicity study were studied. All animals survived to the end of the study. During the experiment period, no abnormal changes were observed in clinical signs, body weight and ophthalmological examinations. No significant differences were found in urinalysis, hematology and serum biochemistry parameters between the treatment and control groups. Necropsy and histopathological examination indicated no treatment-related changes. The NOAEL of *A. cinnamomea* extract was identified to be greater than 3000 mg/kg bw/day in Sprague-Dawley rats. The investigators also stated that using a safety factor of 100, the acceptable daily intake (ADI) of *A. cinnamomea* extract was estimated to be 30 mg/kg bw/day.

In a series studies with *A. cinnamomea* powder consisting of 5% extract of fruiting bodies from cut-log cultivation, 94% mycelium from high-efficient solid state cultivation of *A. cinnamomea* and 1% magnesium stearate (*A. cinnamomea* extract), Lin et al. (2015) investigated toxicity potentials by conducting following studies: (a) acute and repeated dose oral toxicity studies in rats; (b) reproductive and developmental assessment in pregnant female rats; and (c) mutagenicity and genotoxicity by bacterial reverse mutation assay, mammalian chromosomal aberration test, mammalian erythrocyte micronuclei test using CHO-K1 cells, and rat bone marrow erythrocytes.

In the acute toxicity study by Lin et al. (2015), oral administration of *A. cinnamomea* extract at dose levels of 0, 1400, 2800 and 5600 mg/kg bw to Sprague Dawley rats (6/sex/group) produced neither deaths nor treatment-related signs of toxicity in any of the treatment groups during the study. In addition, no weight loss resulted from the extract treatment compared to the control groups in both genders after 1, 4, 8 and 15 days. Based on the findings from this study, the oral LD_{50} of the extract was found to be greater than 5 g/kg bw for both genders. The data generated from this study provided safety information for human exposure and also provided information to establish a dose regimen in further studies.

The 90-day repeat dose (subchronic) toxicity study was performed as per OECD guidelines. In this study, Sprague Dawley rats (12/sex/group) were divided into 4 groups (Group I to IV) and were treated with *A. cinnamomea* extract at dose levels of 0 700 (low), 1400 (mid) and 2800 (high) mg/kg bw/day for 90 consecutive days (Lin et al., 2015). All standard parameters for such a toxicity study and as recommended by OECD were evaluated. No mortalities or ophthalmologic and treatment related signs of toxicity were observed during the study period in any of the treatment groups. *A. cinnamomea* extract did not affect body weights, body weight gain or feed consumption. No treatment related severe clinical signs were observed. There were no statistically significant differences in hematological parameters of male rats, whereas some statistically significant differences were observed in female rats treated with the extract. Particularly, the hematocrit of the high-dose group was statistically lower as compared to vehicle control group (Lin et al., 2015).

Some serum chemistry parameters showed statistically significant changes in both genders. In male rats, the glucose levels in high-dose dose group was statistically higher, while total protein level in the mid dose group was lower than control group. In female rats, a significant increase of total cholesterol in mid and high dose groups was reported. However, there was no statistically significant difference in triglyceride levels. Urine analysis showed there was no significant difference in volume, specific gravity and urobilinogen in all tested groups, whereas the pH of high-dose treated rats were statistically lower as compared to control group in both genders. However, despite those statistical differences, the data were within the normal historical range and without physiological abnormalities (Lin et al., 2015).

The internal organ weights in all treated groups of both genders were not significantly different from those of the control groups with the exception of the liver weight of those male animals in the highest dose (2800 mg/kg bw/day) group. This statistical difference was within normal historical control range and without physiological abnormalities. In females, there was no statistical difference between the vehicle control and the treated groups. Gross necropsy findings revealed no treatment related signs of toxicity noted with respect to gross examination of all organs. Histopathologically, myeloid hyperplasia of mononuclear cells was noted in the liver, spleen and bone marrow in femur. All lesions showed moderate mononuclear cell leukemia. According to the severity and incidence in histopathological evaluation, this lesion was considered to be a spontaneous abnormality and not related to the treatment (Lin et al., 2015).

In the reproductive and developmental toxicity study, 60 male CD (SD) IGS rats and 120 female rats were used. After mating, confirmed-mated females were assigned to the four study groups. Eighty confirmed pregnant female rats were equally divided into 4 groups: and treated with oral gavage at levels of 0, 700, 1400 and 2800 mg/kg bw during the major embryonic organogenesis period (gestation day 6 through 15). All standard investigations of such a study were undertaken. The confirmed pregnancy rates were high (80 to 90%) for all groups. No

maternal mortality or morbidity or significant maternal body weight and weight gain were noted. Maternal evaluation did not reveal significant statistical differences between the groups in gravid uterus weight, implantation number, corpora lutea number, litter size, live or dead fetal number, male or female fetus number, resorption number, fetal sex ratio, pre-implantation loss and post-implantation loss (Lin et al., 2015).

Fetal examination showed no statistical significance in fetal body weight and body length among the test groups. There was no dose response significance noted in the incidence of abnormalities. Skeletal examination of 50% fetuses of each litter showed no statistically significant incidences or abnormalities in tested fetuses suggesting that there was no treatment related abnormalities and teratogenic toxicity with *A. cinnamomea* extract at dose levels up to 2800 mg/kg bw/day, the highest dose tested (Lin et al., 2015).

Lin et al. (2015) studied the mutagenic potential of *A. cinnamomea* extract by a bacterial reverse mutation (Ames) assay, while genotoxicity was examined by a mammalian chromosomal aberration test and mammalian erythrocyte micronuclei test using CHO-K1 cells and rat bone marrow erythrocytes, respectively. In the bacterial reverse mutation test, histidine-requiring *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were used. A plate incorporation assay was employed and performed to detect reverse mutation in bacterial strains. Based on cytotoxicity and range finding studies, following doses: 0.313, 0.625, 1.25, 2.5 and 5 mg/plate were used. Compared to the negative control groups (sterile water), the positive control substances induced generally at least 3-fold increase of the number of reverse mutation colonies, confirmed the validity of the test. The mean number of revertant colonies of the negative control was within the historical range. *A. cinnamomea* extract did not increase the mean number of reverse mutations at dose levels between 0.313 and 5 mg/plate in both normal and metabolically activated bacterial strains. These results suggest that *A. cinnamomea* extract does not induce bacterial reverse mutation within the test doses (Lin et al., 2015).

For the *in vitro* chromosomal aberration test, the Chinese hamster ovary cell line (CHO-K1) was used. The frequency of the cells with chromosome structural aberration was scored by measuring chromosome breakage (csb), chromosome exchange (cse), chromatid breakage (ctb), chromatid exchange (cte), and other abnormalities such as polyploidy, these were scored. Based on the results of the viability test, dosages with over 50% cell viability, selected for use in the chromosome aberration test were 0.625, 1.25 and 2.5 mg/mL for 3-hour treatment group without S9 and those used in the 3 hour treatment group with S9 were 1.25, 2.5 and 5 mg/ml. In the 18-hour treatment group without S9, the doses used in the chromosome aberration test were 0.313, 0.625 and 1.25 mg/ml. The findings for negative and positive control were confirmed for accuracy. The chromosome aberrations in *A. cinnamomea* extract treated cells were 1, 3 and 3 at 0.625, 1.25 and 2.5 mg/mL under 3-hour without S9 and 3, 3 and 3 at 1.25, 2.5 and 5 mg/mL, respectively under 3-hour with S9 metabolic activation. Moreover, the chromosome aberration in 200 observed metaphase cells were 4, 3 and 2 by 0.313, 0.625 and 1.25 mg/mL *A. cinnamomea* extract, respectively under 18-hour without S9 metabolic activation. The findings indicate that exposure to *A. cinnamomea* extract does not significantly induce chromosome aberration in cultured mammalian somatic cells under the test conditions (Lin et al., 2015).

The micronucleus test was performed as per OECD guidelines in mice. In this study, groups of mice received *A. cinnamomea* extract in water orally at dose levels of 0, 700, 1400 or 2800 mg/kg, while positive control group received intraperitoneally 80 mg/kg cyclophosphamide. At 48- and 72-hours post-treatment, peripheral blood samples were obtained from the tail vein

and smeared on acridine orange coated microscopic slides. The percentage of PCE in 1000 erythrocytes was quantified. At least 2000 PCE/animal were scored for the incidence of PCE with micronucleus. After 96 hours post-treatment, no mortalities were recorded, and gross necropsy of the animals revealed no macroscopic findings. The percentage of positive control groups at 48 hours were 0.60±0.36% in female and 1.02±0.19% in male. The percentage of PCE in positive control was significantly decreased after dosing indicating that cyclophosphamide inhibits erythropoiesis. However, the percentage of PCE in *A. cinnamomea* extract treated groups showed no significant decrease as compared to negative control group, suggesting that all the testing doses of *A. cinnamomea* extract did not affect erythropoiesis. The micronucleus frequency in 1000 PCE using fluorescence microscope did not reveal any significant difference between three testing doses of *A. cinnamomea* extract and negative control group in both genders at 48 and 72 hours. Based on these observations, the investigators concluded that *A. cinnamomea* extract does not increase micronucleated PCE under the test condition (Lin et al., 2015).

In summary, the results of acute oral toxicity study of *A. cinnamomea* extract in rats showed that LD_{50} is greater than 5 g/kg bw. The findings from the dose response subchronic toxicity study did not reveal any evidence of toxicity at dose levels up to 2800 mg/kg bw/day supporting safety of *A. cinnamomea* extract for oral consumption. Based on the results of reproductive and developmental toxicity study, there were no observable segment II reproductive and developmental evidences of *A. cinnamomea* extract. In mutagenicity and genotoxicity studies, *A. cinnamomea* extract showed no mutagenic activity in the bacterial reverse mutation test, did not induce micronuclei in mammalian erythrocytes or increase the rates of structural and numerical chromosome aberration of CD mice. The NOAEL under the conditions of this study was 2800 mg/kg bw/day. Taken together, these studies suggest that *A. cinnamomea* extract has a very low order of toxicity. These studies also show that the source material of Antrodia mushroom β-glucan preparation, *A. cinnamomea* is safe for human consumption.

6.1.2.2. Toxicity Studies with other Mushroom β-Glucans

In a series of toxicity studies, Chen et al. (2011) investigated the subchronic toxicity and potential genotoxic effects of mushroom β-glucans derived from *Ganoderma lucidum*. The experimental protocol and methodologies used in this study were similar to those described for the above study by Chen et al. (2018). Hence, the details of protocol are not elaborated in the below description of these studies.

6.1.2.2.1. Repeat Dose Toxicity Study with other Mushroom β-Glucans

In the subchronic toxicity study with mushroom β-glucans derived from *G. lucidum*, Sprague Dawley rats (12/sex/group) were administered (gavage) mushroom β-glucan at dose levels of 0, 500, 1000 and 2000 mg/kg bw/day for 90 consecutive days (Chen et al., 2011). The study was also conducted as per OECD guidelines. The oral (gavage) of mushroom β-glucan at levels up to 2000 mg/kg bw/day to male and female Sprague Dawley rats for 13 weeks was not associated with adverse effects on the general condition and appearance of the animals, growth, feed consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights and terminal necropsy (gross or histopathology findings). There were only a few statistically significant differences between rats treated with mushroom β-glucan and controls which were ascribed to treatment but none of these were regarded to represent adverse effects of mushroom β-glucan.

For example, at 2000 mg mushroom β-glucan/kg bw/day a statistically significant increase in MCV and MCH levels was noted, whereas hematocrit levels showed increase at 500 mg/kg bw/day. Similarly, an increase in sodium levels in males at dose levels of 500 and 2000 mg/kg bw/day was noted without any significant change at 1000 mg/kg bw/day. In female rats, a significant increase in sodium levels was also noted at 1000 and 2000 mg/kg bw/ day dose levels. Additionally, in male rats a statistically significant decrease in testes weight receiving 500 mg/kg bw/day dose and heart weight in female rats receiving 1000 mg/kg bw/day dose was noted. These statistically significant changes noted in clinical pathology parameters and organ weight following administration of the mushroom β-glucan were considered as incidental and not related to the treatment, as they were either limited to one sex, lacked dose-response, were within the normal laboratory ranges, and/or were not supported by any other changes in related clinical parameters or histopathological observations. Based on the results of this study, the NOAEL of the mushroom β-glucans derived from *G. lucidum* is determined as 2000 mg/kg bw/day, the highest dose tested (Chen et al., 2011).

6.1.2.2.2. Genotoxicity Studies with other Mushroom β-Glucans

In the study by Chen et al. (2011), mutagenicity and genotoxicity of mushroom β-glucans derived from *G. lucidum* was investigated by three separate assays such as gene mutations in *S. typhimurium* (Ames assay), *in vitro* chromosome aberrations and *in vivo* micronucleus test in mouse. The experimental methods used in these assays was similar to as those described earlier by Chen et al. (2018).

In the Ames assay, *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were tested by plate incorporation method at concentrations of 0.313, 0.625, 1.25, 2.5, and 5 mg/plate in the presence or absence of a S9 metabolic activation. There was no significant increase in the numbers of revertant colonies. Mushroom β-glucans did not present any genotoxic effect at any of the concentrations tested with or without the presence of S9. The number of revertant colonies in the negative control groups of each strain was within the range of historic control data. The revertant colonies in the positive control group were more than two times (TA98, TA100, and TA102) and three times (TA1535 and TA1537) the negative control groups. There was no significant increase in the number of revertant colonies at any of the concentrations in the presence or absence of S9 mixture, suggesting that mushroom β-glucans was not genotoxic.

In the chromosome aberration assay, the genotoxic potential of mushroom β-glucans in Chinese hamster ovary cells (CHO-K1) was evaluated (Chen et al., 2011) as per OECD guidelines. Five doses (0.313, 0.625, 1.25, 2.5, and 5 mg/ml) of mushroom β-glucans were tested for cytotoxicity using the MTT assay. The positive controls in the different treatments included mitomycin C (3 and 18 hours), benzo(a)pyrene (3 hours). The non-cytotoxic dosages of mushroom β-glucans with or without S9 in short-term (3 hours) and without S9 in long term (18 hours) were selected for the chromosomal aberration test. In the three hour exposure group, cell viability in the absence of S9 metabolic activation at the following concentrations 5, 2.5, 1.25, 0.625, and 0.313 mg/ml were 83.53±2.64%, 85.38±3.60%, 91.96±.77%, 91.54±1.12%, 96.79±3.27%, respectively. In the short-term treatment in the presence of the S9 metabolic mixture, the cell viabilities at 5, 2.5, 1.25, 0.625, and 0.313 mg/ml were 96.17±3.88%, 85.83±1.75%, 93.49±0.39%, 97.39±3.10%, 100.82±2.11%, respectively. In the long-term treatment in the absence of the S9 metabolic mixture, the cell viabilities were 67.05±5.59%,

69.40±1.88%, 81.46±1.92%, 82.01±3.15%, 95.61±3.11%, respectively. Mushroom β-glucans treatment of CHO-K1 cells did not display genotoxicity. The results of this investigation suggest that in comparison with the negative control, mushroom β-glucans treatment did not result in any difference in both the short- and long-term treatments of the chromosomal aberration test (Chen et al., 2011).

The *in vivo* mammalian peripheral blood micronucleus test was conducted in accordance with the OECD guideline (Chen et al., 2011). In this study, mushroom β-glucans was administered orally to CD-1® (ICR) mice (5/sex/group) at dose levels of 0, 1250, 2500, and 5000 mg/kg bw. Cyclophosphamide (80 mg/kg bw) administered via intrapertoneally was chosen as the positive control. The peripheral blood samples from the tail vein were collected at 24, 48, and 72 hours after dosing. There were no abnormal clinical symptoms or mortality observed during the study. No significant differences in mean body weights were noted between the groups. The PCE percentage of the positive control group at 48 hours after dosing in females and males were 1.05±0.23% and 1.25±0.34%, respectively. A decrease in the PCE percentage of the positive control group at 48 hours after dosing, indicated inhibition of erythropoiesis by cyclophosphamide. However, the PCE percentage in all the treatment groups showed no obvious decrease than observed for the negative control group indicating mushroom β-glucans did not inhibit erythropoiesis. The micronucleus frequency in one thousand PCE of the negative control group at 24, 48, and 72 hours after dosing were 1.00±0.71%, 1.20±0.84%, and 0.80±0.84 $^{0}/_{00PCE}$ in females, and 1.00±0.71%, 1.20±0.45%, and 1.00±0.71% in males, respectively. The micronucleus frequency in one thousand PCE of the positive control group at 48 hours after dosing was 21.00±8.49% in females, and 21.00±2.55% in males. After Poisson distribution analysis, there was no significant difference between the three treatment groups and the negative control group, suggesting that mushroom β-glucans was non-genotoxic.

6.1.2.3. Toxicity Studies of β-Glucans from other Sources

In a short-term oral feeding study, Jonker et al. (2010) investigated the safety of β-glucans (>75%) derived from barley. In this study, barley β-glucan was fed to Wistar rats (5/sex/group) at dietary levels of 0 (control), 1, 5 and 10% (equivalent to 0, 500, 2500, 5000 mg/kg bw/day) for 28 days and all common toxicity parameters, including neurobehavioral observations, growth, feed and water consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights, necropsy and histopathological examination were investigated. In the high-dose group male rats exhibited lower plasma cholesterol and phospholipid levels and a higher plasma urea level. These changes were considered of no toxicological significance. In the mid- and high-dose males, full and empty caecum weights were increased, and this was considered to be due to a physiological response to the consumption of high amounts of indigestible carbohydrate. The results of this study show that feeding barley β-glucans at dietary levels up to 10% for 28 days was well tolerated without any signs of toxicity. This dietary level was equivalent to 5800 and 5900 mg barley β-glucans/kg bw/day in male and female rats, respectively (NOAEL = 5800 mg/kg bw/day). In two separate 28-day toxicity studies, Delaney et al. (2003a; 2003b) also reported that consumption of concentrated barley β-glucans (10% in feed) was not associated with any obvious signs of toxicity in rats and mice even following consumption of large quantities.

In another study, Babicek et al. (2007) investigated the acute and subchronic toxicity of a yeast-derived β-glucans ingredient in rats. In the acute study in F344 rats, the LD_{50} value of

yeast-derived β-glucans was found to be greater than 2000 mg/kg bw. In the subchronic study, F344 rats (10/sex/group; 5-6 weeks of age and weighed between 80 and 100 g at the initiation of treatment) were administered (gavage) daily with yeast-derived β-glucans at doses of 0, 2, 33.3, or 100 mg/kg bw/day for 90 consecutive days. In this study, the standard full toxicological monitoring and endpoints were evaluated. Administration of yeast-derived β-glucans did not significantly affect animal weights or feed consumption. No mortality, clinical pathology, functional/behavioral, microscopic, or gross observations indicative of toxicity were noted. Sporadic changes noted in some biochemical and hematological parameters were not considered to be of toxicological significance. Based on the results of this study, the investigator determined a NOAEL of 100 mg/kg bw/day, the highest dose tested.

In a long-term oral toxicity study in rats, Feletti et al. (1992) investigated the effects of yeast β-glucans derived from *Candida albicans* ATCC 20955. In this study, Sprague Dawley rats (20/sex/group) were administered the test material by gavage at doses of 0, 50, 100, or 200 mg total β-glucans/kg bw/day for 52 weeks. No treatment-related deviation from normality was noted in mortality, physical appearance and general behavior. Feed and water intake and body weight gain of β-glucans-fed groups did not differ from those of control groups. No changes in the weight of the main organs was noted. Hematology, clinical chemistry, urinalysis and autopsy findings were within normal ranges. In the 200 mg/kg bw/day group, soft stools or diarrhea and cecal enlargement with variable hyperplasia of the colon mucosa were observed. As stated by the investigators, these symptoms are typical of exposure to substances which are absorbed incompletely in the small intestine and subjected to microbial metabolism in the cecum and colon. In rodents consuming large amounts of polyols, cecal enlargement is a well-established response (Newberne et al., 1988), and such a response is not considered toxicologically significant and not relevant to humans (WHO, 1987). The investigators estimated the NOEL to be 100 mg/kg/day. However, given the changes noted in this study, a NOAEL of 200 mg/kg/day is more appropriate, the highest dose tested.

6.1.2.4. β-Glucans Metabolic Fate

Similar to other prebiotics and other non-digestible/fermentable carbohydrates such as inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), resistant starch, polydextrose, cyclodextrins, and lactulose, β-glucans largely remain undigested in the upper gastrointestinal tract (Macfarlane et al., 2006). Humans are unable to digest carbohydrate polymers with β-glucosidic linkages (Wisker et al., 1998). Hence, absorption by the intestinal epithelium and significant systemic exposure to particulate mushroom β-glucans is unlikely. In the colon, mushroom β-glucans is likely to be fermented by the resident microbiota resulting in the formation of H_2, CO_2 and short chain fatty acids (SCFA) (Park and Floch, 2007; Zhao and Cheung, 2011). Any healthy diet rich in dietary fiber that comprises sources of fermentable fiber leads to the generation, absorption and excretion of the same metabolites (H_2, CO_2, SCFA) as will be the case upon digestion of mushroom β-glucans. Given this, the metabolism of mushroom β-glucans does not raise safety concerns and no systemic toxicity is expected following its ingestion.

In a human study, Lehne et al. (2005) investigated tolerability and absorption of a soluble baker's yeast β-1,3/ β-1,6-D-glucans preparation. In this open label dose-escalation study, 8 healthy volunteers (6/group) were randomized to receive 100, 200, or 400 mg SBG (bakers yeast β-1,3/β-1,6-D-glucans preparation)/person for a period of 4 consecutive days. Plasma concentrations of β-glucans were measured on the first day at -1, +1 hour of treatment and on

days 2, 5, and 8. The detection limit of β-glucans was 5 pg/ml. Plasma concentrations of β-glucans did not differ between the pre-study values and the values recorded on 5 and 8 days, demonstrating that there was no systemic absorption. No statistically significant changes in treatment groups as compared to baseline were noted during intervention including a four-day follow-up period. The results of this study indicate that following short-term oral administration of the baker's yeast preparation no systemic absorption of β-1,3-glucan occurred.

In another study, Zhang and Cheung (2011) evaluated the bifidogenic effect of β-glucans from barley, seaweed, bacteria, and mushroom and compared their *in vitro* fermentation by three bifidobacteria commonly found in the intestinal lumen of humans including *Bifidobacterium infantis* (in nursing infants) and *B. longum* and *B. adolescentis* (both in human adults). In this study, β-glucans were incubated with pure cultures of these bacteria for a 24-hour batch fermentation. Inulin was used as a control. The parameters monitored during the fermentation included changes in pH, microbial proliferation, and SCFA production. The pH value in all culture media was decreased by 0.5-1.5 units and all β-glucans supported the growth of the three bifidobacteria. *B. infantis* produced almost double the amount of total SCFA than the other two bifidobacteria. The SCFA profile of *B. infantis* had a relatively higher proportion of propionic and butyric acid but less acetic acid compared to the other bifidobacteria. The utilization of all the β-glucans from different sources regardless of their differences in glycosidic linkages and molecular weight by all three bifidobacteria was comparable to that of inulin. The results of this study indicate that β-glucans derived from mushroom are fermented similar to those from other sources in the human gastrointestinal tract.

6.1.2.5. Human Studies of β-Glucans

In a Phase I clinical trial, Twardowski et al. (2015) investigated the effects of white button mushroom (WBM; *Agaricus bisporus*- an edible mushroom) powder on serum prostate specific antigen (PSA) in patients with biochemically recurrent prostate cancer. Additionally, the tolerability and biological activity of WBM was determined. In this study, patients (n=36; age 53-80 years) with continuously rising PSA levels were enrolled. Dose escalation was conducted in cohorts of six at 6 dose levels: 4, 6, 8, 10, 12 and 14 g daily. If no Dose Limited Toxicities (DLT) were encountered for a cohort of patients during the first 28 days of treatment, the next highest dose level was tested (up to 14 g/day). Toxicity assessment was performed on all patients who began therapy using the NCI Common Terminology Criteria for Adverse Events. Approximately 90% of fresh WBM weight consists of water. Therefore, 4 g - 14 g mushroom tablets are equivalent to 40 g - 140 g of fresh WBM. The primary objective of the study was to evaluate treatment feasibility and associated toxicity. In this study, 36 patients were treated; no dose limiting toxicities were encountered. Overall PSA response rate was 11%. Two patients receiving 8 and 14 g/day demonstrated a PSA complete response: their PSA declined to undetectable levels that continued for 49 and 30 months. Two patients, receiving 8 and 12 g/day, experienced a PSA partial response. After 3 months of treatment, 36% of patients (13/36) experienced some PSA decrease below baseline. Minimal side effects were noted and mostly limited to Grade 1 abdominal bloating. Mean compliance with protocol-defined mushroom powder treatment was 98.6%. One patient at dose level 3 (8 g/day) experienced grade 3 hyponatremia, possibly related to therapy, and was taken off the protocol for toxicity. This occurred during the second month (cycle 2) of therapy and therefore was not classified as DLT.

In a randomized, double-blind, placebo-controlled, parallel group interventional trial, Bays et al. (2011) investigated the effects of reduced viscosity barley β-glucan on insulin

sensitivity for individuals at risk for diabetes mellitus. In this study, 50 generally healthy (at risk for type 2 diabetes) subjects were administered beverages containing placebo (control), lower dose (3 g/day), or a higher dose (6 g/day) of barley β-glucans extract for 12 weeks. Subjects (68% women; mean age- 56 years; BMI- 32 kg/m^2; and baseline fasting plasma glucose 102 mg/dl) were instructed to follow a weight-maintaining Therapeutic Lifestyle Changes program. The findings from this study suggest that consumption of 6 g/day barley β-glucans in a beverage over 12 weeks may improve insulin sensitivity among hyperglycemic individuals. All beverages were generally well tolerated with no serious adverse experiences and no significant differences between groups were observed. The most common adverse events included diarrhea, abdominal distension and flatulence. These adverse events were typically mild and self-limiting, with no significant differences between the study groups.

In another double-blind, placebo-controlled clinical trial, described in GRN 239 (Biothera, 2008), healthy volunteers (n=20) consumed a single capsule providing 250 mg yeast whole glucan particle (WGP) β-glucan (WGP 3-6) per day for 10 days. No significant differences in WBC differential count, whole blood phenotyping, or natural killer cell activity were noted. The phagocytosis of *Staphylococcus aureus* beads was significantly increased by WGP 3-6 treatment. Serum tumor necrosis factor (TNF)-alpha levels were increased 6-fold relative to baseline levels, but no effects on interleukin (IL)-1 or interferon (INF)-gamma were reported. The blood chemistry profiles were within normal ranges for most subjects with the following exceptions: 6 of 10 subjects had increased potassium levels; the glucose levels increased in one subject and decreased in another, and calcium levels were increased in 1 subject. Overall, WGP 3-6 at a dose of 250 mg/person/day for 10 days was safe and well tolerated and the blood biochemistry parameters were essentially unaffected by β-glucans treatment.

In yet another placebo-controlled, double-blind study, also described in GRN 239 (Biothera, 2008), 62 subjects with common cold (exposure to rhinovirus) were assessed for safety of WGP 3-6 and its impact on immune biomarkers. All volunteers were pre-screened to exclude subjects that exhibited levels of rhinovirus antibodies, and each participant consumed 250 mg of WGP 3-6 twice a day for 10 consecutive days. A number of immune-related hematological biomarkers, including standard safety endpoints were investigated. WGP 3-6 supplementation insignificantly increased the NK cell number relative to the placebo, while no significant effects on T cells or cytokine levels were observed. Overall, WGP 3-6 was well tolerated and no adverse effects attributable to the test article were reported.

In a study in 15 free-living, obese, hypercholesterolemic men, Nicolosi et al. (1999) evaluated the effect of a yeast-derived β-glucans fiber on serum lipids. In this study, after a 3-week period in which subjects ate their usual diet, 15 g β-glucans fiber per day was added to the diet for 8 weeks and then stopped for 4 weeks. Plasma lipids were measured weekly during baseline and at week 7 and 8 of fiber consumption, and again at week 12. Compared to baseline, the consumption of yeast β-glucans decreased plasma total cholesterol levels by 8 and 6% at week 7 and 8, respectively. These values returned to normal following discontinuation of β-glucans diet. However, a significant increase (16%) in high-density lipoprotein (HDL)-cholesterol was reported at week 12. Adverse effects typically reported with fiber consumption, such as diarrhea, nausea, abdominal discomfort, abdominal distension, and flatulence, were minimal. The results of this study indicate that yeast β-glucans at a dose of 15 g/person/day was well tolerated in adults in the general population.

In an open label dose-escalation study, Lehne et al. (2005) investigated the safety of a soluble branched β-l,3-glucan (SBG) derived from *S. cerevisiae*. In this study, in 18 healthy volunteers (6/group) were randomized to receive 100, 200, or 400 mg SBG per person for a period of 4 consecutive days. A series of safety related parameters including hematological, clinical chemistry, urinalysis, immunoglobulin (Ig) A, IgG, IL-6 and TNF-alpha were investigated. No abnormalities in vital signs were observed and no adverse events were considered to be related to SBG administration. Minor mucosal lesions of the oral cavity noted in 7 subjects were considered normal physiological variations. Increased C-reactive protein, fibrinogen and abnormal differential counts of leucocytes were observed in 5 subjects with pre-existing respiratory infections, including 1 with *herpes labialis*. All other hematological and biochemical parameters were within normal physiological ranges. On day 5, a significant increase in the saliva IgA was noted in the 400 mg dose group, but no other significant differences in serum or saliva IgA or IgG values were reported. There were no significant changes in IL-lp, IL-6, or TNF-a between treatment groups. The investigators concluded that SBG was safe and well tolerated by healthy volunteers, when given orally once daily for 4 consecutive days at doses up to 400 mg.

6.1.2.6. Potential Allergenicity to Mushroom β-Glucans

The available information suggests that there are at least 170 foods that have been reported to cause allergic reactions (Boye, 2012). However, there are only eight major food allergens (i.e., milk, egg, peanut, tree nuts, wheat, soy, fish and crustacean shellfish) that are responsible for most of the serious food allergy reactions in the US. It is estimated that up to 15 million Americans have food allergies, including 5.9 million children under the age of 18. Each year in the U.S., it is estimated that anaphylaxis to food results in 30,000 emergency room visits, 2,000 hospitalizations, and 150 deaths[4].

Although mushrooms are considered to be capable of eliciting allergic symptoms, studies on this subject are few and take no account of many of the important mushroom families and their potential to cause allergy. In a review article, Koivikko and Savolainen (1988) reported that the overall extent of mushroom allergy remains unknown. It may be very slight (<1%) from eating, but could, alternatively, be as prevalent as pollen and mold allergy (10-30% of an allergic population). In the published literature no reports of allergy to *Antrodia cinnamomea* mushroom were found. Among the different mushrooms, the genera that produce distinguishable basidiospores are Ganoderma, Boletus, Rhodophyllus, Thelephora, Russula and Lactarius. In the published studies, some reports have appeared on allergenicity of *Ganoderma* species related to the spores from these species. As regards yeast β-glucans' allergic risk, the EFSA Panel noted that the allergenic risk to this ingredient is no greater than the risk from exposure to other products containing baker's yeast (EFSA, 2011).

Bruce (1963) investigated skin and bronchial reactivity in asthmatics to different allergen extracts comprised of basidiospores, including those from *Ganoderma applanatum.* Reactivity was seen with bronchial challenge test and intradermal skin test. In this study, basidiospore extracts gave a higher frequency of positive reactions to bronchial provocations compared to extracts of house dust, or the pollen or spores of rusts, smuts, or molds. Reaction in skin tests were frequent but less than those with molds or common allergens. In another study, Herxheimer

[4] Accessible at: https://www.fda.gov/food/buy-store-serve-safe-food/what-you-need-know-about-food-allergies

et al. (1966) reported three asthmatic subjects testing positive with *Ganoderma applanatum* extract. It should be noted that in this study, spore extracts were used.

Toda et al. (2010) reported a case of a 38-year-old woman with bronchial asthma and hypersensitivity reactions following Matsutake mushroom (*Tricholoma matsutake*) ingestion. The patient showed a positive reaction in the skin prick test (wheal of 5 × 4 mm and flare of 26 × 15 mm at 15 minutes) for Matsutake mushroom, but was negative for Shiitake mushroom. On the other hand, ten healthy volunteers showed negative results to this test. Anaphylaxis caused by Matsutake mushroom is considered rare. In Japan, a total of only 13 cases have been reported.

Huang et al. (2010) investigated the effects of polysaccharide extracted from *G. lucidum* following an alkaline extraction process on immune function in mice. Male NIH mice were gavaged with the extract at dose of 50, 100, and 200 mg/kg/day for up to 30 days. A series of immunological tests such as carbon clearance, delayed hypersensitivity, serum hemolysin and NK cell activity were performed. The results of immunological assays revealed that the alkaline soluble polysaccharides had no noticeable effects on monocyte phagocytosis and immune organ (spleen, thymus) weight of immunocompromised mice at the tested dosages. However, they could restore a delayed-type hypersensitivity reaction to dinitrofluorobenzene (DFNB), hemolysis antibody levels at the three doses applied, and improve the natural killer cell activity at the high-dose and medium dose.

In summary, allergy to *Antrodia cinnamomea* has not been reported from consumption of this mushroom as a food. Mushroom allergies are typically caused by airborne particles or skin contact. Few cases of allergy from consumption of other types of mushrooms have been reported. The residual amount of protein in the Antrodia mushroom β-glucan product the subject of present GRAS assessment is very low (<1%).

6.1.3. Corroborative Information

6.1.3.1. FDA GRAS Notices on β-Glucan

The FDA received three GRAS notifications on β-glucans, one derived from mushroom *G. lucidum* [GRN 413 (SBG, 2012)] and two from yeast [GRN 309 (Glucan, 2010) and GRN 239 (Biothera, 2008)]. In these submissions, extensive data from the published literature on β-glucans were presented by the notifiers. The FDA did not question the acceptability and suitability of the available evidence to support the safe use of β-glucans in its letters that were sent to the notifiers. The discussion presented below suggests that the agency is comfortable with the GRAS status of β-glucans for its proposed use levels in selected foods as presented in the GRNs. As the subject of this GRAS determination is substantially similar to the products of these FDA notifications, the studies described in these notifications can also be utilized to support the safety of use in the present GRAS assessment of Antrodia mushroom β-glucans. Although there are some differences in the chemical structure of the β-glucans between yeast and mushroom, the available information, particularly from a metabolic perspective, indicates that these molecules will be handled similarly in the body. A summary of product similarity between the FDA notified β-glucans and the subject of the present GRAS evaluation is presented in Table 4.

Table 4. Comparison of Mushroom β-Glucans with Yeast β-Glucans from GRAS Notices

Specifications	Mushroom (present GRAS)	Mushroom (GRN 413)*	Black yeast (GRN 309)*	Baker's yeast (GRN 239)*
Description	Fine light beige powder	Fine light beige powder	Reddish yellow powder	Fine beige/tan powder
Source organism	*A. cinnamomia*	*G. lucidum*	*A.. mllulans*	*S. cerevisiae*
Total Carbohydrate (%)	> 90	At least 95	At least 80	At least 80
β-Glucan (%)	Minimum 65	At least 50	At least 40	At least 70
Protein (%)	< 1	<1.0	<10	<10
Fat (%)	< 1	<1.0	<5	<20
Ash (%)	< 3	<3.0	<10	<5
Moisture (%)	< 5	<2.0	<6	<8
Lead (ppm)	<0.5	<0.5	ND	<0.5
Mercury (ppm)	<0.05	<0.05	ND	<0.05
Arsenic (ppm)	<0.5	<0.5	ND	<0.1
Selenium (ppm)	NA	NA	ND	NA
Cadmium (ppm)	<1.0	<1.0	ND	<1
Zinc (ppm)	NA	NA	NA	NA
Aerobic plate count	<15,000	<10,000	<3,000	<20,000
Yeast and molds	<150 combined	≤15	≤20	≤25
Coliform	<10	Negative	<10	<10
Escherichia coli	Negative	Negative	Negative	Negative
Salmonella sp.	Negative	Negative	Negative	Negative

*Adapted from GRN 413, GRN 239 and GRN 309; NA=Not available; ND =Not detected

6.1.3.1.1. GRN 413- Beta glucans derived from *Ganoderma lucidum*

This GRAS notification on β-glucans derived from mushroom *Ganoderma lucidum* (SBG, 2012) was submitted by Super Beta Glucan Inc, the notifier of the present GRAS notice. The ingredient is described as a water soluble, fine, light-beige colored powder, obtained from the cultured mycelium of *G. lucidum* (ATCC 32472). The identity and specifications have been established (Table 4) and are similar to present GRAS. The proposed use levels were at 150 mg/serving in baked goods and baking mixes, beverages and beverage bases, cereal and cereal products, dairy product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, and soft candy. The resulting mean and 90th percentile (users only) EDI for the total population was reported as 291.3 and 583.4 mg/person/day or 6.3 and 14.5 mg/kg bw/day, respectively.

In this GRAS notice, the notifier extensively summarized and discussed published information to support the safety of β-glucans (SBG, 2012). These studies included a specific subchronic toxicity study in Sprague-Dawley rats as well as specific mutagenicity and genotoxicity studies. Based on the results of the subchronic study, the NOAEL for β-glucans derived from *G. lucidum* mycelium was 2000 mg/kg bw/day, the highest dose tested. The source organism, *G. lucidum*, was stated as non-pathogenic and non-toxigenic and does not produce any mycotoxins. Additional published human clinical data as well as published acute, subchronic, and chronic animal studies were described to further support the intended use of β-glucans derived from *G. lucidum* mycelium. These studies utilize β-glucans derived from sources other than mushroom mycelium such as *Saccharomyces cerevisiae* and *Aureobasidium pullulans*. Based on available published information no allergenic reactions in humans following oral

ingestion of either β-glucans or its source organism *G. lucidum* (Reishi) was anticipated. Following its review of the information provided by the notifier, as well as other information available to FDA, the agency provided a no questions letter to the notifier regarding the GRAS status of β-glucans derived from *G. lucidum* mycelium under the intended conditions of use.

6.1.3.1.2. GRN 309- Black Yeast β-Glucans

The subject of this GRAS notification is β-glucans derived from *Aureobasidium pullulans* (Glucan, 2010). In this GRAS notice, the β-glucans was identified as a beta-1,3/1,6-glucan and described as a light brown/beige powder with high solubility in water. The safety of β-glucans derived from *A. pullulans* was supported by published acute toxicity, subchronic oral toxicity, and genotoxicity studies conducted in mice. These acute and subchronic oral toxicity studies did not show any evidence of toxicity. Additionally, the genotoxicity studies also did not show any adverse effects. The notifier also corroborated the safety of β-glucan derived from *A. pullulans* with a published subchronic oral toxicity study conducted in rats (Babicek et al., 2007), and an unpublished study conducted in humans. In the subchronic oral toxicity study, rats showed no systemic or gastrointestinal toxicity at the highest tested level of 2000 mg/kg bw/day (1000 mg/kg bw twice a day) of β-glucans derived from *A. pullulans*. From the human study, the notifier concluded that 400 mg/person/day of β-glucans derived from *A. pullulans* did not reveal adverse effects. The notifier concluded that the results of the published and unpublished studies support the safety of β-glucan derived from *A. pullulans*. The agency reviewed the notice and did not question the notifier's conclusion that β-glucans derived from *A. pullulans* is GRAS under the intended conditions of use.

6.1.3.1.3. GRN 239- Bakers Yeast β-Glucans

In this first GRAS notice on baker's yeast β-glucans (Biothera, 2008), the notifier, Glucan Corporation discussed published and unpublished rodent and human studies, including acute toxicity studies in rats and mice, a subchronic oral toxicity study in rats, and double-blind, placebo-controlled studies for 10 and 30 days in humans. No adverse effects were observed in any of the studies. The notifier concluded that the rodents in the acute toxicity studies had no evidence of adverse effects on clinical chemistry or histopathological observations. In the subchronic oral toxicity study, the rats showed no evidence of systemic or gastrointestinal toxicity at the highest level (100 mg/kg bw/day) of baker's yeast β-glucans. The notifier summarized the results of the human clinical studies, and concluded that levels up to 500 mg/person/day of baker's yeast β-glucans were well-tolerated and that there were no significant differences in blood biochemistry parameters. The FDA reviewed the notice and did not question the notifier's conclusion that baker's yeast β-glucans is GRAS under the intended conditions of use.

6.1.3.2. EFSA Assessment of Yeast β-Glucans Safety

In 2011, the EFSA Panel on Dietetic Products, Nutrition and Allergies, evaluated the safety of yeast β-glucans derived from *S. cerevisiae* for use as a novel food ingredient in a variety of foods and beverages for the general population resulting in a daily intake of up to 600 mg/day (EFSA, 2011). The EFSA Panel noted that the intake scenario for yeast β-glucans is somewhat similar to the background intake of β-glucans from other dietary sources. The data reviewed pertaining to acute and sub-chronic toxicity, absorption, and the limited human data did not raise safety concerns. The Panel considered that the allergenic risk of the yeast β-glucans is no greater than the risk from exposure to other products containing baker's yeast. The Panel

noted that β-glucans from other sources have already been evaluated for safety by EFSA. Following its review, the Panel concluded that on the basis of the nature of yeast β-glucans, the significant history of use of its source (baker's yeast), the provided intake estimate and the supplementary data from human and animal studies, yeast β-glucans is safe for human consumption at the proposed conditions of use.

6.1.4. Expert Panel Review, Summary and Discussion

At the request of Super Beta Glucan (SBG), USA, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[5], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened to evaluate the Generally Recognized As Safe (GRAS) status of Antrodia mushroom β-glucan, derived from *Antrodia cinnamomea*, for use as a food ingredient in selected food categories such as baked goods and baking mixes, beverages and beverage bases, cereal and cereal products, dairy product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, and soft candy at levels up to 150 mg Antrodia mushroom β-glucans *per* serving (reference amounts customarily consumed, 21 CFR 101.12). A comprehensive search of the scientific literature for safety and toxicity information on mushroom β-glucans and related preparations was conducted through April 2020 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by SBG and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred on July 24, 2020 and unanimously agreed to the decision described herein.

SBG ensured that all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, and nutrition. The Expert Panel was selected and convened in accordance with the Food and Drug Administration (FDA)'s guidance for industry on "Best Practices for Convening a GRAS Panel"[6]. Efforts were placed on identifying conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the Expert Panel and no such conflicts of interest or "appearance issues" were identified. The Expert Panel members received a reasonable honorarium as compensation for their time; the honoraria provided to the Expert Panel members were not contingent upon the outcome of their deliberations.

The standardized Antrodia mushroom β-glucans preparation from the species *Antrodia cinnamomea* (ATCC® 200183™) is manufactured according to current good manufacturing practices. Antrodia mushroom β-glucans is a fine beige powder soluble in water with a characteristic mild odor and bland taste. The food grade specifications of Antrodia mushroom β-glucans has been established by SBG. The compositional analysis of Antrodia mushroom β-glucans demonstrated that it primarily contains carbohydrates (>90%) of which β-glucans constitutes >65%. The remaining carbohydrates are primarily monosaccharides and disaccharides. The intended use of Antrodia mushroom β-glucans in the above mentioned selected food categories will result in a mean and 90th percentile estimated daily intake of 291.3 and 583.4 mg/person/day or 6.3 and 14.5 mg/kg bw/day, respectively. The intended use of

[5] Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.
[6] Available at: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm583856.htm

Antrodia mushroom β-glucans is in the same food products and at the same levels mentioned in the GRAS notice GRN 309 and GRN 413.

The source mushroom, *A. cinnamomea,* from which β-glucans is derived is an edible, rare and endemic mushroom native to Taiwan. β-Glucans, the active constituent of Antrodia mushroom preparation, is commonly found in the bran of cereal grains, the cell wall of yeast, certain fungi, seaweed, and bacteria that have been consumed by humans. The FDA has allowed the health claim on a food label for reduction of the cholesterol level when cereal β-glucan is included in such foods. Additionally, the agency has approved the use of bakers yeast glycan for direct addition as a multi-purpose food additive to various food products (21 CFR 172.898). Furthermore, the FDA did not question the conclusions of the GRAS status and safety of the use of yeast and mushroom β-glucans that were the subject of GRAS notifications (GRN 309; GRN 239; GRN 413). All this information suggests that there is a common knowledge of safe consumption of β-glucans from different food sources or products.

From chemical and metabolism point of view, β-glucans is comprised of D-glucose polymers. The primary structure of β-glucans polymers derived from different sources differs, but mainly consists of a linear glucose polymer with β(1,3)-, β(1,4)- or β(1,6)- linkages. Structurally, mushroom β-glucans are similar to yeast β-glucans except that they are comprised of short β(1,6)-branches coming off of a β(1,3)-backbone, thereby lacking the extra β(1,3)-branch extending from the β(1,6)-branch point. In spite of these minor structural differences, the metabolic fate of β-glucans is similar and resembles that of other prebiotics and non-digestible/fermentable carbohydrates. Humans are unable to digest carbohydrate polymers with β-glucosidic linkages and hence systemic exposure to particulate β-glucans, including that from mushroom, is unlikely. However, similar to other dietary fibers, β-glucans will be fermented in the colon by the resident microbiota resulting in the formation of H_2 and CO_2 and SCFA.

In a series of specifically designed studies that followed standard regulatory guidelines, the potential subchronic toxicity and mutagenicity/genotoxicity of Antrodia mushroom β-glucan preparation (the subject of the present GRAS assessment) has been investigated. In the subchronic toxicity study, administration (gavage) of Antrodia mushroom β-glucan preparation at dose levels of 0, 500, 1000 and 2000 mg/kg bw/day for 90 consecutive days did not result in any treatment-related clinical signs of toxicity, mortality or changes in body weights, body weight gain or feed consumption. No toxicologically significant treatment-related changes in hematological, clinical chemistry, urine analysis parameters, and organ weights were noted. In one female from low-dose group focal mass in subcutaneous tissue (mammary gland) was noted at termination. According to severity and incidence based on histopathological evaluation of this lesion (fibroadenoma), the finding was considered as spontaneous abnormality and not related to the β-glucan preparation. No treatment-related macroscopic and microscopic abnormalities were noted at the end of treatment period. The findings from mutagenicity studies, including the Ames assay, *in vitro* chromosomal aberration and *in vivo* micronucleus assay, did not reveal any genotoxicity of Antrodia mushroom β-glucan preparation. All of these toxicity studies are published in a peer-reviewed scientific journal. Based on the subchronic study, the NOAEL for Antrodia mushroom β-glucans was established as 2000 mg/kg bw/day, the highest dose tested. The estimated daily intake (14.5 mg/kg bw/day) of Antrodia mushroom β-glucans from its intended food uses is over 135-fold lower compared to the NOAEL determined from the subchronic toxicity study.

In addition to the specific pivotal studies of Antrodia mushroom β-glucans, the safety of source material, *A. cinnamomea* has been extensively investigated in animal toxicity studies. These studies included three subchronic toxicity studies (one in mice and two in rats), one reproductive and developmental toxicity study in rats, and mutagenicity and genotoxicity studies (Ames Assay, *in vitro* mammalian chromosomal aberration test, and rat bone marrow erythrocytes study). The findings from subchronic oral toxicity studies show that administration of *A. cinnamomea* did not cause toxicity at doses of 1667 mg/kg bw/day in mice and at levels up to 3000 mg/kg bw/day in rats. The results of reproductive and developmental toxicity study did not reveal any observable segment II reproductive and developmental evidences of *A. cinnamomea* extract at levels up to 2800 mg/kg bw/day. *A. cinnamomea* extract showed no mutagenic activity in the bacterial reverse mutation test, did not induce micronuclei in mammalian erythrocytes or increase the rates of structural and numerical chromosome aberration in mice. All these studies suggest that the source material *A. cinnamomea* has a very low order of toxicity and is safe for human consumption.

In a series of studies, subchronic toxicity and potential genotoxic effects of mushroom β-glucans derived from *G. lucidum* has been investigated. Given the similarity between β-glucans derived from *G. lucidum* and from *A. cinnamomea*, findings from these studies are applicable to the present GRAS. In the subchronic toxicity study with β-glucans derived from *G. lucidum,* the NOAEL was established as 2000 mg/kg bw/day The results of mutagenicity studies including the Ames assay, *in vitro* chromosomal aberration and *in vivo* micronucleus assay did not reveal any genotoxicity of β-glucans derived from *G. lucidum*. Furthermore, animal studies such as subchronic and chronic (52 weeks) toxicity studies of yeast β-glucans in rats and clinical trials of yeast β-glucans in human subjects also did not reveal any adverse effects of β-glucans. The safety of Antrodia mushroom β-glucans is supported by the compositional similarity of the ingredient to β-glucans derived from *G. lucidum*, yeast β-glucans and glycan that have been reviewed by the FDA as part of GRAS Notifications.

In summary, on the basis of scientific procedures[7] and history of exposure from natural sources, the consumption of Antrodia mushroom β-glucans derived from *A. cinnamomea* as an added food ingredient is considered safe at the 90th percentile estimated daily intake of 583.4 mg/person/day (14.5 mg/kg bw/day). The intended uses are compatible with current regulations, *i.e.,* Antrodia mushroom β-glucans will be used in baked goods and baking mixes, beverages and beverage bases, cereal and cereal products, dairy product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, and soft candy at use levels up to 150 mg Antrodia mushroom β-glucans *per* serving when not otherwise precluded by a Standard of Identity, and it is produced according to current good manufacturing practices (cGMP).

[7] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

6.1.5. Expert Panel Conclusion

Based on a critical evaluation of the publicly available data summarized above, the Expert Panel members whose signatures appear below, have individually and collectively concluded that Antrodia mushroom β-glucans, meeting the specifications cited above, and when used as a food ingredient in selected food products (baked goods and baking mixes, beverages and beverage bases, cereal and cereal products, dairy product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, and soft candy) at levels up to 150 mg Antrodia mushroom β-glucans/serving (reference amounts customarily consumed, 21 CFR 101.12) when not otherwise precluded by a Standard of Identity as described in this monograph and resulting in the 90th percentile all-user estimated intake of 583.4 mg/person/day (14.5 mg/kg bw/day) is safe and Generally Recognized As Safe (GRAS).

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

Signatures

[redacted]

John A. Thomas, Ph.D., F.A.C.T., F.A.T.S. — Date: July 24, 2020

[redacted]

Robert L. Martin, Ph.D. — Date: July 24, 2020

[redacted]

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.
Advior to Expert Panel — Date: July 24, 2020

Part VII- SUPPORTING DOCUMENTS AND REFERENCES

Babicek, K., Cechová, I., Simon, R.R., Harwood, M., Cox, D.J., 2007. Toxicological assessment of a particulate yeast (1,3/1,6)-β-d-glucan in rats. Food Chem. Toxicol. 45: 1719-1730.

Bashir, K., Choi, J.S., 2017. Clinical and Physiological Perspectives of β-Glucans: The Past, Present, and Future. Inter. J. Molecular Sci. 18(9):1906. https://doi.org/10.3390/ijms18091906

Bays, H., Frestedt, J.L., Bell, M., Williams, C., Kolberg, L., Schmelzer, W., Anderson, J.W., 2011. Reduced viscosity Barley β-Glucan versus placebo: a randomized controlled trial of the effects on insulin sensitivity for individuals at risk for diabetes mellitus. Nutr. Metab. (Lond). 8:58.

Biothera, 2008. GRN 000239. Bakers yeast beta-glucan. GRAS Notification by Biothera Inc. Document available at: http://www.accessdata.fda.gov/scripts/fcn/gras_notices/grn000239.PDF

Borchers, A.T., Keen, C.L., Gershwin, M.E., 2004. Mushrooms, tumors and immunity: An update. Exp. Biol. Med. (Maywood) 229:393-406.

Borchers, A.T., Stern, J.S., Hackman, R.M., Keen, C.L., Gershwin, M.E., 1999. Mushrooms, tumors, and immunity. Proc. Soc. Exp. Biol. Med. 221: 281-293.

Boye, J.I., 2012. Food allergies in developing and emerging economies: need for comprehensive data on prevalence rates. Clinical Translational Allergy 2(1):25. Available at: https://doi.org/10.1186/2045-7022-2-25

Bruce, R.A., 1963. Bronchial and skin sensitivity in asthama. Int. Arch. Allergy 22:294-305.

Chang, J.B., Wu, M.F., Lu, H.F., Chou, J., Au, M.K., Liao, N.C., Chang, C.H., Huang, Y.P., Wu, C.T., Chung, J.G., 2013. Toxicological evaluation of *Antrodia cinnamomea* in BALB/c mice. *In Vivo* 27:739-745

Chen, S.N., Chan, C.S., Chen, S., Soni, M.G., 2018. Subchronic toxicity and genotoxicity studies of Antrodia mushroom β-glucan preparation. Regul. Toxicol. Pharmacol. 92: 429-438.

Chen, T.-I., Chen, C.-C., Lin, T.-W., Tsai, Y.-T., Nam, M.-K., 2011a. A 90-day subchronic toxicological assessment of Antrodia cinnamomea in Sprague-Dawley rats. Food Chem. Toxicol. 49(2):429-433

Chen, S.N., Nan, F.H., Chen, S., Wu, J.F., Lu, C.L., Soni, M.G., 2011. Safety assessment of mushroom β-glucan: Subchronic toxicity in rodents and mutagenicity studies. Food Chem. Toxicol. 49: 2890-2898.

Cheung, P. C. K. (Ed). 2009. Mushrooms as Functional Foods, John Wiley & Sons, Inc., Hoboken, NJ, USA.

Delaney, B., Carlson, T., Frazer, S., Zheng, G.-H., Hess, R., Ostergren, K., Kierzek, K., Haworth, J., Knutson, N., Junker, K., Jonker, D., 2003a. Evaluation of the toxicity of concentrated barley β-glucan in a 28-day feeding study in Wistar rats. Food Chem. Toxicol. 41: 477-487.

Delaney, B., Carlson, T., Zheng, G.-H., Hess, R., Knutson, N., Frazer, S., Ostergren, K., van Zijverden, M., Knippels, L., Jonker, D., Penninks, A., 2003b. Repeated dose oral toxicological evaluation of concentrated barley β-glucan in CD-1 mice including a recovery phase. Food Chem. Toxicol. 41: 1089-1102.

Driscoll, M., Hansen, R., Ding, C., Cramer, D.E., Yan, J., 2009. Therapeutic potential of various beta-glucan sources in conjunction with anti-tumor monoclonal antibody in cancer therapy. Cancer Biol. Ther. 8:218-225.

EFSA, 2009. Scientific Opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 7: 1254.

EFSA, 2011. Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the safety of "Yeast *beta*-glucans" as a Novel Food ingredient. EFSA Journal 9(5):2137.

FDA, 1997. Food labeling: Health claims; oats and coronary heart disease. Fed. Regist. 62: 3584-3601.

FDA, 2005. Food labeling: Health claims; soluble dietary fiber from certain foods and coronary heart disease. Interim final rule. Fed. Regist. 70: 76150-76162.

FDA, 2011. Agency Response Letter GRAS Notice No. GRN 000344 on Barley fiber. Available at: https://wayback.archive-it.org/7993/20171031012231/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm258862.htm

FDA, 2012. Agency Response Letter GRAS Notice No. GRN 000413. Beta glucans derived from *Ganoderma lucidum* mycelium. Available at: https://wayback.archive-it.org/7993/20171031035213/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm319626.htm

Glucan, 2010. GRN 000309. Beta-glucan derived from *Aureobasidium pullulans*. GRAS Notification by Glucan Corporation, Limited. Document available at: https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=309&sort=GRN_No&order=DESC&startrow=1&type=basic&search=309

Feletti, F., De Bernardi di Valserra, M., Contos, S., Mattaboni, P., Germogli, R., 1992 Chronic toxicity study on a new glucan extracted from Candida albicans in rats Arzneimittelforschung 42: 1363-1367.

Herxheimer, H., Hyde, H. A., Williams, D. A., 1966. Allergic asthma caused by fungal spores. Lancet 1 572-573.

Huang, S.Q., Li, J.W., Wang, Z., Pan, H.X., Chen, J.X., Ning, Z.X., 2010. Optimization of alkaline extraction of polysaccharides from Ganoderma lucidum and their effect on immune function in mice. Molecules 15:3694-3708.

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

Jonker, D., Hasselwander, O., Tervild-Wilo, A., Tenning, P.P., 2010. 28-Day oral toxicity study in rats with high purity barley beta-glucan (Glucagel). Food Chem. Toxicol. 48: 422-428.

Kim, J., Lee, S.M., Bae, I.Y., Park, H.G., Gyu, Lee, H., Lee, S., 2011. (1-3)(1-6)-β-Glucan-enriched materials from *Lentinus edodes* mushroom as a high-fibre and low-calorie flour substitute for baked foods. J. Sci. Food Agric. doi: 10.1002/jsfa.4409.

Ko, Y.T., Lin, Y.L., 2004. 1,3-β-glucan quantification by a fluorescence microassay and analysis of its distribution in foods. J. Agric. Food Chem. 52: 3313-3318.

Koivikko, A., Savolainen, J., 1988. Mushroom allergy. Allergy 43: 1-10.

Lehne, G., Haneberg, B., Gaustad, PI Johansen, P.W., Preus, H., Abrahamsen, T.G. 2005. Oral administration of a new soluble branched p-1,3-D-glucan is well tolerated and can lead to increased salivary concentrations of immunoglobulin A in healthy volunteer. Clin. Exp. Immunol. 143: 65-69.

Lin, C.C., Kumar, K., Liao, J.W., Kuo, Y.H., Wang, S.Y. 2015. Genotoxic, teratotoxic and oral toxic assessments of *Antrodia cinnamomea* health food product (Leader Deluxe Antrodia cinnamomea®). Toxicology Reports 2:1409-1417.

Macfarlane, S., Macfarlane, G.T., Cummings, J.H., 2006. Review Article: Prebiotics in the Gastrointestinal Tract. Aliment. Pharmacol. Ther. 24(5):701-714.

Newberne, P M., Conner, M.W., Estes, P., 1988. The influence of food additives and related materials on lower bowel structure and function. Toxicol. Pathol. 16:184-197.

Nicolosi, R., Bell, S J., Bistrian, B R., Greenberg, I., Forse, R.A., Blackburn, G.L., 1999. Plasma lipid changes after supplementation with β-glucan fiber from yeast. Am. J. Clin. Nutr. 70: 208-212.

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

Park, J., Floch, M., 2007. Prebiotics, probiotics, and dietary fiber in gastrointestinal disease. Gastroenterol. Clin. Nutr. Am. 36: 47-63.

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

SBG, 2019. Information on Description, Specifications, Identity, Composition and Manufacturing provided by Super Beta Glucan for the present GRAS. Unpublished.

SBG, 2012. GRN 000413. Beta-glucan derived from *Ganoderma lucidum* mycelium. GRAS Notification by Super Beta Glucan Inc. Document available at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm299311.pdf

Toda, T., Yamaguchi, M., Nakase, Y., Sugimoto, M., Suzukawa, N., Nagase, H., Ohta, K., 2010. A Case of Anaphylactic Reaction Following Matsutake Mushroom Ingestion: Demonstration of Histamine Release Reaction of Basophils. Allergology International 59:417-419.

Twardowski, P., Kanaya, N., Frankel, P., Synold, T., Ruel, C., Pal, S.K., Junqueira, M., Prajapati, M., Moore, T., Tryon, P., Chen, S., 2015. A phase I trial of mushroom powder in patients with biochemically recurrent prostate cancer: Roles of cytokines and myeloid-derived suppressor cells for Agaricus bisporus-induced prostate-specific antigen responses. Cancer, 121(17):2942-2950.

Wasser, S.P., Weis, A.L., 1999. Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. Crit. Rev. Immunol. 19:65-96.

WHO, 1987. Toxicological versus physiological responses. In: Principles for the Safety Assessment of Food Additives and Contaminants in Food. World Health Organization (WHO), International Programme on Chemical Safety (IPCS); Geneva. Environmental Health Criteria, No. 70, p. 82.

Wisker, E., Daniel, M., Feldheim, W., 1998. Fermentation of nonstarch polysaccharides in mixed diets and single fibre sources: comparative studies in human subjects and *in vitro*. Br. J. Nutr. 80: 253-261.

Yan, J., Allendorf, D.J., Brandley, B., 2005. Yeast whole glucan particle (WGP) beta-glucan in conjunction with antitumour monoclonal antibodies to treat cancer. Expert. Opin. Biol. Ther. 5:691-702.

Zhang, B.B., Hu, P.F., Huang, J., Hu, Y.D., Chen, L., Xu, G.R., 2017. Current Advances on the Structure, Bioactivity, Synthesis, and Metabolic Regulation of Novel Ubiquinone Derivatives

in the Edible and Medicinal Mushroom Antrodia cinnamomea. J Agric Food Chem. 65(48):10395-10405.

Zhang, M., Cui, S. W., Cheung, P. C. K., Wang, Q., 2007. Antitumor polysaccharides from mushrooms: a review on their isolation, structural characteristics and antitumor activity. Trends Food Sci. Technol. 18: 4-19.

Zhang, J., Cheung, P.C., 2011. Fermentation of β-glucans derived from different sources by bifidobacteria: evaluation of their bifidogenic effect. J. Agric. Food Chem. 59: 5986-5692.

APPENDIX I

Analytical data from five non-consecutive manufacturing lots of Antrodia mushroom β-glucan

(Included separately)

CERTIFICATE OF ANALYSIS

Product Name: Antrodia Beta Glucan (ABG™) Powder
Manufacturing Date: April 3, 2018
Lot Number: 20180403ABGS
Source of Origin: *Antrodia cinnamomea*

Physical, Chemical and Microbiological Specifications

Parameter	Analysis Results	Specifications	Assay Method
	PHYSICAL PARAMETERS		
Appearance	Conformed to standard	Fine light beige powder	Visual
Odor	Conformed to standard	Mild	Olfactory
Taste	Conformed to standard	Bland	Taste
	CHEMICAL PARAMETERS		
Total Carbohydrate (%)	91.6	>90	By Difference (Calculation)
Beta-Glucan (%)	67.3	Min. 65	Internal Method
Fat (%)	0.7	<1.0	AOAC 996.06
Saturated Fat (%)	0.7	<1.0	AOAC 996.06
Trans Fat (%)	N.D.	N.D.	AOAC 996.06
Protein (%)	0.8	<1.0	AOAC 922.15
Moisture (%)	4.2	<5.0	AOAC 925.45A/V.O
Ash (%)	2.7	<3.0	AOAC 900.02
	HEAVY METALS		
Lead	Negative	<0.5 ppm	ICP-MS
Arsenic	Negative	<0.5 ppm	Cold Vapor
Cadmium	Negative	<1.0 ppm	ICP-MS
Mercury	Negative	<0.5 ppm	ICP-OES
	MICROBIOLOGICAL PARAMETERS		
Aerobic Plate Count (CFU/g)	Conformed to standard	<15,000	FDA BAM
Yeast and Mold (CFU/g)	Conformed to standard	<150 combined	FDA BAM/CMMEF APHA
Total *Coliforms* (MPN/g)	Negative	<10.0	AOAC 966.24
Staphylococcus aureus	Negative	N.D.	AOAC 2003.07/2003.08
E. coli	Negative	N.D.	FDA BAM/AOAC 991.14
Salmonella sp.	Negative	N.D.	FDA BAM

5 Holland 109, Irvine, CA 92618
Office: 949-264-2888 Fax: 626-203-0655

QC: AY- 04/20/2018

CERTIFICATE OF ANALYSIS

Product Name: Antrodia Beta Glucan (ABG™) Powder
Manufacturing Date: March 02, 2018
Lot Number: 20180302ABGS
Source of Origin: *Antrodia cinnamomea*

Physical, Chemical and Microbiological Specifications

Parameter	Analysis Results	Specifications	Assay Method
	PHYSICAL PARAMETERS		
Appearance	Conformed to standard	Fine light beige powder	Visual
Odor	Conformed to standard	Mild	Olfactory
Taste	Conformed to standard	Bland	Taste
	CHEMICAL PARAMETERS		
Total Carbohydrate (%)	91.7	>90	By Difference (Calculation)
Beta-Glucan (%)	65.9	Min. 65	Internal Method
Fat (%)	0.6	<1.0	AOAC 996.06
Saturated Fat (%)	0.6	<1.0	AOAC 996.06
Trans Fat (%)	N.D.	N.D.	AOAC 996.06
Protein (%)	0.8	<1.0	AOAC 922.15
Moisture (%)	4.1	<5.0	AOAC 925.45A/V.O
Ash (%)	2.8	<3.0	AOAC 900.02
	HEAVY METALS		
Lead	Negative	<0.5 ppm	ICP-MS
Arsenic	Negative	<0.5 ppm	Cold Vapor
Cadmium	Negative	<1.0 ppm	ICP-MS
Mercury	Negative	<0.5 ppm	ICP-OES
	MICROBIOLOGICAL PARAMETERS		
Aerobic Plate Count (CFU/g)	Conformed to standard	<15,000	FDA BAM
Yeast and Mold (CFU/g)	Conformed to standard	<150 combined	FDA BAM/CMMEF APHA
Total *Coliforms* (MPN/g)	Negative	<10.0	AOAC 966.24
Staphylococcus aureus	Negative	N.D.	AOAC 2003.07/2003.08
E. coli	Negative	N.D.	FDA BAM/AOAC 991.14
Salmonella sp.	Negative	N.D.	FDA BAM

5 Holland 109, Irvine, CA 92618
Office: 949-264-2888 Fax: 626-203-0655

QC: AY- 04/03/2018

CERTIFICATE OF ANALYSIS

Product Name: Antrodia Beta Glucan (ABG™) Powder
Manufacturing Date: March 12, 2018
Lot Number: 20180312ABGS
Source of Origin: *Antrodia cinnamomea*

Physical, Chemical and Microbiological Specifications

Parameter	Analysis Results	Specifications	Assay Method
	PHYSICAL PARAMETERS		
Appearance	Conformed to standard	Fine light beige powder	Visual
Odor	Conformed to standard	Mild	Olfactory
Taste	Conformed to standard	Bland	Taste
	CHEMICAL PARAMETERS		
Total Carbohydrate (%)	91.8	>90	By Difference (Calculation)
Beta-Glucan (%)	67.0	Min. 65	Internal Method
Fat (%)	0.7	<1.0	AOAC 996.06
Saturated Fat (%)	0.7	<1.0	AOAC 996.06
Trans Fat (%)	N.D.	N.D.	AOAC 996.06
Protein (%)	0.8	<1.0	AOAC 922.15
Moisture (%)	3.8	<5.0	AOAC 925.45A/V.O
Ash (%)	2.9	<3.0	AOAC 900.02
	HEAVY METALS		
Lead	Negative	<0.5 ppm	ICP-MS
Arsenic	Negative	<0.5 ppm	Cold Vapor
Cadmium	Negative	<1.0 ppm	ICP-MS
Mercury	Negative	<0.5 ppm	ICP-OES
	MICROBIOLOGICAL PARAMETERS		
Aerobic Plate Count (CFU/g)	Conformed to standard	<15,000	FDA BAM
Yeast and Mold (CFU/g)	Conformed to standard	<150 combined	FDA BAM/CMMEF APHA
Total *Coliforms* (MPN/g)	Negative	<10.0	AOAC 966.24
Staphylococcus aureus	Negative	N.D.	AOAC 2003.07/2003.08
E. coli	Negative	N.D.	FDA BAM/AOAC 991.14
Salmonella sp.	Negative	N.D.	FDA BAM

5 Holland 109, Irvine, CA 92618
Office: 949-264-2888 Fax: 626-203-0655

QC: AY- 04/05/2018

CERTIFICATE OF ANALYSIS

Product Name: Antrodia Beta Glucan (ABG™) Powder
Manufacturing Date: March 21, 2018
Lot Number: 20180321ABGS
Source of Origin: *Antrodia cinnamomea*

Physical, Chemical and Microbiological Specifications

Parameter	Analysis Results	Specifications	Assay Method
	PHYSICAL PARAMETERS		
Appearance	Conformed to standard	Fine light beige powder	Visual
Odor	Conformed to standard	Mild	Olfactory
Taste	Conformed to standard	Bland	Taste
	CHEMICAL PARAMETERS		
Total Carbohydrate (%)	91.8	>90	By Difference (Calculation)
Beta-Glucan (%)	67.2	Min. 65	Internal Method
Fat (%)	0.7	<1.0	AOAC 996.06
Saturated Fat (%)	0.7	<1.0	AOAC 996.06
Trans Fat (%)	N.D.	N.D.	AOAC 996.06
Protein (%)	0.8	<1.0	AOAC 922.15
Moisture (%)	3.8	<5.0	AOAC 925.45A/V.O
Ash (%)	2.9	<3.0	AOAC 900.02
	HEAVY METALS		
Lead	Negative	<0.5 ppm	ICP-MS
Arsenic	Negative	<0.5 ppm	Cold Vapor
Cadmium	Negative	<1.0 ppm	ICP-MS
Mercury	Negative	<0.5 ppm	ICP-OES
	MICROBIOLOGICAL PARAMETERS		
Aerobic Plate Count (CFU/g)	Conformed to standard	<15,000	FDA BAM
Yeast and Mold (CFU/g)	Conformed to standard	<150 combined	FDA BAM/CMMEF APHA
Total *Coliforms* (MPN/g)	Negative	<10.0	AOAC 966.24
Staphylococcus aureus	Negative	N.D.	AOAC 2003.07/2003.08
E. coli	Negative	N.D.	FDA BAM/AOAC 991.14
Salmonella sp.	Negative	N.D.	FDA BAM

5 Holland 109, Irvine, CA 92618
Office: 949-264-2888 Fax: 626-203-0655

QC: AY- 04/05/2018

CERTIFICATE OF ANALYSIS

Product Name: Antrodia Beta Glucan (ABG™) Powder
Manufacturing Date: April 12, 2018
Lot Number: 20180412ABGS
Source of Origin: *Antrodia cinnamomea*

Physical, Chemical and Microbiological Specifications

Parameter	Analysis Results	Specifications	Assay Method
	PHYSICAL PARAMETERS		
Appearance	Conformed to standard	Fine light beige powder	Visual
Odor	Conformed to standard	Mild	Olfactory
Taste	Conformed to standard	Bland	Taste
	CHEMICAL PARAMETERS		
Total Carbohydrate (%)	91.8	>90	By Difference (Calculation)
Beta-Glucan (%)	67.3	Min. 65	Internal Method
Fat (%)	0.8	<1.0	AOAC 996.06
Saturated Fat (%)	0.8	<1.0	AOAC 996.06
Trans Fat (%)	N.D.	N.D.	AOAC 996.06
Protein (%)	0.7	<1.0	AOAC 922.15
Moisture (%)	4.0	<5.0	AOAC 925.45A/V.O
Ash (%)	2.7	<3.0	AOAC 900.02
	HEAVY METALS		
Lead	Negative	<0.5 ppm	ICP-MS
Arsenic	Negative	<0.5 ppm	Cold Vapor
Cadmium	Negative	<1.0 ppm	ICP-MS
Mercury	Negative	<0.5 ppm	ICP-OES
	MICROBIOLOGICAL PARAMETERS		
Aerobic Plate Count (CFU/g)	Conformed to standard	<15,000	FDA BAM
Yeast and Mold (CFU/g)	Conformed to standard	<150 combined	FDA BAM/CMMEF APHA
Total *Coliforms* (MPN/g)	Negative	<10.0	AOAC 966.24
Staphylococcus aureus	Negative	N.D.	AOAC 2003.07/2003.08
E. coli	Negative	N.D.	FDA BAM/AOAC 991.14
Salmonella sp.	Negative	N.D.	FDA BAM

5 Holland 109, Irvine, CA 92618
Office: 949-264-2888 Fax: 626-203-0655

QC: AY- 04/25/2018

Bonnette, Richard

Subject: FW: [EXTERNAL] GRAS Notification for the intended use of Antrodia mushroom β-glucans as a Food Ingredient

From: Sherwin Chen <sherwin@superbetaglucan.com>
Sent: Wednesday, May 19, 2021 11:45 AM
To: Bonnette, Richard <Richard.Bonnette@fda.hhs.gov>
Cc: Kolanos, Renata <Renata.Kolanos@fda.hhs.gov>; Madhu Soni <SoniM@bellsouth.net>
Subject: Re: [EXTERNAL] GRAS Notification for the intended use of Antrodia mushroom β-glucans as a Food Ingredient

Hi Richard,

Thanks for letting me know. This is to confirm that the notifier is Super Beta Glucan. Also, this email is confirming that the submission was prepared in accordance with 12 CFR Part 170 Subpart E.

Please let me know if there's any questions.

Best Regards

Sherwin
Corporate 949-264-2888 Ext 800
Office 949-707-5385
Cell 626-354-1617
Fax 626-203-0655

On Wed, May 19, 2021 at 7:45 AM Bonnette, Richard <Richard.Bonnette@fda.hhs.gov> wrote:

> Dear Sherwin,
>
> Your submission for submission for β-glucans from *Antrodia cinnamomea* has completed our prefiling evaluation. However, before we can file the submission as a GRAS notice there are some points we need to clarify. Please provide this information by email.
>
> - The cover letter states that it was submitted by Quorum Innovations, Florida. Part 1 of the submission states that the notifier is Super Beta Glucan. Please clarify the identity of the notifier.

- There is no statement in Part 1 regarding Subpart E. Please just note in your email that the submission was prepared in accordance with 12 CFR Part 170 Subpart E.

Let me know if you have any questions,

Richard

Richard E. Bonnette, M.S.
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1235
richard.bonnette@fda.hhs.gov

From: Sherwin Chen
To: Hice, Stephanie
Subject: [EXTERNAL] Re: GRN 000995 - Questions for Notifier
Date: Tuesday, November 16, 2021 2:19:13 PM
Attachments: image002.png
image004.png
image006.png
image008.png
image010.png
image012.png
GRN 995 - FDA Query Response GRN 995 final response 11-16-2021.pdf

---

Dear Dr. Hice,

Attached please find the response to your email of November 8, 2021 regarding additional information and clarifications required for our mushroom β-glucan GRAS notice (GRN 000995). We are providing a point-by-point response to your queries along with some relevant discussion.

Please confirm receipt of this email and let me know if there's any question, thank you very much.

Sincerely,

Sherwin
Super Beta Glucan
Corporate 949-264-2888
Sherwin@superbetaglucan.com

On Mon, Nov 8, 2021 at 7:49 AM Hice, Stephanie <Stephanie.Hice@fda.hhs.gov> wrote:

> Dear Mr. Chen,
>
> During our review of GRAS Notice No. 000995, we noted questions that need to be addressed and are attached to this email.
>
> We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

Stephanie Hice, Ph.D. (they/them/their)

Regulatory Review Scientist & Microbiology Reviewer

Division of Food Ingredients

Office of Food Additive Safety

Center for Food Safety and Applied Nutrition

U.S. Food and Drug Administration

stephanie.hice@fda.hhs.gov

Pronouns: They-Them-Their (what is this?)

Dear Dr. Hice,

RE: Mushroom β-Glucan GRAS Notice (GRN 995)

This responds to your email of November 8, 2021 regarding additional information and clarifications required for our mushroom β-glucan GRAS notice (GRN 000995). We are providing a point-by-point response to your queries along with some relevant discussion.

FDA Query 1: The American Type Culture Collection (ATCC) product sheet for *Antrodia cinnamomea* strain ATCC 200183 states, "This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use. Any proposed commercial use is prohibited without a license from ATCC". Please clarify.

Response: Thank you for bringing this to our attention. There appears to be some mis-understanding as to the role of the ATCC. As you may be aware, the ATCC is a non-profit organization that serves as a depository dedicated to maintaining and distributing authentic reference strains of algae, bacteria, fungi, protozoa, bacteriophages, viruses, etc. as well as cell lines of animal tissues. [See: ATCC: The Global Bioresource Center | ATCC]. **As per our understanding, these statements are standard for some other commercially marketed microorganisms. We have also discussed this with ATCC and was informed that a commercial license is not needed when the strain was subcultured. Only, if we are reselling the original obtained strain, it is prohibited and a commercial license is required. Please note that products derived using this strain or any other strain must meet the safety requirements of FDA before this product can be introduced into commerce. Most of all, we will ensure the safety in production for the strain used.**

FDA Query 2: Please provide detailed description of *A. cinnamomea* strain ATCC 200183 including genotypic (e.g., pathogenicity) and phenotypic characteristics (e.g., production of antimicrobials, production of secondary metabolites and mycotoxins, antifungal resistance), and whether this poses a safety concern.

Response: *A. cinnamomea* strain ATCC 200183 is considered BSL1, guided by the current edition of Biosafety in Microbiological and Biomedical Laboratories (BMBL), published by the U.S. Department of Health and Human Services. In addition, as described in section *6.1.2.1.* Toxicity Studies of Source Mushroom in the GRAS notification dossier, there is no safety concern associated with *A. cinnamomea*. The available information from other strains as well as safety

studies conducted with β-glucan derived from *A. cinnamomea* ATCC 200183 indicate that this strain is unlikely to be pathogenic or toxigenic and does not produce any mycotoxins and antimicrobials. It is also unlikely that the source strain produces any secondary metabolites or has antifungal resistance that can be of safety concern.

Geng et al. (2012) reported that *A. cinnamomea* ATCC 200183 can form arthrospores in the end of liquid fermentation. These investigators analyzed different morphologies of A. *cinnamomea* in submerged culture using scanning electron microscopy. The optimal carbon and nitrogen sources for sporulation were soluble starch and yeast extract. The investigators found that a carbon-to-nitrogen ratio (C/N) of 40:1, $MgSO_4$ (0.5 g/l), maximum production of arthroconidia. KH_2PO_4 (3.0 g/l), an initial pH 5.0, and an inoculum size of 1.5×10(5) spores/ml led to maximum production of arthroconidia.

FDA Query 3: On pages 21-24, you summarize the results from published studies investigating the toxigenicity of *A. cinnamomea* but do not provide a conclusion as to whether the production strain is non-toxigenic. For the administrative record, please state whether A. cinnamomea strain ATCC 200183 is non-toxigenic.

Response: Sorry for our oversight. Based on the available information, we conclude that *A. cinnamomea* strain ATCC 200183 is non-toxigenic.

FDA Query 4: Please state whether the genome of the production strain has been sequenced. If the genome of the production strain has been sequenced, please discuss whether the full genomic sequence is publicly available and provide the corresponding accession number.

Response: The whole genome of the production strain has not been sequenced.

FDA Query 5: For the administrative record, please state whether the production strain is genetically engineered.

Response: The production strain is not genetically engineered.

FDA Query 6: On page 7, you state, "*A. cinnamomea*, a species of the genus *Antrodia* (Polyporaceae), is a parasitic fungus that lives in the inner cavity of the tree *Cinnamomum kanehirai*, which is endemic to Taiwan". Please elaborate on this statement and describe what "parasitic" means in this context.

Response: In this sentence from the dossier, we would like to clarify the word "parasitic" refers to the fact that unlike plants, mushrooms are unable to conduct photosynthesis which synthesize food. Instead, mushroom relies on the nourishments from the host environment in order to dwell. *A. cinnamomea* does not cause infection to human, as such there is no report of any human infection.

FDA Query 7: For the administrative record, please briefly specify how the purity of the production strain is ensured.

Response: The purity of the production strain is ensured by conducting an identification of the subcultured strain as well as properly sterilizing the production enclosure. A cGMP master cell bank (MCB) and working cell bank (WCB) is established for the quality of microbial production. The identity of the strain is validated throughout the production.

FDA Query 8: For the administrative record, please briefly describe how stability of the production strain is ensured.

Response: The stability of the production strain is ensured by well controlled production process with a HACCP (Hazard Analysis Critical Control Point) management system that ensures both the yield and stability. The HACCP plan is provided in the dossier.

FDA Query 9: On page 9, you state, "… the mycelia of *A. cinnamomea* were introduced into the sterile medium and cultured using an incubator at 24-27 °C with relative humidity at 60-70% for 6-8 weeks to allow full growth of the *A. cinnamomea* mushroom culture". For the administrative record, please describe how the production strain is cultivated in more detail (e.g., is the production strain cultivated in an enclosed, sterile environment?)

Response: Utilizing an aseptic technique, the mycelia of *A. cinnamomea* were introduced into the sterile medium (sterilization performed with autoclaving) and cultured using an incubator at the temperature range of 24-27°C with relative humidity at 60-70% for 6-8 weeks to allow full growth of the *A. cinnamomea* mushroom culture. The production strain is cultivated in an enclosed, sterile environment to ensure the purity and stability of the production strain.

FDA Query 10: Please confirm that all starting materials used in the manufacture of β-glucans are food grade and that all processing aids and food contact materials are used in accordance with U.S. regulations.

Response: This is to confirm that all starting materials used in the manufacture of β-glucans are food grade and that all processing aids and food contact materials are used in accordance with U.S. regulations.

FDA Query 11: For the administrative record, please state whether any of the raw materials used in the cultivation media are major allergens or derived from major allergens. If any of the raw materials used are major allergens or derived from major allergens, please discuss why these materials do not pose a safety concern.

Response: This is to confirm that none of the raw materials used in the cultivation media are major allergens or derived from major allergens.

FDA Query 12: In Table 3 (page 9) of your notice, you provide the specifications for β-glucans and the corresponding analytical methods used to analyze for the specification parameters.

a) Please confirm that all analytical methods are validated for their intended purpose. We note that the cold vapor method that you identified as a method used to analyze for arsenic is typically used for mercury (cold vapor generation atomic absorption spectroscopy (CVAAS)). The method that can be used to analyze for elements forming gaseous hydrides (e.g., arsenic) is hydride generation atomic absorption spectroscopy (HGAAS). Please clarify the analytical method used to analyze for arsenic in your ingredient.

Response: The analytical method used to analyze for arsenic in the ingredient is ICP-MS FDA EAM 4.7. Although we mentioned in the GRAS dossier "cold vapor," for all future analysis ICP-MS FDA EAM 4.7 method will be used. These methods are validated consistent with their intended use.

b) The specification established for fat content is described as "N.D." Please clarify what N.D. means. If N.D. means "not detected", please provide the limit of detection (LOD) for the method used.

Response: The trans-fat content is described as N.D. which means "not detected" within the limit of detection. The limit of detection (LOD) for trans-fat by this method is 0.01%.

FDA Query 13: In Appendix I, you provide the results from the analyses of four batches of β-glucans.

a) We note that the results for heavy metals are reported as "negative". On page 8 of the notice, you state, "Extensive analyses of different batches … revealed that these contaminants were not detected within the limits of detection for the method used." Please clarify the meaning of the "negative" results in Appendix I. In addition, please provide the LOD for each analytical method used to analyze for heavy metals. If the LOD is lower than the specification limit, please provide the results of the heavy metal analyses as actual measured values. We note that the LODs for analytical methods such as ICP-MS would be expected to be lower than 0.5 mg/kg.

Response: Thank you for pointing this out. We agree that the ICP-MS method has a lower LOD (limit of detection). Please note that none of the heavy metals were detected at the LOD of the method used. The meaning of the "negative" results in Appendix I indicates that these heavy metal contaminants were not detected within the limits of detection for the method used. The LOD for all heavy metal assays are 10 ppb (parts per billion).

b) We note that the specification limits for cadmium and mercury listed in Table 3 (page 9) and Appendix I differ (<0.5 mg/kg vs. <1 mg/kg for cadmium and <0.05 mg/kg vs. 0.5 mg/kg for mercury). Please address these discrepancies and state the correct specification limits for cadmium and mercury.

Response: Thank you for bringing this to our attention and we are sorry for the oversight. Please note that specification limits for cadmium and mercury given in Table 3 are correct and all certificates of analysis will comply with the standard product specifications.

FDA Query 14: In Table 3 (page 9), you list the following specifications and/or methods:

a. *Staphylococcus aureus, Escherichia coli*, and *Salmonella* serovars are listed as "negative" (page 9). Please clarify what this means, and provide units as appropriate (e.g., negative in 25 grams).

Response: *Staphylococcus aureus, Escherichia coli,* and *Salmonella serovars* are listed as "negative" which means not defected within the limit of detection, which is <10 CFU/gram.

b. The methods used to detect *E. coli* is AOAC 966.24 (page 9), which per AOAC corresponds to detection of coliforms and E. coli in nuts and nut products/tree nut meats. Please clarify if this method is appropriate and fit for purpose.

Response: We are sorry for the incorrect citation. The correct method used for reference should be specified as FDA BAM ONLINE CHP 4, AOAC991.14.

c. The methods used to detect *S. aureus* is AOAC 2003.07 (page 9), which per AOAC corresponds to detection of *S. aureus* in frozen lasagna, custard, frozen mixed vegetables, frozen hash browns, and frozen batter-coated mushrooms. Please clarify if this method is appropriate and fit for purpose.

Response: The method used for reference should be specified as FDA BAM CHP 12, AOAC 2003.08, 2003.11 and AOAC 2003.07 which we consider is fit for its purpose.

d. The methods used to detect *S. aureus* is AOAC 2003.08 (page 9), which per AOAC corresponds to detection of S. aureus in ice cream, raw milk, yogurt, whey powder and cheese. Please clarify if this method is appropriate and fit for purpose.

Response: The method used for reference should be specified as FDA BAM CHP 12, AOAC 2003.08, 2003.11 and AOAC 2003.07 which we consider is fit for its purpose. This method has been modified in-house and has been validated and determined to be acceptable for this use in non-frozen samples.

e. The methods used to detect aerobic plate count, yeast and mold, *E. coli* and *Salmonella serovars* are listed as "FDA BAM" (page 9). For the administrative record, please provide the chapter numbers from the FDA Bacteriological Analytical Manual used for these referenced methods.

Response: For the administrative record, please find the following reference chapter numbers associated with each method of detections:

- **For aerobic plate count, Chapter 3 from FDA BAM**
- **For Yeast and Mold, Chapter 18 from FDA BAM**
- **For *E. Coli*, Chapter 4 from FDA BAM**
- **For *Salmonella serovars* Chapter 5 from FDA BAM**
- **The reference method used to detect yeast and mold is also listed as "CMMEF APHA", which stands for the abbreviation for "Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Chapter 20."**

f. One of the methods used to detect yeast and mold is listed as "CMMEF APHA" (page 9). For the administrative record, please provide the complete citation for this referenced method.

Response: The reference method abbreviation "CMMEF APHA", stands for the "Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Chapter 20."

FDA Query 15: The description in Section 2.4 indicates that the ingredient is primarily composed of β-glucans (≥65%) with a total carbohydrate content of >90% and approximately 1% fat, 1% protein, 3% ash and 5% moisture. Please characterize the other components of the mycelia (e.g., triterpenes/triterpenoids, nucleosides, carbohydrates other than β-glucans) and discuss whether they may be present in the final ingredient.

Response: The non-β-glucans portion of the preparation consists of primarily monosaccharides and disaccharides (approximately 25%), small amounts of cellulose, chitin, and chitosan (< 1%), trace amount of triterpenes and triterpenoids (<0.1%), and nucleosides (<0.1%).

Any presence of triterpenes/triterpenoids, nucleosides, carbohydrates is unlikely to be of safety concern as the final product has been tested for subchronic toxicity and mutagenicity/genotoxicity that did not reveal adverse effects. Thus suggesting that presence of these other components is unlikely to cause any adverse effects at the intended use levels.

FDA Query 16: On page 12, you state that the intended uses and use levels of your ingredient are identical to those described in GRNs 000413 and 000309 for β-glucans derived from other sources (*Ganoderma lucidum* and *Aureobasidium pullulans*, respectively). You also cite the mean and 90th percentile dietary exposures reported in GRNs 000413 and 000309 and conclude that the intended use of your ingredient is not expected to affect dietary exposure to β-glucans. We note that the use levels in GRN 000309 are for the ingredient that contains ≥40% of the β-glucan component and that the content of this component in your ingredient is ≥65%. Please clarify how the use levels were determined for your ingredient. We note that if the use level is determined based on the ingredient, we would expect that the dietary exposure to the β-glucan component will be higher than the reported in GRNs 000413 and 000309. Please discuss the dietary exposure to the β-glucan component from the intended use of your ingredient and provide the revised dietary exposure as appropriate.

Response: This is a good point and thank you for bringing this to our attention. As summarized in Table 4 of our GRAS notice, the comparison of yeast derived (GRN 309 and GRN 239) and mushroom derived (GRN 413 and GRN 995-current GRAS notice) β-glucans shows that the β-glucans component levels in these products range from 40% to 70%. Please note that the use levels were determined on the basis of complete product and not for its β-glucan component. Please also note that the dietary exposure for the present GRAS is taken from

previous GRAS notice GRN 309 and from GRN 413. In the previous GRAS notices (GRN 309 and GRN 413) the β-glucans component content was reported as ≥ 40% and 50%, while the β-glucans content of the subject of present GRAS is 65%, relatively higher. However, it is lower than GRN 239 that reported the β-glucans component content of 70%. A comparison of estimated mean and 90th percentile daily intake of product containing β-glucan and the intake of βglucan component is provided in below Table.

As described in the GRAS notice (GRN 995), the intended use of Antrodia mushroom β-glucans in the selected food categories will result in a mean and 90th percentile estimated daily intake of 291.3 and 583.4 mg/person/day or 6.3 and 14.5 mg/kg bw/day, respectively. It should be noted this intake of the final product is same to that of the subject of GRN 309 and GRN 413. As the subject of present GRAS (GRN 995) contains 65% β-glucans, the resulting exposure to the β-glucans component from all proposed uses at mean and 90th percentile will be 189.34 and 379.21 mg/person/day or 4.09 and 9.42 mg/kg bw/day, respectively.

Intake Estimate Comparison of Mushroom and Yeast β-Glucans from GRAS Notices

	Mushroom (GRN 995)	**Mushroom (GRN 413)**	**Black yeast (GRN 309)**	**Baker's yeast (GRN 239)**
Mean EDI mg/p/day (mg/kg bw/day)	291.3 (6.3)	291.3 (6.3)	291.3 (6.3)	413 (8.90)
90th percentile EDI mg/p/day (mg kg bw/day)	583.4 (14.5)	583.4 (14.5)	583.4 (14.5)	885 (20.66)
β-Glucan (%)	Minimum 65	At least 50	At least 40	At least 70
Mean EDI of β-Glucan component mg/p/day (mg/kg bw/day)	189.34 (4.09)	145.65 (3.15)	116.52 (2.52)	289.1 (6.23)
90th percentile EDI of β-Glucan component mg/p/day (mg/kg bw/day)	379.21 (9.42)	291.7 (7.25)	233.36 (5.8)	619.5 (14.46)
EDI = Estimated daily intake; mg/p/day = mg/person/day				

In the first GRAS notice (GRN 239) that has β-glucans content of at least 70%, the resulting 90th percentile intake from all proposed uses was estimated as 827 mg/person/day (20.66 mg/kg bw/day), while for the present GRAS (GRN 995) that contains 65% β-glucans, the 90th percentile intake from all proposed uses is estimated as 583.4 mg/person/day (14.5 mg/kg bw/day). Also as described in the above table the intake of β-glucans component in these two GRAS notices is 379.21 and 619.5 mg/person/day (or 9.42 and 14.46 mg/kg/bw/day), respectively. This shows that the intake of β-glucans from the proposed uses in GRN 995 is much lower as compared with GRN 239 and is unlikely to be of any safety concern. Additionally, the specific safety studies conducted with the subject of present GRAS did not reveal any adverse effects suggesting that the resulting

intake higher levels of β-glucan component from the proposed uses is unlikely to cause any adverse effects.

FDA Query 17: We note that according to 21 CFR 101.12, the current reference amount customarily consumed per eating occasion for yogurt is 170 g, not 225 g as provided in GRNs 000309 and 000413. Please revise the use level of your ingredient in yogurt. In addition, please provide a table with the intended food categories and corresponding use levels expressed on a percent basis.

Response: Thank you for bringing this to our attention that the reference amount of yogurt has been changed. We are providing a revised table that includes food categories and use levels expressed as mg/serving and on a percent basis.

Proposed Food Uses and Use Levels of Mushroom β-Glucans

Food category	Proposed food use	RACC (g or ml)	Mushroom β-Glucans	
			mg/serving	Percent
Baked goods and baking mixes	Cookies	30 to 40	150	0.5 to 0.375
Beverages and beverage bases	Meal replacement beverages (not milk based)	240	150	0.0625
Cereal and cereal products	Nutritional bars (breakfast, granola, protein)	40	150	0.375
Dairy product analogs	Soy milk	240	150	0.0625
Milk and milk products	Meal replacement beverages	240	150	0.0625
	Probiotic beverages	240	150	0.0625
	Yogurt	**170**	**150**	**0.088**
	Yogurt beverage	240	150	0.0625
Plant protein products	Soy protein bars	40	150	0.375
Processed fruits and fruit juices	Fruit beverages (drinks, juices, smoothies)	240	150	0.0625
Soft candy	Chocolate confections	40	150	0.375
Soup and soup mixes	Soups	245	150	0.061

FDA Query 18: You state, "Additionally, the source material of Antrodia mushroom β-glucans, *A. cinnamomea* is an edible mushroom" (page 14). Please elaborate on this statement, and provide relevant references, as applicable.

Response: There are over 2000 known species of edible fungi worldwide, several of which have been commercially cultivated (Chuang et al., 2020). *A. cinnamomea*, known as Niu-Chang-Chih, is an aboriginal and edible mushroom in Taiwan. This is considered as important natural resources and has also been used traditionally for therapeutic purposes. Given its slow growth rate and rarity of parasitic host tree *Cinnamomum kanehirai* Hay, it is difficult to obtain

fruiting bodies of *A. cinnamomea* in the wild (Zhang et al., 2017). Hence, artificial cultivation, including basswood cultivation, solid-state fermentation, and submerged fermentation, has been applied as a substitute for the wild fruiting body of *A. cinnamomea* to meet the increasing consumption demand.

One of the traditional food preparations for *A. cinnamomea* is via slow cooking along with chicken and vegetables to make soups.

(Recipes listed in Traditional form of Chinese including:

Antrodia Mushroom Broth: https://health.udn.com/health/story/6036/2692964

Antrodia Mushroom Stew: https://www.o-yes99.com/about.asp?in=262

Other means of traditional consumptions include cooking along with honey and berries to make a healthful Antrodia tea. (Recipe listed in Traditional form of Chinese: http://www.htac.com.tw/edcontent_d.php?lang=tw&tb=4&id=41)

FDA Query 19: Please provide an updated literature search including the date (month and year) the literature search was performed and discuss the identity and safety of *A. cinnamomea*.

Response: In an updated search conducted for the period of April 2020 to October 2020, no significant related to safety were found except for the one clinical study described below.

In a recent, nonrandomized, open-label, single-arm study in healthy adults, Chen and Chang (2021) examined changes in blood biochemical parameters before and after *A. cinnamomea* consumption. In this study, 32 healthy men and women (20 and 60 years of age) ingested capsules containing 380 mg *A. cinnamomea* solid-state cultivated mycelium (LAC) powder twice a day for three months. The subjects were monitored during the study and one month after the study end-point. At each monthly visit samples or blood examinations, blood biochemical analysis, and urine examination were collected.

Physical examination included temperature, heartbeat, and blood pressure. Blood examination includes Hemoglobin (Hb), WBC with classification count (WBC with differential), red blood cells (RBC), platelets, hematocrit (Hct), mean hematocrit Volume ratio (MCV), average red blood cell hemoglobin amount (MCH), and average red blood cell hemoglobin concentration (MCHC). Blood biochemical analysis included alkaline phosphatase (Alk-P), alanine transaminase (ALT), aspartate aminotransferase (AST), albumin, blood urea nitrogen (BUN), alanine transferase (γ-GT), total bilirubin, total cholesterol (TC), triglyceride (TG), total protein, creatinine, fasting blood glucose, uric acid, ACTH, cortisol. Urine examination included urine pH, white blood cells (WBC), red blood cells (RBC), urine protein (Protein), urine cylinder (Cast) test (Chen and Chang, 2021).

After 12 weeks of intervention, the hepatobiliary indices of subjects, including AST, ALT, total bilirubin, ALP, and gamma GT were not significantly different from

baseline, except for albumin, which decreased by 1.1% although the value was still within the normal range. There were no significant differences in the indicators of renal function, including BUN, creatinine, and uric acid levels before and after intervention. Taken together, these results confirmed the safety of LAC-Capsule on liver and kidney functions. LAC consumption did not significantly change fasting blood glucose, blood pressure, and triglyceride levels or liver and renal function indices. No adverse events occurred during the trial. A significant change from baseline in total cholesterol levels was observed; men and women had decreases of 5.7% and 5.3%, respectively. Based on these findings, the investigators concluded that ingestion of LAC-capsule has a considerable degree of safety (Chen and Chang, 2021).

FDA Query 20: References to "*Salmonella typhimurium*" on pages 19, 23, and 25 should read *Salmonella* Typhimurium as serovars are not italicized. Please make a statement that corrects this reference.

Response: We agree with FDA that serovars are not italicized and references to *Salmonella typhimurium* on pages 19, 23, and 25 should read as *Salmonella* Typhimurium.

We hope the above information and clarification addresses your queries. If you have any questions or need additional explanation, please let us know.

Thank you for the opportunity to provide this explanation.

Best regards

Sherwin Chen

References:

Chen WJ, Chang FW., 2020. A Pilot Study to Assess Food Safety and Potential Cholesterol-Lowering Efficacy of *Antrodia cinnamomea* Solid-State Cultivated Mycelium in Healthy Adults. Evidence-based complementary and alternative medicine: eCAM, 2020, 5865764. https://doi.org/10.1155/2020/5865764

Chuang WY, Hsieh YC, Lee TT., 2020. The Effects of Fungal Feed Additives in Animals: A Review. Animals (Basel). 2020;10(5):805. doi:10.3390/ani10050805

Ganesan N, Baskaran R, Velmurugan BK, Thanh NC., 2019. Antrodia cinnamomea-An updated minireview of its bioactive components and biological activity. J Food Biochem. 43(8):e12936.

Geng Y, He Z, Lu ZM, Xu HY, Xu GH, Shi JS, Xu ZH., 2013. *Antrodia camphorata* ATCC 200183 sporulates asexually in submerged culture. Appl Microbiol Biotechnol. 97(7):2851-2858.

Zhang BB, Hu PF, Huang J, Hu YD, Chen L, Xu GR., 2017. Current Advances on the Structure, Bioactivity, Synthesis, and Metabolic Regulation of Novel Ubiquinone Derivatives in the Edible and Medicinal Mushroom *Antrodia cinnamomea*. J Agric Food Chem. 65(48):10395-10405.

From: Sherwin Chen
To: Hice, Stephanie
Subject: [EXTERNAL] Re: GRN 000995 - Questions for Notifier
Date: Thursday, December 23, 2021 5:21:29 PM
Attachments: image013.png
image014.png
image015.png
image016.png
image017.png
image018.png
GRN 995 - FDA Aditional Queries- Response GRN 995-2.pdf

Dear Dr. Hice,

Attached please find the response to your email of December 14, 2021 regarding additional information and clarifications required for our mushroom β-glucan GRAS notice (GRN 000995). We are providing a point-by-point response to your queries along with some relevant discussion.

Please confirm receipt of this email and let me know if there's any question, thank you and wishing you happy holidays.

Sincerely,

Sherwin
Super Beta Glucan
Corporate 949-264-2888
Sherwin@superbetaglucan.com

On Tue, Dec 14, 2021 at 2:39 PM Hice, Stephanie <Stephanie.Hice@fda.hhs.gov> wrote:

> Dear Mr. Chen,
>
> During our review of GRAS Notice No. 000995, we noted additional questions that need to be addressed and are attached to this email.
>
> We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

Stephanie Hice, Ph.D. (they/them/their)

Regulatory Review Scientist & Microbiology Reviewer

Division of Food Ingredients

Office of Food Additive Safety

Center for Food Safety and Applied Nutrition

U.S. Food and Drug Administration

stephanie.hice@fda.hhs.gov

Pronouns: They-Them-Their (what is this?)

Dear Dr. Hice,

RE: Mushroom β-Glucan GRAS Notice (GRN 995) – Additional queries

This responds to your email of December 14, 2021 related to additional questions for our mushroom β-glucan GRAS notice (GRN 000995). We are providing a point-by-point response to your queries along with some clarification and discussion.

FDA Query 1: In your response to question 13, you state that the limit of detection (LOD) for all heavy metal assays is 10 µg/kg (ppb). We note that the specifications for these heavy metals are significantly higher. In order to keep the exposure to heavy metals as low as possible, please lower the specifications for heavy metals so that they align with the results from your batch analyses.

Response: Given your recommendations, we would like to lower the specification for heavy metals, lead, arsenic and cadmium (individually) from 0.5 ppm to 0.1 ppm, without changing mercury specification that has been established at 0.05 ppm.

FDA Query 2: In your November 16, 2021 amendment, in response to question 14(e) and (f), you state that the "… reference method used to detect yeast and mold is also listed as "CMMEF APHA", which stands for the abbreviation for "Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Chapter 20"". We note that in the fifth edition of the Compendium of Methods for the Microbiological Examination of Foods, Chapter 20 corresponds to "Probiotics", while Chapter 21 corresponds to "Yeasts and Molds". Please see: https://ajph.aphapublications.org/doi/book/10.2105/MBEF.0222

For the administrative record, please clarify.

Response: Sorry for the oversight. We agree that it should be Chapter 21.

FDA Query 3: In your response to question 16, you indicate in the provided table that the 90th percentile estimated daily intake (EDI) for GRN 000239 is 885 mg/person (p)/day (d). We believe that this is a typographical error, and that the 90th percentile EDI should be 827 mg/p/d. Please confirm the 90th percentile EDI for GRN 000239.

Response: Thank you for bringing this to our attentions. We are sorry for the typographical error. We confirm that the 90th percentile EDI for GRN 000239 is 827 mg/p/d.

FDA Query 4: In your response to question 17, you provide a revised table that includes the intended food categories and use levels for your ingredient. You also state that the intended use is the same as that in GRNs 000309 and 000413. However, we note that soup and soup mixes were not included in the uses for these GRNs. This category was removed in an amendment to GRN 000309. If you do not intend to use your ingredient in soup and soup mixes, please remove this food categories from your intended uses. However, if you would like to include this food category, you should indicate that the intended use in soup and soup mixes does not include products under the jurisdiction of the USDA and provide a revised dietary exposure to include this use.

Response: We request to remove the soup and soup mixes from the proposed uses.

FDA Query 5: In your November 16, 2021 amendment, in response to question 19, you state that an updated literature search was performed through October 2020. The updated literature search included a clinical study published in 2021. For the administrative record, please clarify if the literature search was performed through October 2021.

Response: Sorry for the incorrect statement, please note that the literature search was performed through October 2021.

We hope the above information and clarification addresses your queries. If you have any questions or need additional explanation, please let us know.

Thank you for the opportunity to provide this explanation.

Best regards

Sherwin Chen

From: Sherwin Chen
To: Hice, Stephanie
Subject: [EXTERNAL] Re: GRN 000995 - Questions for Notifier
Date: Thursday, April 14, 2022 1:08:37 AM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png
GRN 995 - FDA Additional Queries-2 - Responses GRN 995 final.pdf

---

Dear Dr. Hice,

Attached please find the responses to your email of April 6, 2022 regarding additional information and clarifications required for GRN 000995. We are providing a point-by-point response to your queries along with some relevant discussion.

Please confirm receipt of this email and let me know if there's any question.

Thank you very much once again.

Sincerely,

Sherwin Chen
Super Beta Glucan
Corporate 949-264-2888
Sherwin@superbetaglucan.com

On Wed, Apr 6, 2022 at 1:51 PM Hice, Stephanie <Stephanie.Hice@fda.hhs.gov> wrote:

> Dear Mr. Chen,
>
> During our review of GRAS Notice No. 000995, we noted additional questions that need to be addressed and are attached to this email.
>
> We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)

Regulatory Review Scientist & Microbiology Reviewer

Division of Food Ingredients

Office of Food Additive Safety

Center for Food Safety and Applied Nutrition

U.S. Food and Drug Administration

stephanie.hice@fda.hhs.gov

Pronouns: They-Them-Their (what is this?)

Dear Dr. Hice,

RE: Mushroom β-Glucan GRAS Notice (GRN 995) – Additional queries-2

This responds to your email of April 6, 2022 related to additional questions for our mushroom β-glucan GRAS notice (GRN 000995). We are providing a point-by-point response to your queries along with some clarification and discussion.

FDA Query 1: On page 12 of the notice, you state that the intended use and use levels are the same as those in GRN 000413 and estimate mean and 90th percentile dietary exposures to β-glucans from *Antrodia cinnamomea* strain ATCC 200183 to be 291.3 mg/person (p)/day (d) and 583.4 mg/p/d, respectively. In your amendment dated November 16, 2021, you provide a table that compares the dietary exposure of β-glucans from *A. cinnamomea* strain ATCC 200183 with the dietary exposures to β-glucans from uses in GRNs 000239, 000309, and 000413. In the table you also compare the β-glucans component of your ingredient with the aforementioned GRNs and note that the dietary exposure to β-glucans from the intended uses is "much lower" compared to GRN 000239 and is therefore unlikely to be a safety concern. Please clarify if the use β-glucans from *A. cinnamomea* strain ATCC 200183 is substitutional for the current uses.

Response: We confirm that the proposed use of β-glucans from *Antrodia cinnamomea* strain ATCC 200183 is substitutional to that of the current uses. Sorry we forgot to mention this.

FDA Query 2: The GRAS Final Rule published in 2016 (81 FR 54960) requests the notifier to provide data and information about dietary exposure to the ingredient (21 CFR 170.235) from all sources (added and natural). Please provide a narrative supported by updated literature to address the cumulative dietary exposure to β-glucans from all dietary sources including the intended uses of β-glucans from *A. cinnamomea* strain ATCC 200183 in GRN 000995.

Response: It is well recognized that β-glucan is a polysaccharide in the form of fiber and the main element of fiber in oats, barley, yeast and mushrooms. Since prehistoric times, foods high in glucans forming structural components of cell walls, have been commonly consumed. Food sources of β-glucan include edible mushrooms, yeast, and grains, such as oat, barley, and wheat. Among cereals, the highest content of β-glucan has been reported for barley: 2-20 g (65% is water-soluble fraction) and for oats: 3-8 g (82% is water-soluble fraction). Other cereals

also contain β-glucan but in much lower amounts: sorghum 1.1-6.2 g, rye 1.3-2.7 g, maize 0.8-1.7 g, triticale 0.3-1.2 g, wheat 0.5-1.0 g, durum wheat 0.5-0.6 g, and rice 0.13 g (Bacic et al., 2009). Fractions rich in β-glucans are readily obtained from cereal grains by dry milling followed by sieving and air classification processes or by wet milling followed by sieving and solvent extractions (Lazaridou and Biliaderis, 2007). These approaches result in concentrates or isolates containing 8-30% and 95% β-glucans, respectively.

Among soluble fibers, β-glucan is the most frequently consumed. The available information suggests that worldwide there is great interest in the application of β-glucans in the food and beverage sectors. Food Marketing Industry data showed that in 2016, the global β-glucan market was worth $307.8 million with a prediction by Markets and Markets to reach $476.5 million in 2022 (Bai et al., 2019). Innova Market Insights reports a 75% jump in global food and beverage launches featuring β-glucan between 2019 and 2020, albeit from a small base.

Recommendations for fiber intakes range from 25-38 g/day depending on country-specific guidelines As regards the intake of fiber (soluble plus insoluble combined), the Institute of Medicine provides adequate intake levels for total fiber based on age and gender. For women ages ≥50 years, the adequate intake levels for total fiber are 21 g/day, while for younger women (<50 years) it is 25 g/day. For men ≥50 years, it is 30 g, while for men ages 50 and older it is 38 g. These recommendations are based on calorie requirements for people in each age and gender category. However, in spite of widespread knowledge of the role of fiber in a healthy diet, the average intakes are well below the recommended amount (Stephen et al., 2017).

As such, there are no specific dietary reference intake recommendations for soluble fiber. However, the U.S. Department of Health and Human Services' therapeutic lifestyle changes, or TLC, diet for lowering cholesterol provides recommendations specifically for soluble fiber intake (DHHS, 2005). The TLC diet recommends consumption of 10 to 25 g of soluble fiber each day to reduce your LDL cholesterol. Soluble fiber is found in psyllium seeds, legumes, fruits, some vegetables, barley and oats. Nuts and seeds are also rich in soluble fiber. Also, as described in GRN 995 (Part III, section 3.3.), FDA approved a health claim that β-glucan intakes of 3 g or more per day that were reported to be effective in lowering serum lipids. 50 g whole grain oat or barley provides 2.5 and 3.5 g β-glucan, respectively. The available information suggests that most individuals habitually consume less than 25 g of fiber/day in total and there is a need to increase the intake of soluble fiber.

A thorough search of the literature did not reveal any references on cumulative exposure data on β-glucan. As β-glucan is found in many commonly consumed

dietary sources, it is difficult to calculate the cumulative intake of dietary exposure to β-glucan from all sources (added and natural). Also, such an exercise will be quite an expensive undertaking. However, as discussed above and also extensively described in Part III section 3.3. of GRN 995, there is a need to further increase the intake of β-glucan. As β-glucan from *A. cinnamomea* (subject of GRN 995) manufactured by Super Beta Glucan will serve as an alternative source of β-glucan to existing GRAS sources of β-glucan described in GRNs 000239, 000309, and 000413, the introduction of β-glucan by Super Beta Glucan is unlikely to further increase dietary intake of β-glucan in an additive manner.

Similarly, compared to many other common dietary sources, such as grains, yeast, vegetables, etc., that contains relatively high levels of β-glucan, the proposed use levels of Antrodia mushroom β-glucans of 150 mg/serving and resulting 90th percentile intake of 583.4 mg/person/day is very small. The TLC diet recommends consumption of 10 to 25 g of soluble fiber that primarily composed of β-glucans. As subject of GRN 995 contains 65% β-glucans, the resulting intake of β-glucans from the proposed uses will be 379.2 mg (0.38 g)/person day. All this also suggest that any addition of β-glucans (0.38 g/day) to the existing uses or recommended daily intake (10-25 g/day) is very small or negligible. The proposed uses of β-glucan by Super Beat Glucan will serve as an alternative to existing GRAS sources and is very small. Hence, it will not change the current dietary exposure to β-glucan among U.S. consumers of foods to which β-glucan may be added. Any additional intake is considered as safe.

We hope the above information and discussion addresses your queries. Thank you for the opportunity to provide this explanation.

Best regards

Sherwin Chen

References:

Bacic, A., Fincher, G.B., Stone, B.A., 2009. Chemistry, Biochemistry, and Biology of (1-3)-[beta]-Glucans and Related Polysaccharides, Academic Press, Amsterdam, The Netherlands, 1st edition, 2009.

Bai, J., Ren, Y., Li, Y., Fan, M., Qian, H., Wang, L., Wu, G., Zhang, H., Qi, X., Xu, M., 2019. Physiological functionalities and mechanisms of β-glucans. Trends Food Sci. Technol. 88:57-66.

De Marco Castro, E., Calder, P.C., Roche, H.M., 2021. β-1,3/1,6-Glucans and Immunity: State of the Art and Future Directions. Mol Nutr Food Res. 65(1):e1901071. doi: 10.1002/mnfr.201901071.

DHHS, 2005. US Department of Health and Human Services, National Institutes of Health, and National Heart, Lung, and Blood Institute. Your Guide to Lowering Your Cholesterol with TLC. NIH Publication No. 06-5235.

Lazaridou, A., Biliaderis, C.G., 2007. Molecular aspects of cereal β-glucan functionality: physical properties, technological applications and physiological effects. Journal of Cereal Science 46(2):101-118.

Stephen AM, Champ MM, Cloran SJ, Fleith M, van Lieshout L, Mejborn H, Burley VJ., 2017. Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. Nutr Res Rev. 30(2):149-190.