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Division of Biotechnology and GRAS Notice Review

December 5, 2011

Dr. Antonia Mattia, Ph.D.
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
4300 River Road
College Park, MD 20740

RE: Notification of GRAS Determination for β-glucans derived from mushroom

Dear Dr. Mattia:

In accordance with proposed 21 CFR § 170.36 (Notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18939-18964), I am submitting in triplicate, as the agent to the notifier, Super Beta Glucan Inc., a GRAS Notification for β-glucans derived from mushroom.

Mushroom β-glucans, primarily comprised of β-glucans (minimum 50%) derived from *Ganoderma lncidum* is intended for use 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, soft candy, and soup and soup mixes at level of 150 mg mushroom β-glucans *per* serving (reference amounts customarily consumed, 21 CFR 101.12).

Please let me know if you have any questions.

Sincerely,

(b) (6)

Edward A. Steele
President

Enclosures

EAS Consulting Group, LLC
1940 Duke Street, Suite 200, Alexandria, Virginia 22314
(877) 327-9808 Toll Free • (703) 684-4408 Local • (703) 684-4428 Fax

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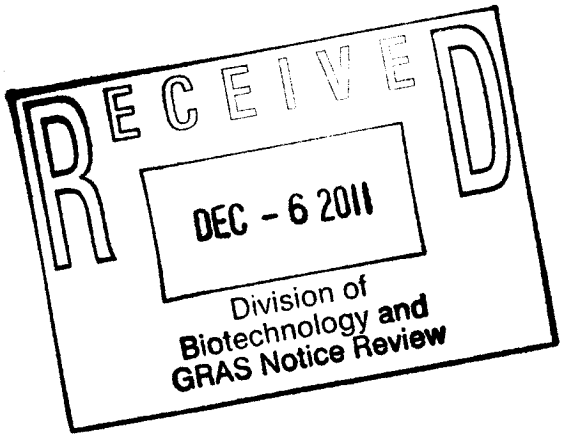

December 5, 2011

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

RE: Notification of GRAS Determination for Wheat Bran Extract

Dear Sir/Madam:

In accordance with proposed 21 CFR § 170.36 (Notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18939-18964), I am submitting in triplicate, as the agent to the notifier, Super Beta Glucan Inc., a GRAS Notification for β-glucans derived from mushroom.

Mushroom β-glucans, primarily comprised of β-glucans (minimum 50%) derived from *Ganoderma lncidum* is intended for use 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, soft candy, and soup and soup mixes at level of 150 mg mushroom β-glucans *per* serving (reference amounts customarily consumed, 21 CFR 101.12).

Please let me know if you have any questions.

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GRAS Notice Review

GRAS NOTIFICATION

I. Claim of GRAS Status

A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

Super Beta Glucan Inc. (the notifier) has determined that its β-glucans derived from mushroom is Generally Recognized As Safe, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food. Therefore, the use of mushroom β-glucans is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Date 12/5/11

Edward A. Steele

Agent for:

Super Beta Glucan Inc.,
5 Holland, Suite 109
Irvine, CA 92618

B. Name and Address of Notifier:

Sherwin Chen,
Head, Regulatory Affairs
Super Beta Glucan Inc.
5 Holland, Suite 109
Irvine, CA 92618

Phone: +1 626 354 1617
Fax: +1 626 203 0655
E-mail: sherwin@superbetaglucan.com

C. Common or usual name of the notified substance:

Mushroom β-glucans

D. Conditions of use:

Mushroom β-glucans, primarily comprised of β-glucans (minimum 50%) derived from *Ganoderma lucidum* is intended for use 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, soft candy, and soup and soup mixes at a level of 150 mg mushroom β-glucans *per* serving (reference amounts customarily consumed, 21 CFR 101.12). The intended use of mushroom β-glucans is in the same food products and at the levels mentioned in the GRN 309[1]. The proposed food categories are also identical to another GRAS notification (GRN 239[2]) on this subject. The intended use of mushroom β-glucans in the above mentioned food categories is estimated to result for "users only" at mean and 90th percentile intakes of 338.7 and 678.4 mg/person/day or 7.3 and 16.4 mg/kg body weight/day, respectively. Mushroom β-glucans is not intended to be marketed for infant and toddler foods.

E. Basis for GRAS Determination:

In accordance with 21 CFR 170.30, the intended use of mushroom β-glucans has been determined to be generally recognized as safe (GRAS) based on scientific procedures. A comprehensive search of the scientific literature was also conducted for this review.

β-Glucans derived from yeast was the subject of two separate GRAS notifications (Footnotes 1 and 2). For these GRAS notifications (GRN 309- 2010 and GRN 000239- 2008), FDA responded that the agency had no questions regarding the conclusions that the use of β-glucans derived from yeast is GRAS under the conditions of use described in these notices. There is sufficient qualitative and quantitative scientific evidence, including human and animal data, to determine safety-in-use for mushroom β-glucans. The safety determination of mushroom β-glucans is based on the totality of available evidence, including specifically designed animal toxicity studies as well as other human, animal, and *in vitro* studies, further

[1] Accessible at: http://www.accessdata.fda.gov/scripts/fcn/gras_notices/grn_309.pdf
[2] Accessible at: http://www.accessdata.fda.gov/scripts/fcn/gras_notices/grn000239.PDF

corroborating its safety. The results from a subchronic study in rats conducted according to OECD and FDA guidelines show that mushroom β-glucans had no adverse effects at dose levels up to 2000 mg mushroom β-glucans/kg bw/day, the highest dose tested. This study suggests a no-observed-adverse-effect level (NOAEL) of 2000 mg of mushroom β-glucans/kg bw/day. The estimated daily intake of mushroom β-glucans from the intended uses at the 90th percentile is determined as 678.4 mg/person/day (16.4 mg/kg bw/day). The estimated intake is over 120-fold lower than the NOAEL in animal study. The estimated daily intake, if ingested daily over a lifetime, is determined as safe.

F. Availability of Information:

The data and information that serves as the basis for this GRAS determination will be available for the Food and Drug Administration's review and copying at the following address or will be provided to FDA at their request:

Edward A. Steele, EAS Consulting Group, LLC, 1940 Duke Street, Suite 200, Alexandria, VA 22314; Phone: (703) 684-4408; Fax: (703) 684-4428; E-mail: esteele@easconsultinggroup.com.

The primary toxicologist, Dr. Madhusudan G. Soni, responsible for the preparation of this GRAS monograph and who is also a member of the expert panel can also be contacted for the data and information that serves as the basis for this GRAS determination at the following address: Madhusudan G. Soni, Ph.D., 749 46th Square, Vero Beach FL, 32968; Phone: (772) 299-0746; E-mail: sonim@bellsouth.net.

II. Detailed Information About the Identity of the Notified Substance:

A. Chemical name

Mushroom β-glucans. The product is composed mainly of β-glucans.

B. Common/Trade Name:

The subject of this notification will be marketed as Mushroom Beta Glucan/MBG® and Beta136®.

C. Chemical Abstract Registry Number:

Not available

D. Chemical Formula:

(1 -3),(1-6)-β-D-glucan, Poly-(l-6)-β-D-glucopyranosyl-(l,3)-β-D-glucopyranose

E. Structure:

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

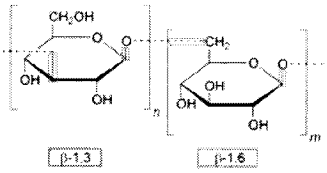

Diagram showing orientation and location of different β-glucan linkages.

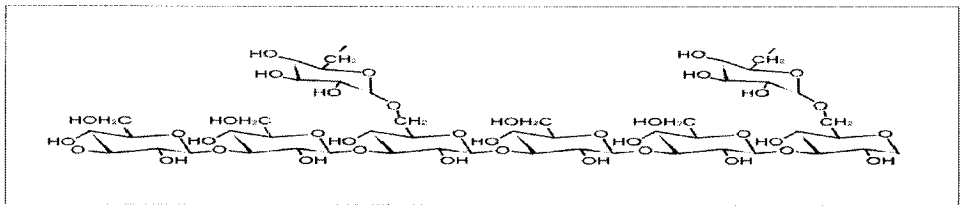

Fig. 1 Chemical Structure of Mushroom β-glucan.

F. Molecular Weight

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

G. Physical Characteristics

Mushroom β-glucans is a fine light beige colored powder.

H. Typical Composition and Specifications

Typical food grade specifications of mushroom β-glucans are presented in Table 1. It is a fine light beige powder soluble in water. Analyses of 5 independently produced, and representative non-consecutive batches (Appendix I) of 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 specification. The product is composed of β-glucans (50%) with a total carbohydrate content of over 95%. Mushroom β-glucans also contains approximately 1% fat, 1% protein, 3% ash and 2% moisture. As shown in Table 1, the sum of all analyzed components demonstrates that mushroom β-glucans is fully characterized (almost 100%) for its constituents. Extensive analyses of different batches for potential external contaminants of 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 1. Physical, chemical and microbiological specifications of 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 (%)	> 95	By Difference (Calculation)
B-Glucan (%)	Minimum 50	Internal Methods
Fat (%)	<1.0	CNS 5036
Saturated Fat (%)	<0.5	AOAC
Trans Fat (%)	<0.5	AOAC
Protein (%)	<1.0	LECO 2000
Moisture (%)	<2.0	AOAC
Ash (%)	<3.0	AOAC
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)	<10,000	FDA III
Yeast and Mold (CFU/g)	<15	FDA XVIII
Total Coliforms	Negative	FDA IV USDA/3M PETRIFILM
E. coli	Negative	FDA IV USDA/3M PETRIFILM
Salmonella sp.	Negative	FDA V

I. Manufacturing process

Mushroom β-glucans is manufactured according to current Good Manufacturing Practices (cGMP) using appropriate food grade ingredients. The mushroom β-glucans production process is schematically represented in Figure 2. The *Ganoderma lucidum* mushroom used in the production of mushroom β-glucan is a standard species (*G. lucidum* ATCC 32472). Mycelium from the *G. lucidum* strain was subcultured and maintained in sterile agar medium under controlled conditions. The manufacturing process is initiated by preparing a culture medium containing glucose, galactose, sucrose, mannose and yeast extract. Following a sterilization procedure at a selected temperature and pressure for a specific time, the mycelia of *G. lucidum* were introduced into the sterile medium and cultured using an incubator at a range of temperatures for 3~5 weeks to allow full growth of the mushroom culture. Subsequently, mushroom β-glucans in mycelium was extracted using a high speed homogenizer and ultrasonic vibration. The resulting solution is then filtered and separated using a ceramic membrane to strip most of the residual small carbohydrate molecules. The concentrated mushroom β-glucans are then pooled, dried and ground into the powder form. Upon completion, the mushroom β-glucans is packaged into food grade bags. The sample from each batch is analyzed for the presence of carbohydrate molecules using high pressure liquid chromatography (HPLC). All equipment and materials used in the production process have a history of use in food processing. All materials used in the processing steps are in compliance with FDA regulations.

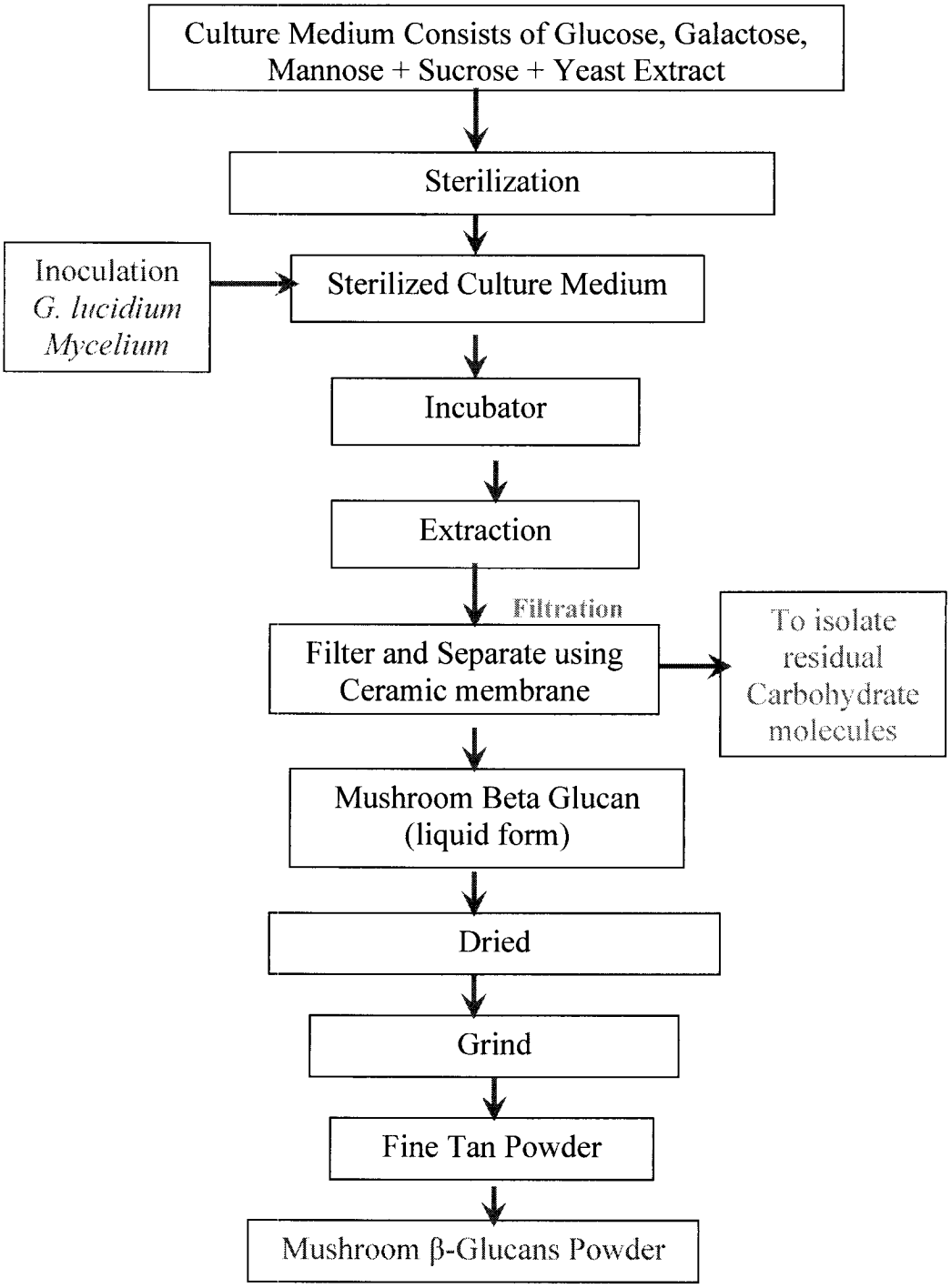

Figure 2. Manufacturing process of mushroom β-glucans

III. Summary of the Basis for the Notifier's Determination that Mushroom β-Glucans is GRAS

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Super Beta Glucans Inc to determine the Generally Recognized As Safe (GRAS) status of mushroom β-glucans intended for use as a food ingredient, particularly as a source dietary fiber. A comprehensive search of the scientific literature was also conducted to identify relevant safety studies for this assessment.

Based on a critical evaluation of the pertinent data and information summarized herein, the Expert Panel members have individually and collectively determined by scientific procedures that the addition of mushroom β-glucans at a level of up to 150 mg mushroom β-glucans *per* serving to 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, soft candy, and soup and soup mixes, meeting the specifications cited above and manufactured according to current Good Manufacturing Practice, is Generally Recognized As Safe (GRAS) under the conditions of intended use in selected foods, as specified herein.

In coming to its decision that mushroom β-glucans is GRAS, the Expert Panel reviewed published toxicology and clinical studies and other relevant corroborative information relating to the safety of the product and concluded that neither mushroom β-glucans nor any of its degradation products pose any toxicological hazards or safety concerns at the intended use levels. It is also their opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

In addition, the Expert Panel reviewed the production and safety data in GRNs 239 and 309 for yeast-derived β-glucans. The information in these GRNs is incorporated in their entirety by reference into this document.

IV. Basis for a Conclusion that Mushroom β-Glucans is GRAS for its Intended Use.

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MUSHROOM β-GLUCANS AS A FOOD INGREDIENT

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DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MUSHROOM β-GLUCANS AS A FOOD INGREDIENT

1. INTRODUCTION

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[3], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened at the request of Super Beta Glucans Inc., USA, to determine the Generally Recognized As Safe (GRAS) status of 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, soft candy, and soup and soup mixes at use levels of up to 150 mg mushroom β-glucans *per* serving when not otherwise precluded by a standard of identity. A comprehensive search of the scientific literature for production, safety and toxicity information specifically on mushroom β-glucans and its main component β-glucans was conducted through October 2011 and made available to the Expert Panel. Super Beta Glucan Inc. assures that all unpublished information in its possession and relevant to the subject of this determination have been provided for this assessment and have been summarized in this GRAS monograph. The Expert Panel independently and critically evaluated materials submitted by Super Beta Glucans Inc., USA, and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred on November 11, 2011 and unanimously agreed to the decision described herein.

1.1. Background

The main sources of β-glucans include barley, oats, yeast and mushrooms. β-Glucans derived from mushrooms have been used in many countries in Asia for health purposes for a long time. Several mushroom species have been reported to contain bioactive or functional compounds such as polysaccharides, polysaccharides-peptides, nucleosides, and triterpenols that are reported to possess hypoglycemic, immunomodulatory, anti-inflammatory, antiviral, antibacterial or antiparasitic activities (Wasser and Weis, 1999; Cheung, 2009). Among these compounds, the polysaccharides are considered as potent active compounds (Borchers et al., 1999). Polysaccharide-rich fungi and plants have been employed for centuries by cultures around the world for their dietary and therapeutic benefits. In recent years, polysaccharides such as β-glucans extracted from mushroom have been examined for their potential anti-cancer/tumor inhibitory effect (Wasser and Weis, 1999; Ooi and Liu, 2000; Ling and Zhang, 2004). The anti-cancer effects of these polysaccharides have been suggested to be due to immunomodulatory effects rather than having direct cytotoxic activity. One of these mushrooms, *Ganoderma lucidum* (also known as Lingzhi in Chinese or Reishi in Japanese) has been widely used and investigated for its potential therapeutic benefits (Ling and Zhang, 2004). The therapeutic benefits of mushrooms have been claimed to be due to the polysaccharide β-glucans. In several countries, marketing claims for reduction of cholesterol for cereal fibers, particularly those containing β-glucans from oat and barley have been recognized by governmental agencies (FDA, 1997). Given the potential health benefits of β-glucans, Super Beta Glucan Inc intends to market

[3]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.

selected conventional food products containing mushroom β-glucans extracted from mushroom (*G. lucidum*).

1.2. Chemistry and Biological Activity

β-Glucans, comprised of D-glucose polymers, is primarily produced in fungi, yeast and plants (grains) but not in mammalian cells (Driscoll et al., 2009). It is one of the main components of the cell wall in most fungi and plants. As a carbohydrate polymer, β-Glucans exists as a chain of glucose molecules linked together by β-glycosidic bonds (Figure 1). 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. The primary chemical structure of β-glucans polymers differs from source to source, but 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., 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).

Recently, 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. 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}CNMRand FTIR spectroscopy. 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.

1.3. Description, Specifications and Manufacturing Process

Mushroom β-glucans, derived from *G. lucidum,* is a fine light beige color powder with a mild odor and bland taste. Specifications of mushroom β-glucans manufactured by Super Beta Glucan Inc are summarized in Table 1 (Section II- H). Analytical results of five independently produced, representative batches demonstrate that the mushroom β-glucans consistently meets these specifications (Appendix I). Extensive analysis for potential external contaminants of mushroom β-glucans such as heavy metals [cadmium, arsenic, mercury, lead], and microbes (including Aerobic Plate Count, Yeast and Mold, Total Coliforms, *E. coli, Salmonella* sp.) that are generally associated with food products, from five batches revealed either the absence of these contaminants or their presence at very low levels that are considered as safe. Mushroom β-glucans from *G. lucidum* is manufactured according to current Good Manufacturing Practices (cGMP).

1.4. Intake of β-Glucan from Food

β-Glucans present in cereal bran such as oat and barley are commonly produced as agricultural by-products. Given their health benefits, the FDA (1997) permitted a health claim on a food 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). Even though β-glucans derived from different sources differ slightly in structure, they are expected to behave in the same manner in biochemical systems. This is discussed in more detail below.

No specific numbers are available on the pre-existing exposure to mushroom β-glucans. However, per 21 CFR 101.81, 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). Therefore, it can be estimated that a diet aiming to reduce the risk of coronary heart disease provides at least 3 g β-glucan/day. Given that 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. The FDA has approved the use of bakers yeast glycan (sometime 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 (21 CFR 172.898). 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.

Ko and Lin (2004) estimated the levels of β-1,3-glucans in 17 items from six food categories including legumes, cereals, tubers, vegetables, fruits, and mushrooms. 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. It has been reported that Snow mushroom (dry weight) had the highest β-1,3-glucans content (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).

1.5. Intended Use Levels and Food Categories

Super Beta Glucan Inc intends to use mushroom β-glucans obtained from *G. lucidum* 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, soft candy, and soup and soup mixes at a level of 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 mushroom β-glucans are intended for foods without a standard of identity. The intended food use categories and use levels of mushroom β-glucans are summarized in Table 2. 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 by PeopleAndTechnologies LLC for the black yeast β-glucans in GRN 309.

1.5.1. Estimated Daily Intake from the Intended Uses

The intended use of mushroom β-glucans (50% concentration) in the same foods and at the same levels as those in GRN 309 (FDA, 2010) 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 309 notice, it was estimated that the mean and 90th percentile all users intakes for the total population would be 338.7 and 678.4 mg/person/day or 7.3 and 16.4 mg/kg bw/day, respectively. The dietary analysis described in GRN 309 was not questioned by FDA in the response letter of June 14, 2010. The relevant dietary analysis data from GRN 309 is presented in Table 2. The analysis reported in GRN 309 was performed using the United States Department of Agriculture (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996) survey. The data presented in Table 2 shows that, except for infants and children, the daily intake of β-glucans expressed on body weight basis is below 17 mg/kg bw/day. While infants were included in the daily estimated intake estimates, mushroom β-glucans is not intended for inclusion in infant formula or baby foods.

Table 2. Intake of Mushroom β-Glucans (mg/person/day or mg/kg bw/day) based on USDA data[a]

Population Group	Age Group (Years)	% Users	Actual # of Users	All-Person Consumption (mg)		All-Users Consumption (mg)	
				Mean	90th Percentile	Mean	90th Percentile
Infants[b]	0 to 2	71.6	2,526	224.1[c] (17.8)[d]	521.6 (41.0)	287.3 (22.9)	568.4 (44.2)
Children	3 to 11	98.4	6,205	389.8 (16.1)	712.6 (31.1)	397.7 (16.5)	713.9 (31.2)
Female Teenagers	12 to 19	91.5	642	330.9 (6.1)	702.6 (14.3)	361.8 (6.7)	726.6 (14.5)
Male Teenagers	12 to 19	91.7	638	436.2 (72.)	930.3 (15.1)	476.4 (7.8)	972.9 (16.3)
Female Adults	20 and Up	85.0	3,887	238.7 (3.7)	526.9 (8.4)	278.2 (4.3)	551.9 (8.9)
Male Adults	20 and Up	81.6	3,876	290.6 (3.6)	678.8 (8.6)	355.1 (4.4)	743.7 (9.5)
Total Population	**All Ages**	**86.4**	**17,812**	**292.7 (6.3)**	**644.7 (15.4)**	**338.7 (7.3)**	**678.4 (16.9)**

[a]Adapted from GRN 309. [b]While infants are included in these estimates, mushroom β-glucans is not intended for inclusion in infant formula or baby foods. [c]Values in mg/person/day. [d]Values in parenthesis represent intake as mg/kg bw/day

2. SAFETY OF β-GLUCAN

2.1. Common Knowledge of Safe Use

Although Super Beta Glucan Inc is basing their conclusion that mushroom-derived β-glucans is GRAS based on scientific procedures, there is a long history and wealth of evidence and information related to the use of these substances in food. This information is summarized below.

There is common knowledge of a long history of human consumption of mushrooms as a food. As a popular edible mushroom, *G. lucidum* (Reishi), the source organism of β-glucans in this notice, has been widely consumed in Asian countries for the general promotion of health and longevity (Sliva, 2003). *G. lucidum* is used in dietary preparations, particularly to make teas or soups (Ghorai et al., 2009). *G. lucidum* are used as food supplements and several benefits of this mushroom have been reported (Ogbe et al., 2010). The usefulness of the edible mushroom *G. lucidum* as a preventive/therapeutic supplement has been suggested in The Chinese Medicine (TMC) for thousands of years and has recently gained interest in Western countries. As indicated earlier, several beneficial effects of *G. lucidum* have been claimed. The majority of these claims have not been studied in controlled clinical trials, although there has been an abundance of clinical use, as well as *in vitro* and animal data supporting its safety. Safety is further supported by common use of *G. lucidum* (Reishi) as an edible mushroom and broad exposure to consumers with no adverse effects reported, indicating that the constituents of this mushroom are well tolerated. Thus, the available evidence provides support for common knowledge of safe human exposure to the β-glucans found in *G. lucidum* mushroom.

2.2. GRAS Notices on β-Glucan

Recently, the FDA received two separate GRAS notifications on β-glucans derived from yeast [GRN 309 (FDA, 2010 and GRN 239 (FDA, 2008)]. In these submissions, extensive data from the published literature on β-glucans were presented by these 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 determination 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 determination in the present GRAS assessment of 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 3.

2.2.1. GRN 309- Black Yeast β-Glucans

The subject of the most recent GRAS notification is β-glucans derived from *Aureobasidium pullulans* (FDA, 2010). 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 notifier stated that the safety of β-glucans derived from *A. pullulans* is supported by published acute toxicity, subchronic oral toxicity, and genotoxicity studies conducted in mice. These acute toxicity 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.

Table 3. Comparison of mushroom β-glucans with yeast β-glucans from GRAS notices

Specifications	Black yeast (GRN 309)*	Baker's yeast (GRN 239)*	Mushroom (Present GRAS)
Description	Reddish yellow powder	Fine beige/tan powder	Fine light beige powder
Source organism	*A.. mllulans*	*S. cerevisiae*	*G. lucidum*
Total Carbohydrate (%)	At least 80%	At least 80%	At least 95%
B-Glucan	At least 40%	At least 70%	At least 50%
Protein (%)	<10	<10	<1.0
Fat (%)	<5	<20	<1.0
Ash (%)	<10	<5	<3.0
Moisture	<6	<8	<2.0
Lead	ND	<0.5	<0.5
Mercury	ND	<0.05	<0.05
Arsenic	ND	<0.1	<0.5
Selenium	ND	NA	NA
Cadmium	ND	<1	<1.0
Zinc	NA	NA	NA
Aerobic plate count	<3000	<20000	<10000
Yeast and molds	≤20	≤25	≤15
Coliform	<10	<10	Negative
Escherichia coli	Negative	Negative	Negative
Salmonella sp.	Negative	Negative	Negative
*Adapted from GRN 309			

2.2.2. GRN 239- Bakers Yeast β-Glucans

In this submission on bakers yeast β-glucans (FDA, 2008), the notifier 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 toxicity. 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 bakers yeast β-glucans. The notifier summarized the results of the human clinical studies, and concluded that levels up to 500 mg/person/day of bakers 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 bakers yeast β-glucans is GRAS under the intended conditions of use.

2.3. EFSA Safety Assessment of Yeast β-Glucans

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 which resulted in a daily intake at dose levels up to 600 mg/day β-glucans (EFSA, 2011). The 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 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 bakers yeast. The Panel noted that β-glucans from other sources have already been evaluated for safety by EFSA. 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 at the proposed conditions of use.

2.4. Biological Data

The safety determination of mushroom β-glucans is based on the totality of available evidence, particularly from experimental studies of mushroom β-glucans and those conducted on β-glucans from other sources in animals, as well as from human trials. The experimental studies of mushroom β-glucans were designed to evaluate its safety as a dietary supplement. The relevant biological and toxicological studies on mushroom β-glucans and other similar dietary fibers are included in the following section to provide support for the conclusions reached in this determination. Subsequent to the recent FDA evaluations of the GRAS notices on yeast β-glucans 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. Recently, EFSA (EFSA, 2011) also evaluated the safety of yeast β-glucan and concluded that consumption at levels up to 600 mg per day is safe. 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.

2.4.1. Absorption Metabolism

The metabolic fate of β-glucans resembles that of other prebiotics and other non-digestible/fermentable carbohydrates such as inulin, fructooligosaccharides (FOS), galactooligosaccharides, resistant starch, polydextrose, cyclodextrins, and lactulose, which all remain largely 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). Therefore, absorption by the intestinal epithelium and significant systemic exposure to particulate mushroom β-glucans is unlikely. Similar to other non-digestible polysaccharides, mushroom β-glucans is resistant to digestion in the upper part of the gastrointestinal tract down to the distal ileum. However, in the colon, it 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. Therefore, the metabolism of mushroom β-glucans does not raise safety concerns and no systemic toxicity is expected following its ingestion.

In a human study, tolerability and absorption of a soluble bakers yeast ß-1,3/ ß-1,6-D-glucans preparation was investigated (Lehne et al., 2005). In this open label dose-escalation study, 8 healthy volunteers (6/group) were randomized to receive 100, 200, or 400 mg SBG (bakers yeast ß-1,3/ ß-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 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 no systemic absorption of β-1,3-glucan occurred following short-term oral administration.

In a recent 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 *Bifidobacterium longum* and *Bifidobacterium adolescentis* (both in human adults). 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 than 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.

2.5. Toxicological Information

2.5.1. Specific Toxicity Studies

In a series of toxicity studies, Chen et al. (2011) investigated the subchronic toxicity and potential genotoxic effects of mushroom β-glucans (the subject of this GRAS determination). For both the subchronic toxicity study and the mutagenicity studies, the mushroom β-glucans used was produced by Super Beta Glucan Inc. These studies are described in the following sections.

2.5.2. Subchronic Study of Mushroom β-Glucan

Chen et al. (2011) evaluated the safety of mushroom β-glucans extracted from *G. lucidum* at doses up to 2000 mg/kg bw/day in rats. In this study, 96 Sprague-Dawley rats (12/sex/group; 6 week old) were administered (gavage) once daily with mushroom β-glucans dissolved in sterile water at dose levels of 0 (control), 500 (low dose), 1000 (mid dose), or 2000 (high dose) mg/kg bw for 90 consecutive days. 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. Throughout the study period the animals were observed for clinical signs of toxicity and mortality/morbidity (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. Hematology, serum chemistry, and urinalysis measurements were performed for surviving animals after 13 weeks of treatment (at termination). At termination, necropsy was performed and tissue weights were recorded. 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 mushroom β-glucans. One female in the low-dose group was euthanized on day 82 due to accidental trauma unrelated to the mushroom β-glucans treatment. There were no treatment-related clinical signs of toxicity noted. Ophthalmological examinations did not reveal any abnormalities. The body weights of the treatment groups were comparable to the control group animals throughout the treatment period and no statistically significant ($p < 0.05$) difference was noted, with a few exceptions. In females, the mean body weight gain was statistically lower in the low-dose group at the week 9 measurement and in the high-dose group at week 13. These changes were not considered to be treatment-related were within the normal laboratory control range. The mean feed consumption (g/rat/day) was comparable in all the dose groups of both the sexes. The urinalysis parameters such as volume, pH, specific gravity, and urobilinogen did not show any significant difference from the respective control groups (Chen et al., 2011).

There were no treatment-related biologically significant adverse effects of mushroom β-glucans on hematology parameters in male and female rats. However, some statistically significant differences were noted. In female rats, a statistically significant increase in MCV and MCH levels was noted in the high dose treatment group (2000 mg/kg/day). Similarly, a significant increase in hematocrit levels was noted in the low dose treated group (500 mg/kg/day). These changes were not observed in both sexes, lacked correlating changes in other red blood cell parameters, were of small magnitude, and/or were not noted in a dose-related manner; hence, they were considered as incidental changes/biological variations and not treatment-related adverse effects (Chen et al., 2011).

There were no treatment-related biologically significant adverse effects of the mushroom β-glucans on serum chemistry parameters. However, some statistically significant differences were noted. In male rats, a significant increase in serum sodium level was observed in the low (500 mg/kg/day) and the high (2000 mg/kg/day) dose groups as compared to the control group. Similarly in female rats, compared to the control group, a significant increase in sodium levels was noted in mid- (1000 mg/kg/day) and high (2000 mg/kg/day) dose groups. These changes noted in serum sodium levels were well within the normal laboratory control range; hence, they were considered as incidental changes/biological variations and not as treatment-related effects (Chen et al., 2011).

No toxicologically significant changes in organ weights were observed when compared to the control group. However, in males, testes weight was statistically lower in the low-dose group, and heart weight was lower in the high-dose group. These changes were of small magnitude, and/or were not noted in a dose-related manner hence they were not considered as test article-related. There were no treatment-related macroscopic findings in any of the groups. All macroscopic changes noted were considered to be spontaneous and/or incidental in nature,

without a dose-related trend, and without histopathological abnormality. There was no mushroom β-glucans treatment-related histopathological findings in any organs. All the histopathological changes observed were considered as spontaneous and incidental to rats of this particular strain and age. Based on the results of this study, the no-observed effect level (NOAEL) of the mushroom β-glucans is determined as 2000 mg/kg bw/day, the highest dose tested (Chen et al., 2011).

2.5.3. Genotoxicity Studies

2.5.3.1. Ames Assay

The mutagenic potential of mushroom β-glucans was assessed using the Bacterial Reverse Mutation Assay, also known as the Ames test (Chen et al., 2011). *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. The following concentrations of β-glucans were tested: 0.313, 0.625, 1.25, 2.5, and 5 mg/plate. The chemicals used as positive control for assays without or with metabolic activation included mitomycin C and benzo(a)pyrene. 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.

2.5.3.2. Chromosome aberrations

The genotoxic potential of mushroom β-glucans to induce chromosome aberrations in Chinese hamster ovary cells (CHO-K1) was evaluated (Chen et al., 2011). The test was performed in accordance with the OECD guideline for this assay. 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 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 0.313 mg/ml were 83.53 ± 2.64%, 85.38 ± 3.60%, 91.96 ± 0.77%, 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).

2.5.3.3. *In vivo* Micronucleus Assay

The mammalian peripheral blood micronucleus test was conducted in accordance with the OECD guideline for such a study (Chen et al., 2011). 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) was chosen as the positive control and administered intraperitoneally. 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 polychromatic erythrocytes (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.

2.6. Other Toxicity Studies

Babicek et al. (2007) investigated the acute and subchronic toxicity of a yeast-derived β-glucans ingredient (WGP 3-6) in rats. In the acute study in F344 rats, the LD_{50} value of WGP 3-6 was found to be greater than 2000 mg/kg. 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 WGP 3-6 at doses of 0, 2, 33.3, or 100 mg/kg bw/day for 90 days. The standard full toxicological monitoring and endpoints were investigated. Administration of WGP 3-6 did not significantly affect animal weights or feed consumption. No mortality, clinical pathology, functional/behavioral, microscopic, or gross observations indicative of toxicity were observed. 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 52 week oral toxicity study in rats, Feletti et al. (1992) investigated the effects of β-glucans extracted from *Candida albicans* ATCC 20955. 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 alteration of the weight of the main organs was observed. Hematology, blood chemistry, urinalysis and autopsy findings were within normal ranges. In the 200 mg/kg group, soft stools or diarrhea and cecal enlargement with variable hyperplasia of the colon mucosa were observed. As sated by the authors, 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 an 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.

In a 28-day oral feeding study of barley β-glucans, Jonker et al. (2010) investigated the safety of a high purity (>75%) barley β-glucans. In this study, barley β-glucan was fed to Wistar rats (5/sex/group) at dietary levels of 0 (control), 1, 5 and 10% (0, 500, 2500, 5000 mg/kg bw/day) for 28 days. Clinical and neurobehavioral observations, growth, feed and water consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights, necropsy and histopathological examination revealed no adverse effects of barley β-glucans. Male rats in the high-dose group exhibited lower plasma cholesterol and phospholipid levels and a higher plasma urea level. These changes were considered of no toxicological significance. Lowering of cholesterol is a known effect of β-glucan in animals and is an intended effect in humans. The lower plasma lipid levels of high-dose males were within the normal range for rats of this strain and age. Additionally, the changes in plasma lipids were not accompanied by any relevant changes in other end-points including histopathology, the lower plasma lipid levels in high-dose males were considered not to be toxicologically relevant. 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 5.8 and 5.9 g barley β-glucans/kg bw/day in male and female rats, respectively (NOAEL = ~5.85 g/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.

2.7. Potential Allergenicity

It is a popular belief that mushrooms are capable of eliciting allergic symptoms. However, 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 mould allergy (10-30% of an allergic population). The majority of the published studies on the allergenicity of *Ganoderma* species were related to the spores from these species. Among the different mushrooms, the genera that produce distinguishable basidiospores are Ganoderma, Boletus, Rhodophyllus, Thelephora, Russula and Lactarius. 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 bakers 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 moulds. Reaction in skin tests were frequent but less than those with moulds or common allergens. In another study, Herxheimer 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: a total of only 13 cases have been reported in Japan.

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, mushroom allergies are typically caused by airborne particles or skin contact. Allergy to *G. lucidum* spores has been reported, however the available information did not reveal any case of allergy occurring from consumption *G. lucidum* mushroom as a food. Few cases of allergy from consumption of other types of mushrooms such as Matsutake have been reported. A case with bronchial asthma with hypersensitivity reactions following Matsutake mushroom (*Tricholoma matsutake*) ingestion has been reported. The residual amount of protein in the mushroom β-glucan product the subject f present GRAS assessment is very low (<1%; Table 1). Additionally, as the ingredient will be labeled as mushroom β-glucan, it is anticipated that individuals with mushroom or β-glucan allergy or sensitivity will avoid ingestion of this product.

2.8. Human Studies

In a recent randomized, double-blind, placebo-controlled, parallel group interventional trial, 50 generally healthy (at risk for type 2 diabetes) subjects (age 30-70 years; BMI 25-40) were administered beverages containing placebo (control), lower dose (3 g/day), or a higher dose (6 g/day) of reduced viscosity barley β-glucans extract for 12 weeks (Bays et al., 2011). 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 results of this study suggested 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 a double-blind, placebo-controlled clinical trial described in GRN 239 (FDA, 2008), healthy volunteers (n=20) consumed a single capsule providing 250 mg WGP 3-6/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 patient. 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 the second placebo-controlled, double-blind study also reported in GRN 239 (FDA, 2008), 62 subjects with the 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.

Nicolosi et al. (1999) evaluated the effect on serum lipids of a yeast-derived β-glucans fiber in 15 free-living, obese, hypercholesterolemic men. In this study, after a 3-week period in which subjects ate their usual diet, 15 g β-glucans fiber /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. 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.

Lehne et al. (2005) investigated the safety of a soluble branched β-l,3-glucan (SBG) derived from *S. cerevisiae* in an open dose-escalation study in 18 healthy volunteers. In this study, volunteers (6/group) were randomized to receive 100, 200, or 400 mg SBG/person for a period of 4 consecutive days. A series of safety related parameters including hematological data, clinical chemistry data, 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.

3. SUMMARY

Super Beta Glucan Inc has developed a process to manufacture a standardized β-glucans product from the mushroom species *Ganoderma lucidum*. Mushroom β-glucans is a fine light beige powder soluble in water with a characteristic mild odor and bland taste. The compositional analysis of mushroom β-glucans demonstrated that it primarily contains carbohydrates (>95%) of which β-glucans constitutes >50%. Super Beta Glucan Inc intends to use β-glucans at concentrations up to 150 mg/serving (reference amounts customarily consumed, 21 CFR 101.12) 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, soft candy, and soup and soup mixes. The intended use of mushroom β-glucans in the above mentioned food categories will result in a mean and 90th percentile estimated daily intake of 338.7 and 678.4 mg/person/day or 7.3 and 16.4 mg/kg body weight/day, respectively. The intended use of mushroom β-glucans is in the same food products and at the levels mentioned in the GRN 309.

The source mushroom from which β-glucans is derived is an edible species which has been consumed for centuries. Additionally, β-glucans is commonly found in the bran of cereal grains, the cell wall of yeast, certain fungi, seaweed, mushrooms 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 β-glucans that was the subject of two GRAS notifications (GRN 309; GRN 239). All this information suggests that there is a common knowledge of safe consumption of β-glucans from different food sources or products.

From a chemical perspective, 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. 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 of mushroom β-glucans was investigated. In the subchronic toxicity study, administration (gavage) of mushroom β-glucans 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. No treatment-related macroscopic and microscopic abnormalities were noted at the end of treatment period. The results of mutagenicity studies including the Ames assay, *in vitro* chromosomal aberration and *in vivo* micronucleus assay did not reveal any genotoxicity of mushroom β-glucans. Based on the

subchronic study, the no-observed-adverse-effect level (NOAEL) for mushroom β-glucans was determined to be 2000 mg/kg bw/day, the highest dose tested. The estimated daily intake of mushroom β-glucans from its intended food uses is over 120-fold lower compared to the NOAEL determined from the subchronic toxicity study.

In addition to the specific studies of mushroom β-glucans, other investigations that included 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 mushroom β-glucans is supported by the compositional similarity of the ingredient to yeast β-glucans and glycan that have been reviewed by the FDA as part of GRAS Notifications. Moreover, significant levels of 1,3- β-glucans also occur naturally in a number of foods, including edible mushrooms resulting in current exposure.

In summary, on the basis of scientific procedures[4] and history of exposure from natural sources, the consumption of mushroom β-glucans derived from *G. lucidum* (Reishi) as an added food ingredient is considered safe at the 90th percentile estimated daily intake of 678.4 mg/person/day (16.4 mg/kg bw/day). The intended uses are compatible with current regulations, *i.e.,* 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, soft candy, and soup and soup mixes at use levels of 150 mg 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).

[4] 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.

4. 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 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, soft candy, and soup and soup mixes) at levels up to 150 mg 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 678.4 mg/person/day (16.4 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 mushroom β-glucans, when used as described, is safe and GRAS based on scientific procedures.

Signatures

(b) (6)

17 November 2011

Stanley M. Tarka, Jr., Ph.D. — Date

(b) (6)

Nov. 18, 2011

Robert L. Martin, Ph.D. — Date

(b) (6)

November 21, 2011

Madhusudan G. Soni, Ph.D., F.A.C.N. — Date

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5. APPENDIX I

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

Specifications of mushroom β-glucan (Super Beta Glucan Inc, 2011)

Parameter	**Standard***	**Lot # 110106**	**Lot # 110208**	**Lot # 110307**	**Lot # 110522**	**Lot # 110616**
Physical specifications						
Appearance	Fine light beige powder	Complies	Complies	Complies	Complies	Complies
Odor	Mild	Mild	Mild	Mild	Mild	Mild
Taste	Bland	Bland	Bland	Bland	Bland	Bland
Chemical parameters						
Total Carbohydrate (%)	>95	96.9	97.0	96.3	96.5	96.7
B-Glucan (%)	Minimum 50	52.5	56.0	53.0	51.5	53.0
Fat (%)	<1.0	<0.4	<0.4	<0.4	<0.4	<0.4
Saturated Fat (%)	<0.5	<0.1	<0.1	<0.1	<0.1	<0.1
Trans Fat (%)	<0.5	<0.3	<0.3	<0.3	<0.3	<0.3
Protein (%)	<1.0	0.4	0.4	0.5	0.5	0.4
Moisture (%)	<2.0	0.9	0.8	0.7	0.8	0.8
Ash (%)	<3.0	1.8	1.8	2.4	2.2	2.0
Heavy metals						
Lead	<0.5 ppm	N.D.	N.D.	N.D.	N.D.	N.D.
Arsenic	<0.5 ppm	N.D.	N.D.	N.D.	N.D.	N.D.
Cadmium	<0.5 ppm	N.D.	N.D.	N.D.	N.D.	N.D.
Mercury	<0.05 ppm	N.D.	N.D.	N.D.	N.D.	N.D.
Microbiological assay						
Aerobic Plate Count (CFU/g)	<10,000	5.8×10^1	4.5×10^1	4.8×10^1	5.2×10^1	4.5×10^1
Yeast and Mold (CFU/g)	<15	10	11	10	9	11
Total Coliforms	Negative	Negative	Negative	Negative	Negative	Negative
E. coli	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella sp.	Negative	Negative	Negative	Negative	Negative	Negative
*Standard specifications for marketed product						

SUBMISSION END