SUPER BETA GLUCAN

December 2, 2022

Dr. Paulette Gaynor
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 Lion's Mane mushroom β-glucans as a Food Ingredient

Dear Dr. Gaynor:

In accordance with 21 CFR part 170, subpart E, Super Beta Glucan Inc., USA, hereby submits the enclosed notice of a claim that the food ingredient Lion's Mane mushroom β-glucans, derived from *Hericium erinaceus,* 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

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

LION'S MANE MUSHROOM β-GLUCAN

AS A FOOD INGREDIENT

Prepared for:
Super Beta Glucan
5 Holland, Unit 109
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USA

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November, 2022

EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF LION'S MANE MUSHROOM β-GLUCAN AS A FOOD INGREDIENT

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Part I- SIGNED STATEMENT AND CERTIFICATION

1.1. Basis of Conclusion

This GRAS conclusion for the use of Lion's Mane mushroom β-glucans, derived from *Hericium erinaceus,* as a food ingredient, has been reached in accordance with the requirements as defined in 21 CFR 170.220. The submission is prepared in accordance with 21 CFR Part 170 Subpart E.

Please note that, in 2012 and subsequently in 2021, Super Beta Glucan (SBG) Inc. submitted two GRAS notices (GRN 413 and GRN 995) to the FDA on β-glucans derived from *Ganoderma lucidum* and from *Antrodia cinnamomea* that received "FDA has no questions" letters[1,2]. As discussed below, the subject of this present GRAS notification is similar to GRN 413 and GRN 995, except that the β-glucan is derived from yet another species of mushroom, i.e., *Hericium erinaceus*.

1.2. Name and address of organization:

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

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

1.3. Name of substance:

The name of the substance of this GRAS assessment is Lion's Mane Beta Glucan, LMBG, and Lion's Mane Extract.

1.4. Intended conditions of use:

Lion's Mane mushroom β-glucans derived from *Hericium erinaceus* 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 Lion's Mane mushroom β-glucans *per* serving (reference amounts customarily consumed, 21 CFR 101.12). The intended use of Lion's Mane mushroom β-glucans is in the same food products and at the identical levels cited in the GRN 413[1] and GRN 995[2], β-glucans derived from *G. lucidum* mycelium and *A. cinnamomea*, respectively. The proposed food categories are also similar to other GRAS notifications on β-glucans, i.e., GRN 309[3] (β-glucan

[1] Accessible at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm299311.pdf
[2] Accessible at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=995
[3] Accessible at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm269476.pdf

derived from *Aureobasidium pullulans*) and are similar to GRN 239[4] (Baker's yeast β-glucan). The intended use of Lion's Mane mushroom β-glucans in the above-mentioned food categories is estimated to result for "users only" at the mean and 90th percentile intakes of 291.3 and 583.4 mg/person/day, respectively. Lion's Mane 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 Inc. (SBG) has concluded that Lion's Mane mushroom β-glucans is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on our conclusion that Lion's Mane 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 Lion's Mane 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 the FDA upon request by contacting Dr. Chen at the below address. The data and information will be made available to the 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).

Dr. Sherwin Chen
Vice-President
Super Beta Glucan
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Irvine, CA 92618

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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.

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

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 Lion's Mane mushroom β-glucan. SBG accepts responsibility for the GRAS conclusion that has been made for Lion's Mane 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 Lion's Mane 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, Lion's Mane mushroom β-glucans, derived from *Hericium erinaceus,* is a standardized powder. The preparation is a fine light beige powder with characteristic mild odor and bland taste. It is extracted from *H. erinaceus* culture. General descriptive characteristics of Lion's Mane mushroom β-glucans are summarized in Table 1. The active constituents of the extract are β-glucans.

H. erinaceus gets its common name "Lion's Mane" from its physical appearance, where the white fruiting bodies appear as a downward cascading mane-like (or scruff of a lion) structure (Figure 1) (Friedman, 2015; Grace and Mudge, 2015). These mushrooms have long, dangling spines that are usually greater than a centimeter in length. Unlike most mushroom species, which have spines that project from a branch, the spines of Lion's Mane project outward, giving it that unique look of a lion's mane. Lion's mane mushrooms grow on both living and dead broadleaf trees and are common in the late summer and fall months.

Lion's mane mushroom's synonyms include Monkey head Mushroom and Bear's Head Tooth. The fruiting body is called hóu tóu gū ("monkey head mushroom") in Chinese and yamabushitake ("mountain monk mushroom") in Japanese. The *Audubon Society Field Guide to North American Mushrooms* lists four species of *Hericium* mushrooms including *H. erinaceus* (Fr.) Pers.; the edibility of all four *Hericium* species is characterized as "edible and very good."

Table 1. General Descriptive Characteristics of Lion's Mane Mushroom β-Glucans

Parameter	Description (SBG, 2022)*
Source microorganism	*Hericium erinaceus*
Other names of source	Mountain-priest mushroom or Bearded tooth fungus
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 (2022)

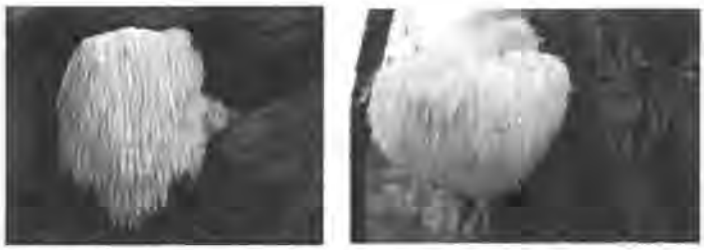

Figure 1. Photographs of Lion's Mane mushroom

2.2. Identification of Source Organism

The hierarchical classification of the source material, *Hericium erinaceus* is presented in Table 2. *Hericium* is a genus of edible mushrooms in the family Hericiaceae. As a commonly

consumed mushroom, *H. erinaceus* belongs to the tooth fungus group, occurs on hardwoods, and is native to North America, Europe and Asia. The *H. erinaceus* strain used in the production of Lion's Mane mushroom β-glucans was obtained from Bioresources Collection and Research Center (BCRC) in the Food Industry Research and Development Institute (Hsinchu, Taiwan). The strain is deposited with BCRC under the number BCRC 35669. Mycelium from the *H. erinaceus* strain was subcultured and maintained in YM agar medium. The whole genome of the production strain has not been sequenced. The production strain is not genetically engineered. Based on the available information, we conclude that *H. erinaceus* strain 35669 is non-pathogenic and non-toxigenic.

Table 2. Taxonomical Classification of *Hericium erinaceus*

Rank	Scientific Name and Common Name
Kingdom	Fungi
Division	Basidiomycota
Class	Agaricomycetes
Order	Russulales
Family	Hericiaceae
Genus	*Hericium*
Species	*Hericium erinaceus*
Strain	*Hericium erinaceus* BCRC 35669

2.3. Identity of Notified Substance

A. Chemical name:

Lion's Mane mushroom β-glucans. The product is composed mainly of β-glucans. β-D-Glucan; (1-3), (1-6)-β-D-Glucan; and/or β-Glucosylglucan.

B. Common/Trade Name:

The subject of this notification will be marketed as Lion's Mane Beta Glucan, Monkey head Mushroom Beta Glucan, LMBG, or Mountain Monk Mushroom Beta Glucan.

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 2.

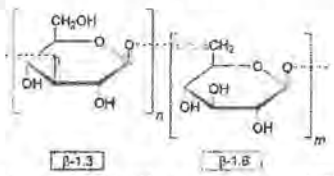

Figure 2. Orientation and Location of Different β-Glucan Linkages.

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 Lion's Mane 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 Lion's Mane 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%. Lion's Mane 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 Lion's Mane mushroom β-glucans is fully characterized (approximately 100%) for its constituents. All analytical methods used in establishing specification parameters are validated for their intended purpose. Erinacine A, a type of diterpenoids (triterpenes/triterpenoids), found in Lion's Mane mushroom is not present in the Lion's Mane β-glucan preparation, the subject of this present GRAS.

Table 3. Physical, Chemical and Microbiological Specifications of Lion's Mane 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
Protein (%)	<1.0	AOAC 922.15
Moisture (%)	<5.0	AOAC 925.45A/V.O
Ash (%)	<3.0	AOAC 900.02
Heavy metals		
Lead	<0.1 ppm	ICP-MS
Arsenic	<0.1 ppm	ICP-MS, FDA EAM 4.7
Cadmium	<0.1 ppm	ICP-MS
Mercury	<0.05 ppm	ICP-OES
Microbiological parameters		
Aerobic Plate Count (CFU/g)	<15,000	FDA BAM Chapter 3
Yeast and Mold (CFU/g)	<150 combined	FDA BAM Chapter 18; CMMEF APHA Chapter 21
Total Coliforms (MPN/G)	<10	AOAC 966.24
Staphylococcus aureus	Negative	FDA BAM CHP 12, AOAC 2003.08, 2003.11 and AOAC 2003.07
Escherichia coli	Negative	FDA BAM Chapter 4; AOAC991.14.
Salmonella sp.	Negative	FDA BAM Chapter 4

Negative = not defected within the limit of detection, which is <10 CFU/gram; CMMEF APHA = Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association

Among the carbohydrates (over 90%) present in Lion's Mane mushroom β-glucans, β-glucans comprises 65%, the remaining carbohydrates 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. 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 amounts 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 the presence of these other components is unlikely to cause any adverse effects at the intended use levels.

Extensive analyses of different batches for potential external contaminants of Lion's Mane 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 (LOD) 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, is unlikely to cause any adverse effects.

Based on the available published studies, Rodrigues et al. (2015) analyzed the composition and nutritive values of five mushroom species, including *H. erinaceus*. Protein, sugar, and fat contents were in the ranges of 16.22-26.6, 52.7-64.9, and 2.3-3.5 g/100 g dry mushrooms, respectively. In another study, Cohen et al. (2014) quantitatively analyzed Lion's Maine mushrooms dried at 50°C and then ground into a powder. The dried powder of *H. erinaceus* fruiting body contained 20.8% protein, 61.1% total carbohydrate, 5.1% fat, 6.8% ash, 6.2% water, and 374 kcal/100 g energy, whereas the mycelia biomass contained 42.5% protein, 42.9% total carbohydrate 6.3% fat, 4.4% ash, 3.9% water, and 398 kcal/100 energy.

2.4. Manufacturing Process

The production process for Lion's Mane mushroom β-glucans is illustrated in Figure 3. Lion's Mane 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. The mycelia of *Hericium erinaceus* were introduced into the sterile medium (sterilization performed with autoclaving) utilizing an aseptic technique, 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 *H. erinaceus* mushroom culture. The production strain is cultivated in an enclosed, sterile environment to ensure the purity and stability of the production strain.

Subsequently, Lion's Mane mushroom β-glucans in mycelium was extracted using a high-speed homogenizer (12,500 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 Lion's Mane mushroom β-glucans were then pooled, dried and grounded into powder form. All starting materials used in the manufacture of β-glucans are food grade and all processing aids and food contact materials are used in accordance with U.S. regulations. None of the raw materials used in the cultivation media are major allergens or derived from major allergens.

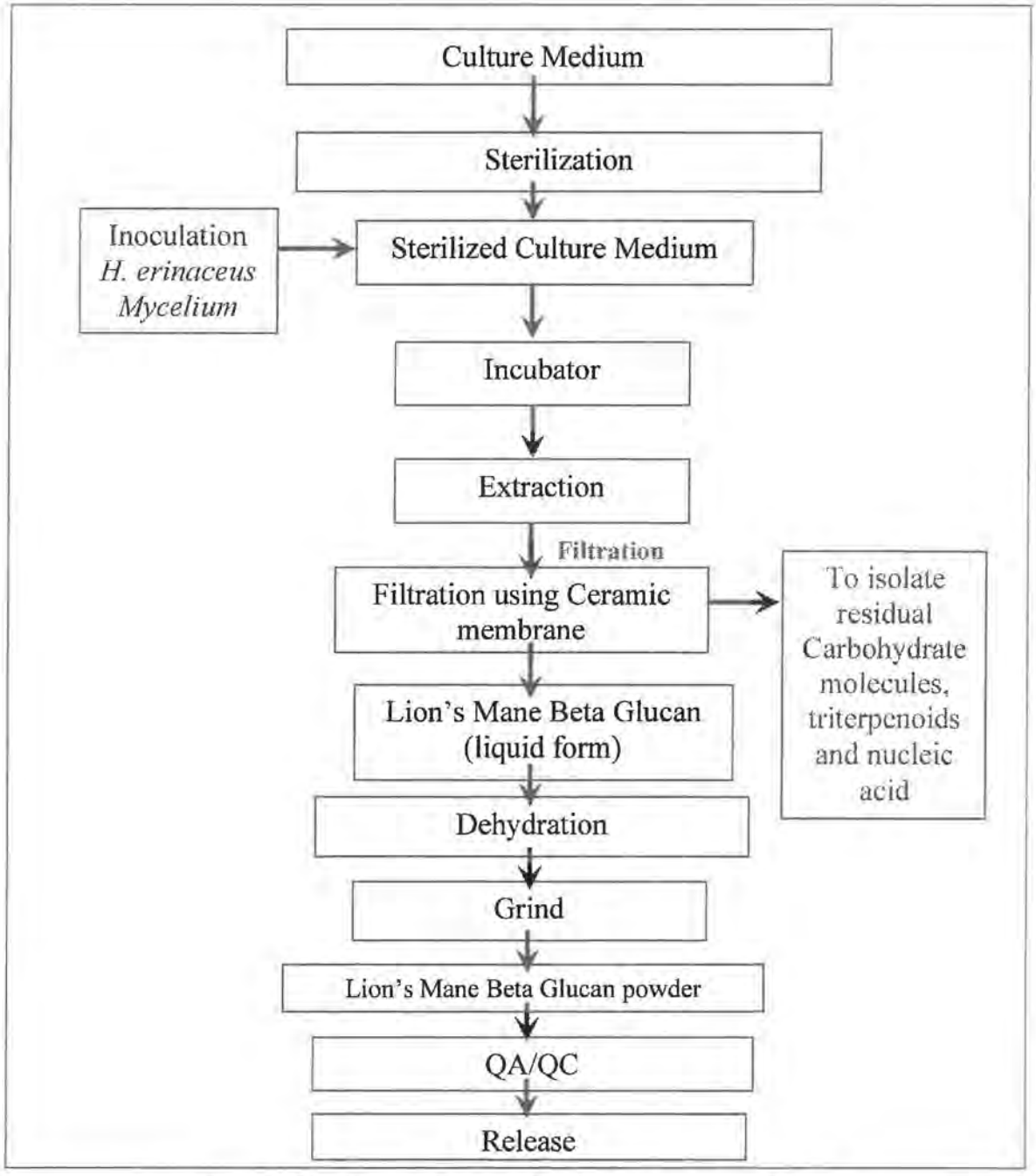

Figure 3. Manufacturing Process of Lion's Mane mushroom β-glucans

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

2.4. Chemistry and Biological Activity

Chemically, β-glucans are defined as linear molecules of β-1,3-and β-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-glucan 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, they 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.

Depending on the source, the biological and physiochemical properties of β-glucans differ. 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 down 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).

In a review article, Friedman (2015) extensively summarized the chemistry, nutrition and health effects of Lion's Mane mushroom fruiting body mycelia and other bioactive compounds. Polysaccharides from *H. erinaceus* have been of particular interest because of the bioactivities that have been attributed to them. Given this, a number of studies have appeared on polysaccharides isolation and analysis. Wu et al. (2015), using several chemical analysis techniques to identify polysaccharides isolated from *H. erinaceus* fruiting bodies from different parts of China, reported similarity in their profiles, including molecular weights, the composition of monosaccharides, and the glycosidic linkages in the polysaccharides, suggesting that the described experimental approach could be used for quality control of polysaccharides in edible and medicinal mushrooms as well as in commercial mushroom products. As described by Friedman (2015), in several other studies identification and characterization of *H. erinaceus,* polysaccharides (β-D-glucans) has been extensively investigated.

Part III- DIETARY EXPOSURE

3.1. Proposed Use Levels and Food Categories

Lion's Mane mushroom β-glucans derived from *Hericium erinaceus* is proposed 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, and soft candy at levels up to 150 mg mushroom β-glucans *per* serving (reference amounts customarily consumed, 21 CFR 101.12). The proposed food categories and use levels expressed as mg/serving and on a percentage basis are summarized in Table 4. Although some foods with standards of identity are included in the list of foods, the uses of Lion's Mane mushroom β-glucans are intended for foods without a standard of identity.

Lion's Mane mushroom β-glucans 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 *Antrodia cinnamomea* (GRN 995) by Super Beta Glucan (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 Lion's Mane mushroom β-glucans.

Table 4. 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

3.2. Estimated Daily Intake

The intended use of Lion's Mane mushroom β-glucans in the same foods and at the same levels as those in GRN 995 (FDA, 2022; SBG, 2020) is not expected to 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 995 and GRN 413 notices, 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 995 and GRN 413, the dietary analysis described in GRN 309 was utilized. The intake analysis described in GRN

309, in GRN 413 and in GRN 995 was not questioned by the FDA in the response letters of June 14, 2010, August 10, 2012, and June 14, 2022, respectively.

As regards the resulting intake of β-glucans from current and previous GRAS notices, the comparison of yeast derived (GRN 309 and GRN 239) and mushroom derived (GRN 413 and GRN 995) β-glucans shows that the β-glucans component levels in these products range from 40% to 70%. The dietary exposure for the present GRAS as well as for GRN 995 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 identical (65%) to that of GRN 995 and is lower than GRN 239 that reported the β-glucans component content of 70%. As the subject of this present GRAS and the recent GRAS notice (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.

In summary, the proposed use of Lion's Mane 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. 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. The proposed use of β-glucans from Lion's Mane mushroom β-glucan is substitutional to that of the current uses described in different GRAS notices and it will not increase the current dietary exposure to β-glucan.

3.3. Exposure to β-Glucans from Food

The available published 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). Chemical structure of β-glucans derived from different food sources are known to differ slightly. However, biologically these molecules are expected to behave in the same manner.

The FDA approved the health claim on the association of soluble fiber from rolled oats and reduced risk of heart disease in 1997 (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 baker's 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.

Ko and Lin (2004) quantitatively analyzed the levels of β-glucans in six food categories including legumes, cereals, tubers, vegetables, fruits, and mushrooms, and from 17 total items. The findings from this study 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. *Hericium erinaceus*, the source material of Lion's Mane mushroom β-glucans, has attracted attention around the world as an edible and therapeutic mushroom.

In summary, the available information suggest that humans are regularly exposed to β-glucans from the 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. As described in Section VI. Narrative, the source material of Lion's Mane mushroom β-glucans, *Hericium erinaceus* is an edible mushroom.

3.4. Cumulative Dietary Exposure to β-Glucans from all Dietary Sources

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 different soluble dietary fibers, β-glucan is the most frequently consumed soluble fiber. 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.

Depending on country-specific guidelines, recommendations for fiber intakes range from 25-38 g/day. 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).

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 above, the 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. Fifty gram of whole grain oat or barley provides 2.5 and 3.5 g β-glucan, respectively. The available information suggest 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, there is a need to further increase the intake of β-glucan. As β-glucan from Lion's Mane mushroom (subject of this GRAS) manufactured by Super Beta Glucan will serve as an alternative source of β-glucan to existing GRAS sources of β-glucan described in GRN 239, GRN 309, GRN 413 and GRN 995, 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 Lion's Mane 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 this GRAS 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 information also suggests 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. Secondly, 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.

Part IV- SELF LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with the intended use of Lion's Mane mushroom β-glucans. It should be noted that Super Beta Glucan does not intend to add Lion's Mane 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 Lion's Mane 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 - Lion's Mane 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, the effects of Lion's Mane mushroom β-glucans was investigated in animals and *in vitro* experimental systems. The findings from all these investigations are published in the journal *Current Reasearch in Toxicology* (Chen et al., 2022). In the following section, relevant toxicological and other studies of Lion's Mane mushroom β-glucans and similar substances are summarized in the order of their importance and in support of the conclusions drawn in this GRAS 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 Lion's Mane mushroom and similar preparations.

The safety determination of Lion's Mane mushroom β-glucans is based on the totality of the available evidence, particularly from experimental studies of Lion's Mane mushroom β-glucans and those conducted on β-glucans from other sources in animals, as well as from human trials. The experimental studies of Lion's Mane mushroom β-glucans were designed to evaluate its safety as a dietary ingredient. Subsequent to the recent FDA evaluations of the GRAS notices on yeast β-glucans and mushroom 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. 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 Lion's Mane Mushroom β-Glucans

6.1.1.1. Subchronic Toxicity Study of Lion's Mane Mushroom β-Glucans

In a recent study, Chen et al. (2022) investigated adverse effects of a standardized Lion's Mane mushroom β-glucan preparation in a repeat-dose subchronic toxicity study in 96, CD® (SD) IGS strain rats. The study was conducted in accordance 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 (6 weeks old), divided into four groups (12/sex/group), were administered (gavage) once daily with Lion's Mane 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.

The animals were observed throughout the study period 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 were performed. 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 Lion's Mane mushroom β-glucan preparation (Chen et al., 2022). Some clinical signs were observed due to housing behavior (wounds: male - 4/12 in Group II and; females - 3/12 in Group II and 8/12 in Group IV) or individual animal difference (hair loss: male - 4/12 and 1/12 in Group II and III, respectively; female 3/12 and 8/12 in Group I and III, respectively). The severities of these clinical signs were slight (wounds and hair loss). The ophthalmological examinations did not reveal any abnormalities in any group before dosing and necropsy. The mean body weights and body weight gains of treatment groups were comparable to control group animals throughout the treatment period and no statistically significant difference was noted. Thus, no treatment-related biologically significant effects of the β-glucan extract preparation were noted on body weight or body weight gain at dose levels up to 2000 mg/kg bw/day. In addition, 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 9, male rats in the high-dose group showed statistically significant reduction in food intake. At week 7, all female rats in treatment group, showed significantly higher feed intake. These significant changes were not considered as related to the administration of *H. erinaceus* β-glucan extract preparations (Chen et al., 2022).

The results of urinalysis parameters in male and female rats following administration of *H. erinaceus* β-glucan extract preparation at dose levels of 0, 500, 1000, and 2000 mg/kg bw/day for 90 consecutive days did not reveal any significant differences or physiological abnormalities in males and females in the control and treatment groups. The urine analysis parameters such as volume, pH, specific gravity, and urobilinogen did not show any significant difference from the respective control groups (Chen et al., 2022).

Administration of *H. erinaceus* β-glucan extract preparation did not result in any biologically significant adverse effects in hematology parameters in male and female rats. However, some statistically significant differences were noted when the control and treatment groups were compared. In male rats, a statistically significant increase in blood levels of eosinophil were noted in the mid-dose treated group (1000 mg/kg bw/day; Group III). This significant change following administration of the β-glucan extract preparation was not observed in both sexes, lacked correlating changes in other red cell parameters, was of small magnitude, and/or was not noted in a dose-related manner. Hence, this change was considered as incidental variation and not a treatment-related adverse effect. There were no other statistically significant differences when the respective control and treatment groups were compared (Chen et al., 2022).

There were no treatment-related biologically significant adverse effects of the *H. erinaceus* β-glucan extract preparation on serum chemistry parameters in male and female rats. However, some statistically significant differences were noted when the respective control and treatment groups were compared. In male rats, as compared to the control group, a significant increase in serum sodium levels in mid- (Group III; 1000 mg/kg bw/day) and the high-dose (Group IV; 2000 mg/kg bw/day) groups; serum albumin in the high-dose group (Group IV; 2000 mg/kg bw/day); and serum chloride in mid-dose (Group III; 1000 mg/kg bw/day) group was noted. Similarly, in female rats, compared to the control group, a statistically significant decrease in serum levels of total protein in the mid-dose group (Group III; 1000 mg/kg bw/day); increase

in alkaline phosphatase in the low-dose (Group II; 500 mg/kg bw/day) and high-dose group (Group IV; 2000 mg/kg bw/day); and increase in serum sodium levels in the low- and mid-dose groups (Group II and III; 500 and 1000 mg/kg bw/day) was noted. There were no other statistically significant differences when the respective control and/or treatment groups were compared. These changes in serum sodium, alkaline phosphatase and proteins were well within the normal laboratory control range and, hence, was considered as incidental changes or biological variations and not as *H. erinaceus* β-glucan treatment-related effects (Chen et al., 2022).

The changes in organ weights following treatment with Lion's Mane β-glucan extract preparation administration did not reveal any 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 extract preparation to rats. At terminal euthanasia, only one female from low-dose group showed focal mass in subcutaneous tissue (mammary gland). According to the severity and incidence based on histopathological evaluation of this lesion (fibroadenoma), the finding was considered as a spontaneous abnormality and not related to treatment. 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. It was inferred that there were no pathological changes in the organs that could be attributed to Lion's Mane mushroom β-glucan extract preparation treatment. All findings observed were consistent with normal background lesions in clinically normal rats of the age and strains used in this study, and were considered spontaneous and/or incidental in nature and unrelated to the treatment (Chen et al., 2022).

In summary, the findings of this subchronic (90-day) toxicity study suggest that oral administration of the Lion's Mane 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 Lion's Mane mushroom β-glucan preparation can be established as 2000 mg/kg bw/day, the highest dose tested (Chen et al., 2022). 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 Lion's Mane mushroom β-glucan is over 135-fold lower as compared to the NOAEL. The findings from this study support the safe use of Lion's Mane mushroom β-glucan preparation by humans.

6.1.1.2. Genotoxicity Studies of Lion's Mane Mushroom β-glucans Preparations

6.1.1.2.1. Ames Assay

The potential mutagenic effects of Lion's Mane mushroom β-glucans was conducted using the Bacterial Reverse Mutation Assay, also known as the Ames test (Chen et al., 2022). 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. In the initial dose range finding study at doses of 5.0, 2.5, 1.25, 0.625 and 0.313 mg of Lion's Mane mushroom β-glucan preparation/plate revealed significant colony growth that interfered with the counting of revertant colony. Hence, the following concentrations of the β-glucan extract preparations were tested: 0.039, 0.078, 0.157, 0.313 and 0.625 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.

In the Bacterial reverse mutation test, the analysis of results of the five different strains showed that, irrespective of the presence or absence of S9, the number of revertant colonies did not increase significantly. The number of revertant colonies in the negative control groups of each strain was within the range of historical 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. These results show no significant increase in the number of revertant colonies at all concentrations of the test article in any of the strains whether with or without S9 mixture. Thus, the results of the study indicated that the Lion's Mane mushroom β-glucan extract preparation was non-mutagenic.

6.1.1.2.2. Chromosome aberrations

The potential genotoxic effects of Lion's Mane mushroom β-glucans to induce chromosome aberrations were investigated in Chinese hamster ovary cells (CHO-K1) (Chen et al., 2022). 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 Lion's Mane 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 Lion's Mane 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 (short-term) treatment group, cell viability in the absence of S9 metabolic activation at the Lion's Mane mushroom β-glucan concentrations 5, 2.5, 1.25, 0.625, and 0.313 mg/ml were 98.23 ± 2.15, 98.14 ± 1.21, 97.88 ± 2.21, 96.37 ± 1.92, 94.87 ± 1.31% 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 117.24 ± 5.85, 114.58 ± 6.46, 109.44 ± 5.14, 129.57 ± 6.47, 131.02 ± 10.52% respectively. In the 18-hour (long-term) treatment in the absence of S9 metabolic mixture, the cell viabilities were 93.72 ± 1.24, 94.25 ± 1.04, 95.52 ± 1.48, 96.21 ± 0.76, 98.45 ± 6.53%, respectively. As the cell viability was greater than 50%, the Lion's Mane mushroom β-glucan extract preparation was not considered as cytotoxic. The results of this test suggested that Lion's Mane mushroom β-glucan extract preparation do not cause significant structural and numerical aberrations under the experimental conditions described (Chen et al., 2022).

6.1.1.2.3. *In vivo* Micronucleus Assay

The mammalian peripheral blood micronucleus test to investigate the effects of Lion's Mane mushroom β-glucan preparation was conducted in accordance with the OECD guideline for the testing of chemicals #474: mammalian erythrocyte micronucleus test (Chen et al., 2022). Lion's Mane 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. For each time-point and each animal, over 1000 erythrocytes were counted the polychromatic erythrocytes (PCE) and the percentage of PCE in erythrocytes (PCE%) were calculated.

There were no abnormal clinical symptoms noted in the animals in any group during the study, and there were no mortalities observed during the course of the study. No significant difference in mean body weights were noted between the groups. The PCE% of the positive control group at 48 h after dosing in females was 1.51 ± 0.52% and males was 1.63 ± 0.45% respectively. A decrease in the PCE% of the positive control group, at after 48 hours, 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 Lion's Mane mushroom β-glucan extract did not inhibit erythropoiesis (Chen et al., 2022).

The micronucleus frequency in thousand PCEs of the negative control group at 48 hours after dosing was 0.18 ± 0.26 $^{0}/_{00\ PCE}$, and 72 h was 0.18 ± 0.66 $^{0}/_{00\ PCE}$ in females, and 0.64 ± 0.52, and 0.60 ± 0.45 $^{0}/_{00\ PCE}$ in males respectively. The micronucleus frequency in thousand PCEs of the positive control group at 48 hours after dosing was 21.00 ± 5.55$^{0}/_{00\ PCE}$ in females, and 21.50 ± 6.50 $^{0}/_{00\ PCE}$ in males. After Poisson distribution analysis, there was no significant difference between the three treatment groups and the negative control group in both males and females, which indicated that Lion's Mane mushroom β-glucan preparation exhibited no genotoxicity in the testing system applied in the study (Chen et al., 2022).

6.1.2. Secondary Published Studies with Other products

6.1.2.1. Toxicity Studies of Source Mushroom

6.1.2.1.1. Acute Toxicity Studies

In an acute study, Wong et al. (2013) investigated the effects of an aqueous extract of erinacine A-enriched *H. erinaceus* fruiting body. In this study, a total of 18 rats were divided into three groups (n=6). The groups were designated control (no extract administered), low-dose (2 g/kg bw) and high-dose (5 g/kg bw). Over the course of a 14-day period, the rats were observed for signs of toxicity, behavior, and mortality. No mortality was noted at any dose level tested, including the high dose of 5 g/kg bw. The findings suggest that aqueous extract of *Hericium erinaceus* is relatively non-toxic.

In another study, Li et al. (2018) investigated potential adverse effects of erinacine A-enriched *H. erinaceus* mycelia following oral administration in both acute and prenatal developmental toxicity tests. For the acute toxicity study, rats (10/sex) were randomly assigned to a treatment or control group. The *H. erinaceus* was prepared as an ethanol extract with 1000 ml of absolute alcohol added to 40 g of powder. The tested dosage was 5000 mg/ kg bw. The study was conducted over 14 days and the rats were observed for signs of toxicity, mortality, morbidity, and body weight changes. Upon conclusion of the 14 days, the rats were euthanized and the blood and organs were used for hematology, clinical biochemistry, and pathological examination. The results of the acute toxicity study did not reveal any demonstrable changes in general symptoms, clinical signs, gross pathology, and mortality rates at dose levels of up to 5000 mg/kg bw.

6.1.2.1.2. Short-term (28 day) Toxicity Studies

Li et al. (2014) investigated the toxicity of *H. erinaceus* mycelium in rats. For this study, *H. erinaceus* liquid concentrations were made from harvested mycelia that was dried, powdered (containing 5 mg/g erinacine A), and dissolved into distilled water with vigorous shaking for final concentrations of 50, 100, and 150 mg/mL. Different concentrations were prepared for a constant volume of 20 mL/kg bw/day at all dose levels. The rats (40/sex) were divided into four groups. The low dose group received 1000 mg/kg bw, mid dose group received 2000 mg/kg bw, and high dose group received 3000 mg/kg bw. The control group received distilled water. The study was carried out as per OECD Guideline 407. During the course of study, weekly body weight measurements, weekly average measurement of water and food intake, and daily signs of toxicity, mortality, and morbidity were recorded. Sixteen-hour urine specimen samples were collected for urinalysis. At termination, blood samples were collected for analysis for hematology, coagulation time and serum biochemistry values, and internal organs were removed and weighed.

Daily oral administration at all tested doses for a 28-day period did not induce any clinical signs of toxicity, morbidity or mortality in both sexes of rats. There was no significant difference between the control and the dosed groups regarding body weight, hematocrit, hemoglobin, red blood cell, white blood cell, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, lymphocyte, neutrophil, monocyte, eosinophil, and basophil. Some hematological analysis parameters revealed a significantly higher value of prothrombin time in males of the high dose group and a significantly lower value of reticulocyte in females of the mid dose group, as compared to the control group. However, such values were found to be within the normal range of rats, thus indicating the result of normal variation among animal groups. Serum biochemistry analysis revealed that globulin levels were statistically higher in male rats treated at dose levels of 2 g/kg when compared to the control group. However, such values were in the range of normal values, which suggested normal function of the organs. There were no significant changes in urinalysis. Histopathological examination of control and high dose groups did not reveal any treatment-related findings in organ (brain, heart, kidney, liver, spleen, adrenal gland, and testis/ ovary). In conclusion, the 28-day sub-chronic toxicity test in Sprague-Dawley rats showed no evidence of systemic toxicity attributable to erinacine A-enriched *H. erinaceus* administration. Thus, based on these findings, the NOAEL is greater than 3000 mg/kg bw/day.

In another study, Park et al. (2008) investigated the effects of a water extract mixture of *Panax ginseng* and *H. erinaceum* containing 50% water (also known as Munophil, prepared as per OTC Korean monograph; additional details of product not provided), following daily oral administration to rats for 28 days. In this study, Sprague Dawley rats (40/sex) were divided into four groups and were treated with 0, 1250, 2500 and 5000 mg/kg bw/day for 28 days. The rats were checked daily for mortality, signs of gross toxicity and behavioral changes, and body weights and food and water consumption were recorded weekly. At termination, blood was collected, animals were euthanized and internal organs were removed, weighed and gross findings were recorded. Hematology, clinical chemistry, urinalysis and histopathological examinations were carried out.

The results revealed no mortality, no statistically significant changes in food and/or water consumption, no statistically significant changes in body weight and organ weight compared to the control group, and no statistically significant differences in urinalysis and most hematological analysis. There was an observed increase in chloride present in the blood in some

of the rat groups receiving the extracts. However, the differences were not considered biologically relevant and were not considered to be treatment related as they did not show a consistent relationship to the dose. The investigators reported that Munophil appears to be safe and non-toxic in this study and the NOAEL in rats was established at 5000 mg/kg bw/day (Park et al., 2008). Although the test article used in this study was a mixture, the findings from this study indicate that Lion's Mane mushroom is unlikely to cause adverse effects.

6.1.2.1.2. Subchronic Toxicity Study

Lakshmanan et al. (2016) investigated potential toxic effects of water extracted fruiting bodies of *H. erinaceus* aqueous extract in rats. Fresh fruiting bodies of *H. erinaceus* were collected, freeze-dried and powdered. The freeze-dried powder was boiled with triple-distilled water at a ratio of 1:10. Residues were removed by filtration and centrifuged. The supernatant was freeze-dried and the aqueous extract powder was stored at 4°C. Doses for the rat groups were established at 0 (control), 250 (low dose), 500 (mid dose) and 1000 (high dose) mg/kg bw/day and were orally administered for 90 days. Body weights were recorded on a weekly basis and general behavioral changes were observed. The blood samples were subjected to hematological, biochemical, serum electrolyte, and antioxidant enzyme estimations. The rats were sacrificed and organs were processed and examined for histopathological changes.

The findings from this 90-day study did not reveal any mortality, no significant changes were noted general behavior and no significant differences in body weight between the control group and the various *H. erinaceus* aqueous extract dosed groups. There were no significant differences in red blood cell count, hemoglobin, hematocrit, mean cell volume, mean corpuscular hemoglobin, platelets, and total white blood cell count between the treated and control groups of rats. Furthermore, there was no significant effects observed between the control group and treated groups on serum electrolytes, organ weight (including heart, liver, kidneys, lung, spleen and brain). Histological examination did not reveal treatment related toxicological changes in the liver, kidney, brain, heart, lung and spleen.

Statistically significant results of the study included observations in some serum biochemical parameters. However, these changes were not considered as treatment. Glucose levels decreased from that of the control group in the low dose (250 mg/kg bw/day) and the mid dose (500 mg/kg bw/day). Total cholesterol decreased in the high dose (1000 mg/kg bw/day), while HDL increased and LDL decreased. At the mid dose, a statistically significant increase in HDL and decrease in LDL without a statistically significant effect on total cholesterol were noted. The low dose group showed a statistically significant decrease in albumin levels. The difference in glucose levels noted in rats treated with 250 and 500 mg/kg bw/day was not observed in rats treated with 1000 mg/kg bw/day. Similarly, the difference in albumin level in the low dose group, but not in the mid or high dose groups. These observations suggest that the statistically significant changes were not treatment related. The safety of oral administration of *H. erinaceus* aqueous extract for 90 days was also supported by relative organ weights and histopathological observations. The NOAEL was determined as >1000 mg/kg bw/day for aqueous extract of *H. erinaceus*.

6.1.2.1.4. Prenatal Toxicity Study

As mentioned earlier, in addition to acute toxicity, Li et al. (2018) also studied the prenatal developmental toxicity of erinacine A-enriched *H. erinaceus* mycelia preparation. In this study, 88 pregnant Sprague-Dawley rats were divided into four test groups, one group being

the control group, and the three test groups that were orally administered with *H. erinaceus* preparation at dose levels of 875, 1750 and 2625 mg/kg bw/day, during the gestation period of 6-15 days, respectively. On the 20th day, the female rats were euthanized and fetuses were delivered via cesarean section. The fetuses were examined for any morphological abnormalities. The findings from this study did not reveal any statistical differences in weight of uterus, fetal body weight, number of corpora lutea, implantation sites, pre-implantation loss, and post-implantation loss of the treatment groups and the control group. In addition, no significant differences were observed in the fetal external, organ, and skeletal examinations. The results of the prenatal developmental study also revealed that administration of erinacine A-enriched *H. erinaceus* mycelia to pregnant rats had no adverse effect on maternal toxicity. Uterus, ovary, and fetal examination showed no significant differences between treatment groups and the control group. The investigators concluded that erinacine A-enriched *H. erinaceus* mycelia preparation is safe and practically nontoxic for consumption within the appropriate doses and investigation period in this study.

In summary, the results of acute oral toxicity study of Lion's Mane mushroom extract in rats showed that LD_{50} is greater than 5 g/kg bw. Findings from a 28-day dose-response toxicity study suggest the NOAEL of erinacine A-enriched *H. erinaceus* as 3000 mg/kg bw/day, while in another study, a mixture of *Panax ginseng* and *H. erinaceum* did not cause toxicity at dose levels up to 5000 mg/kg bw/day. In a subchronic toxicity study conducted as per OECD guidelines, the NOAEL was determined as >1000 mg/kg bw/day for the aqueous extract of *H. erinaceus.* Taken together, these studies indicate that Lion's Mane mushroom has a very low order of toxicity. These studies also show that the source material of Lion's Mane mushroom β-glucan preparation, *H. erinaceus* 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; 2018) investigated the subchronic toxicity and potential genotoxic effects of mushroom β-glucans derived from *Ganoderma lucidum* and from *A. cinnamomea.* The experimental protocol and methodologies used in both these studies were similar to those described for the above study by Chen et al. (2022). Hence, the details of the 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 a 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.

Among the statistically significant changes noted, at 2000 mg/kg bw/day an increase in MCV and MCH levels was noted, whereas hematocrit levels showed an 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).

In another similar study, Chen et al. (2018) investigated adverse effects, if any, of β-glucan (~65% pure) derived from *A. cinnamomea* in subchronic toxicity and genotoxicity studies. In the subchronic toxicity study conducted as per OECD guidelines, Sprague Dawley rats (12/sex/group) were administered (gavage) Antrodia mushroom β-glucan preparation at dose levels of 0, 500, 1000 and 2000 mg/kg bw/day for 90 days. Administration of β-glucan preparation did not result in any toxicologically significant treatment-related changes in clinical observations, ophthalmic examinations, body weights, body weight gains, feed consumption, and organ weights. The clinical pathology as evaluated by hematology, serum chemistry, urinalysis or terminal necropsy (gross or histopathology findings) did not reveal any treatment-related adverse effects. Based on the subchronic study, the no observed-adverse-effect level (NOAEL) for β-glucan preparation from Antrodia mushroom was determined as 2000 mg/kg bw/day, the highest dose tested. The findings from mutagenicity studies are described below.

6.1.2.2.2. Genotoxicity Studies with other Mushroom β-Glucans

In two separate publications, Chen et al. (2011; 2018) also studied the mutagenicity and genotoxicity potentials of mushroom β-glucans derived from *G. lucidum* as well as from Antrodia mushroom, by three separate assays that included gene mutations in *Salmonella typhimurium* (Ames assay), *in vitro* chromosome aberrations and *in vivo* micronucleus test in mouse. The experimental methods used in these assays was similar to the earlier (please see section 6.1.1.2.) methods described in studies by Chen et al. (2022).

In the Ames assay, *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were tested by a plate incorporation method at concentrations of 0.313, 0.625, 1.25, 2.5, and 5 mg mushroom β-glucans derived from *G. lucidum*/plate in the presence or absence of a S9 metabolic activation (Chen et al., 2011). 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 (Chen et al., 2011).

In the similarly conducted study with β-glucans derived from Antrodia mushroom, there was no significant increase in the number of revertant colonies at all concentrations of test article (0.039, 0.078, 0.157 and 0.313 mg/plate) in any of the *Salmonella typhimurium* strains (TA98,

TA100, TA102, TA1535, and TA1537), whether with or without S9 mixture, suggesting that Antrodia mushroom β-glucan preparation was not genotoxic (Chen et al., 2018).

In the chromosome aberration assay, the genotoxic potential of mushroom β-glucans, derived from *G. lucidum*, 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. 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).

In the similarly conducted chromosome aberration assay, Chen et al. (2018) investigated the genotoxic effects with β-glucans derived from Antrodia mushroom. 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. Mushroom β-glucans treatment of CHO-K1 cells did not display genotoxicity.

The *in vivo* mammalian peripheral blood micronucleus test was conducted in accordance with the OECD guideline (Chen et al., 2011; 2018). In these studies, β-glucans derived from *G. lucidum* and from Antrodia mushroom was administered orally to mice (5/sex/group) at dose levels of 0, 1250, 2500, and 5000 mg/kg bw. There were no abnormal clinical symptoms noted in the animals in any group during the course of the experiment in both the studies, and there were no mortalities observed. No significant differences in mean body weights were noted between the groups. The micronucleus frequency in one thousand PCE of the negative control group at 24, 48, and 72 hours after dosing was determined. The PCE% in all the treatment groups showed no significant decrease as compared to the respective negative control group indicating β-glucan preparation derived from *G. lucidum* or from Antrodia mushroom did not inhibit erythropoiesis. There were no significant differences between the three treatment groups and the negative control group in both the studies, suggesting that mushroom β-glucans derived from *G. lucidum* and from Antrodia mushroom was non-genotoxic.

In summary, the findings of mutagenicity studies, as evaluated by gene mutations in *Salmonella typhimurium*, *in vitro* chromosome aberrations and *in vivo* micronucleus test in mice, did not reveal any genotoxicity of β-glucan preparation derived from *G. lucidum* and from Antrodia mushroom.

6.1.2.3. Toxicity Studies of β-Glucans from other Sources

Jonker et al. (2010) investigated the toxicity potentials of β-glucans (>75%) derived from barley in a 28-day study. In this study, Wistar rats (5/sex/group) were fed barley β-glucan 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.

Babicek et al. (2007) investigated the acute and subchronic toxicity of yeast-derived β-glucans ingredient in F344 rats. In the acute study, the LD_{50} value of yeast-derived β-glucans in rats was found to be greater than 2000 mg/kg bw. In the subchronic study, rats (10/sex/group; 5-6 weeks of age) 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.

Feletti et al. (1992) investigated the long term toxic effects of yeast β-glucans derived from *Candida albicans* ATCC 20955 in Sprague Dawley rats. In this study, 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

β-Glucans largely remain undigested in the upper gastrointestinal tract. This is similar to other prebiotics and non-digestible/fermentable carbohydrates such as inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), resistant starch, polydextrose, cyclodextrins, and lactulose, (Macfarlane et al., 2006). Humans are unable to digest carbohydrate polymers with β-glucosidic linkages (Wisker et al., 1998). Thus, 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 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. (2006) 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 (baker's yeast β-1,3/β-1,6-D-glucans preparation)/person for a period of four 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, Zhao and Cheung (2011) evaluated the bifidogenic effect of β-glucans from barley, seaweed, bacteria, and mushroom and compared their *in vitro* fermentation by three Bifidobacterium 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 randomized, double-blind, placebo-controlled parallel-group comparative study, Saitsu et al. (2019) investigated the effects of taking supplements containing fruiting body of *H. erinaceus* for 12 weeks. All participants were randomly divided into two groups: the *H. erinaceus* group (n=16) taking four *H. erinaceus* supplements in a day, and the placebo group (n=15) taking four placebo supplements in a day that did not contain *H. erinaceus* for 12 weeks. One *H. erinaceus* supplement contained 0.8 g of the powdered fruiting body of *H. erinaceus* (total daily intake of 3.2 g) and a placebo supplement did not contain any powder from *H. erinaceus*. Oral intake of *H. erinaceus* significantly improved cognitive functions and prevented deterioration. These investigators speculated that various chemical compounds, including hericenones, in the mushroom have multiple effects to the brain neural networks and cognitive functions. These investigators stated that oral intake of *H. erinaceus* is safe.

Twardowski et al. (2015) studied the effects of white button mushroom (WBM; *Agaricus bisporus*- an edible mushroom) powder in a Phase I clinical trial. In this study, effects of WBM

on serum prostate specific antigen (PSA) in patients with biochemically recurrent prostate cancer were investigated. 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 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. No dose limiting toxicities were encountered. 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 Dose Limited Toxicities.

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 randomized, double-blind, placebo-controlled, parallel group interventional trial, 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. 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.

As 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. In this double-blind, placebo-controlled clinical trial, 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 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. In this placebo-controlled, double-blind study, 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 of a yeast-derived β-glucans fiber on serum lipids 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 per day was added to the diet for 8 weeks and then stopped for four 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 the 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 addition to the above described absorption study, Lehne et al. (2006) also investigated the safety of a soluble branched β-l,3-glucan derived from *S. cerevisiae*. In this open label dose-escalation study, 18 healthy volunteers (6/group) were randomized to receive 100, 200, or 400 mg β-glucan per person for a period of four 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 β-glucan administration. Minor mucosal lesions of the oral cavity noted in seven subjects were considered normal physiological variations. Increased C-reactive protein, fibrinogen and abnormal differential counts of leucocytes were observed in five subjects with pre-existing respiratory infections, including one 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-1β, IL-6, or TNF-a between treatment groups. The investigators concluded that soluble branched β-l,3-glucan was safe and well tolerated by healthy volunteers, when given orally once daily for four consecutive days at doses up to 400 mg.

6.1.2.6. Potential Allergenicity to Mushroom and its β-Glucans

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, 2000 hospitalizations, and 150 deaths.

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). 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 baker's yeast (EFSA, 2011).

In an early study, Bruce (1963) investigated skin and bronchial reactivity in asthmatics to different allergen extracts comprised of basidiospores. 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.

Numerous *in vitro*, laboratory animal, and clinical studies indicate the anti-allergic function of *beta*-glucans (Mironczuk-Chodakowska et al., 2021). The majority of studies conducted so far have confirmed that oral administration of polysaccharides, mainly β-glucans isolated from *Basidiomycetes*, may prevent allergies by decreasing the level of immunoglobulin class E (IgE) and increasing the production of IFN-γ (interferon-gamma).

There have been rare adverse incidences coinciding with consumption of Lion's Mane; however, they present in individuals who have a pre-existing allergy to this mushroom. In one case report (Maes et al., 1999), a 53-year-old man was cultivating Lion's Mane mushroom developed contact dermatitis on hands, forearms and legs. Patch test with the European standard series with Lion's Mane was positive in the man, but negative in 20 control patients. A ROAT (repeat open application test, a skin test used to confirm or rule out the presence of allergic contact dermatitis) was also conducted and confirmed highly positive for the man experiencing symptoms and as a control ROAT was also conducted on his wife, for which the result was negative. Maes et al. (1999) concluded that allergic contact dermatitis such as presented in this one case is rare and they have found no previous reports of allergy to *H. erinaceum* or other edible mushrooms of the same family.

Nakatsugawa et al. (2003) suggested a causal relationship between acute respiratory distress syndrome and *H. erinaceum* extract, which is commercialized as a diet food. A 63-year-old man, with untreated diabetes mellitus, was admitted to hospital for intensive care of severe acute respiratory failure with diffuse infiltration in both lungs. Bronchoalveolar lavage fluid revealed a high percentage of lymphocytes. Lymphocyte stimulation test showed a strong reactivity against extract formulation of *H. erinaceum*, which the subject had taken four months before onset. The man had been taking *Hericium erinaceum* extract supplement for four months prior to diagnosis. The subject recovered with successful steroid pulse therapy under mechanical ventilation. The categories used in this case study for classification of drug therapy adverse reaction are: definite, probable, possible, or doubtful. This reaction was deemed a probable case of adverse reaction against *H. erinaceum*. To the knowledge of the authors, this is the first probable case report of acute lung injury associated with *H. erinaceum* extract. Notable limitations to this case report, there is no mention of daily dosage consumed, type of extraction method performed on the mushroom, nor if the extract was made of the fruiting body, mycelium, or both in combination. There is also no conducted analysis of allergy testing for this mushroom or other mushrooms. The investigators judged the present patient a probable case of adverse drug reaction against *H. erinaceum*.

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.

In summary, allergy to Lion's Mane mushroom is rare. Mushroom allergies are typically caused by airborne particles or skin contact. In the published literature two case reports indicating allergic reaction to *H. erinaceum* have been found. Lion's Mane has been consumed widely for hundreds of years and to the best of our knowledge, no other *H. erinaceum*-related allergic reactions have been reported. Thus, these reactions appear to be extremely rare. The residual amount of protein in the Lion's Mane 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 four GRAS notifications on β-glucans, one derived from mushroom *A. cinnamomea* [GRN 995, SBG, 2020)], another one from *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 these GRNs. As the subject of this present GRAS assessment is substantially similar to the products of existing FDA notifications, the studies described in these notifications can also be utilized to support the safety of use in the present GRAS assessment of Lion's Mane 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 5.

Table 5. Comparison of Mushroom β-Glucans with Yeast β-Glucans from GRAS Notices

Specifications	Mushroom (present GRAS)	Mushroom (GRN 995)*	Mushroom (GRN 413)*	Black yeast (GRN 309)*	Baker's yeast (GRN 239)*
Description	Fine light beige powder	Fine light beige powder	Fine light beige powder	Reddish yellow powder	Fine beige/tan powder
Source organism	*H. erinaceum*	*A. cinnamomia*	*G. lucidum*	*A.. mllulans*	*S. cerevisiae*
Total Carbohydrate (%)	> 90	> 90	At least 95	At least 80	At least 80
β-Glucan (%)	Minimum 65	Minimum 65	At least 50	At least 40	At least 70
Protein (%)	<1.0	< 1	<1.0	<10	<10
Fat (%)	<1.0	< 1	<1.0	<5	<20
Ash (%)	<5.0	< 3	<3.0	<10	<5
Moisture (%)	<3.0	< 5	<2.0	<6	<8
Lead (ppm)	<0.1	<0.5	<0.5	ND	<0.5
Mercury (ppm)	<0.05	<0.05	<0.05	ND	<0.05
Arsenic (ppm)	<0.1	<0.5	<0.5	ND	<0.1
Cadmium (ppm)	<0.1	<1.0	<1.0	ND	<1

Aerobic plate count	<15,000	<15,000	<10,000	<3,000	<20,000
Yeast and molds	<150 combined	<150 combined	≤15	≤20	≤25
Coliform	<10	<10	Negative	<10	<10
Escherichia coli	Negative	Negative	Negative	Negative	Negative
Salmonella sp.	Negative	Negative	Negative	Negative	Negative

*Adapted from GRN 995, GRN 413, GRN 309 and GRN 239; ND =Not detected

6.1.3.1.1. GRN 995- Beta-glucans derived from *Antrodia cinnamomea*

In 2020, Super Beta Glucan Inc. submitted a GRAS notice on β-glucans derived from mushroom *Antrodia cinnamomea*. The use of β-glucans from *A. cinnamomea* was determined to be GRAS through scientific procedures. The ingredient is described as a fine light-beige powder, obtained from the cultured mycelium of *A. cinnamomea* (ATCC 200183). The specifications of this product are similar to the subject current GRAS notice and are also similar to those of its previous GRAS notice (GRN 413) on β-glucans derived from mushroom *G. lucidum*. The typical composition of β-glucans from *A. cinnamomea* is reported to consist of >65% β-D-Glucan, (1-3), (1-6)-β-D-Glucan, and/or β-glucosylglucan. The molecular weight of β-glucans from *A. cinnamomea* ranged from 9.6 kDa to 298 kDa. β-Glucans derived from *A. cinnamomea* ATCC 200183 is manufactured under cGMP using food-grade starting materials, processing aids and food-contact materials, which are used in accordance with U.S. regulations. The proposed uses at levels of 150 mg/serving in different foods and the resulting intake are also similar to the current GRAS notice and the previous GRAS notice (GRN 413). The use of β-glucans from *A. cinnamomea* was determined to be GRAS through scientific procedures. The proposed uses were reported to be substitutional and will not increase the current dietary exposure to β-glucan.

In GRN 995, Super Beta Glucan Inc. extensively summarized the available safety related information on β-glucans derived from *A. cinnamomea*. The available metabolism related information of β-glucans suggest that humans are unable to digest carbohydrate polymers with β-glycosidic linkages and, therefore, absorption by the intestinal epithelium and significant systemic exposure to particulate mushroom β-glucans is unlikely. In a published pivotal subchronic study (Chen et al., 2018), toxicity of β-glucans from *A. cinnamomea* was investigated in Sprague Dawley rats. The findings from the study, performed in accordance with OECD guidelines, suggest that oral administration of Antrodia mushroom β-glucans at levels up to 2000 mg/kg bw/day the highest dose tested, for 90 days did not cause adverse effects in male or female rats. This study supports the safety of estimated dietary exposure at the 90th percentile of 14.5 mg/kg bw/day from the proposed uses of β-glucans from *A. cinnamomea*. The results of published genotoxicity studies (Ames assay, in vitro chromosomal aberration assay and in vivo micronucleus assay) suggest that β-glucans from *A. cinnamomea* was neither genotoxic nor mutagenic in any of these assays. The available *in vivo* and *in vitro* studies conducted on *A. cinnamomea* support the safety of the source material. In the GRAS notice, available human studies on β-glucans are described as corroborative support. The results from these studies demonstrate that β-glucans are safe and well-tolerated. Allergy to *A. cinnamomea* has not been reported and the residual amount of protein in β-glucans from *A. cinnamomea* is very low (<1%). Based on the information described in the GRAS notice, as well as other information available to the FDA, the agency issued a no questions letter and agreed with the conclusion that β-glucans from *A. cinnamomea* is GRAS under its intended conditions of use is safe.

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

In a previous GRAS notice also submitted by Super Beta Glucan Inc. (SBG, 2012), use of β-glucans derived from *Ganoderma lucidum* was determined to be GRAS. 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 5) and are similar to the subject of this 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 GRN 413, the available published safety related information has been extensively summarized and discussed 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 to be 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 the 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 summarized in the GRAS notice, as well as other information available to the FDA, the agency issued a no questions letter agreeing with the GRAS status of β-glucans derived from *G. lucidum* mycelium under the intended conditions of use (FDA, 2012).

6.1.3.1.3. GRN 309- Black Yeast β-Glucans

In addition to the above described GRAS notices with β-glucans derived from mushrooms, the FDA also received two GRAS notices on β-glucans derived from yeast. The subject of GRN 309 is β-glucans derived from *Aureobasidium pullulans* (Glucan, 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 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 reveal 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 total highest tested level of 2000 mg/kg bw/day of β-glucans derived from *A. pullulans*. Based on the findings from a human study, it was concluded that 400 mg/person/day of β-glucans derived from *A. pullulans* did not cause 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.

6.1.3.1.4. GRN 239- Baker's Yeast β-Glucans

In this first GRAS notice on β-glucans derived from baker's yeast (Biothera, 2008), 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 trials in humans for 10 and 30 days. 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

The EFSA Panel 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 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 Inc. (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 Lion's Mane mushroom β-glucan, derived from Lion's Mane (*Hericium erinaceus*), 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 Lion's Mane 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 October 2022 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 November 30, 2022 and unanimously agreed to the decision described herein.

[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.

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 preparation of Lion's Mane mushroom β-glucans, derived from *Hericium erinaceus* (BCRC 35669), is a fine light beige powder with characteristic mild odor and bland taste. It is manufactured according to current good manufacturing practices. The food grade specifications of Lion's Mane β-glucans has been established by SBG. The compositional analysis of Lion's Mane β-glucans demonstrated that it primarily contains over 90% as total carbohydrates of which β-glucans constitutes >65%. The remaining carbohydrates are primarily monosaccharides and disaccharides. The intended use of Lion's Mane β-glucans in the above mentioned 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 resulting intake of β-glucans at mean and 90th percentile is estimated as 189.34 and 379.21 mg/person/day or 4.09 and 9.42 mg/kg bw/day, respectively. The intended use of Lion's Mane β-glucans is in the same food products and at the same levels mentioned in the GRAS notices GRN 309, GRN 413 and GRN 995.

The source organism of Lion's Mane β-glucans, *H. erinaceus,* is an edible, rare and endemic mushroom that is native to North America, Europe and Asia. β-Glucans, the active constituent of Lion's Mane 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 for centuries. 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 baker's 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; GRN 995). All of this information suggests that there is a common knowledge of safe consumption of β-glucans from different food sources or products.

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. Thus, β-glucans is comprised of D-glucose polymers. 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

[6] Available at: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm583856.htm

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.

The toxicity of Lion's Mane mushroom β-glucan preparation (the subject of the present GRAS) has been investigated in a series of specifically designed subchronic toxicity and mutagenicity/genotoxicity studies that followed standard regulatory guidelines. In the subchronic toxicity study in rats, administration (gavage) of Lion's Mane 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. No treatment-related macroscopic lesions were observed at the end of treatment period. There were no treatment-related histopathological findings. The findings from mutagenicity studies, including the Ames assay, *in vitro* chromosomal aberration and *in vivo* micronucleus assay, did not reveal any genotoxicity of Lion's Mane mushroom β-glucan preparation. All of these toxicity studies are published in a peer-reviewed scientific journal (Chen et al., 2022). Based on the subchronic study, the NOAEL for Lion's Mane 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 Lion's Mane 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 Lion's Mane mushroom β-glucans, the safety of source material, *H. erinaceus* has been investigated in animal toxicity studies. The results of acute oral toxicity study in rats showed that LD_{50} of Lion's Mane mushroom extract is greater than 5 g/kg bw. In a 28 day dose-response toxicity study, the NOAEL of erinacine A-enriched *H. erinaceus* was determined as 3000 mg/kg bw/day, while in another study, a mixture of *Panax ginseng* and *H. erinaceum* did not cause toxicity at dose levels up to 5000 mg/kg bw/day. In a subchronic toxicity study, the NOAEL was determined as >1000 mg/kg bw/day for the aqueous extract of *H. erinaceus*. These studies indicate that Lion's Mane mushroom has a very low order of toxicity. These studies also show that the source material of Lion's Mane mushroom β-glucan preparation, *H. erinaceus* is safe for human consumption.

In a series of studies, subchronic toxicity and potential genotoxic effects of mushroom β-glucans derived from *G. lucidum* as well as that from *A. cinnamomea* have been investigated. Given the similarity between β-glucans derived from *H. erinaceus*, and from *G. lucidum* and *A. cinnamomea*, findings from these studies are applicable to the present GRAS. In the subchronic toxicity study with β-glucans derived from *G. lucidum* and *A. cinnamomea,* 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* and *A. cinnamomea*. 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 Lion's Mane mushroom β-glucans is supported by the compositional similarity of the ingredient to β-glucans derived from other mushrooms such as *G. lucidum* and *A. cinnamomea*, as well as 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 Lion's Mane mushroom β-glucans derived from *H. erinaceus* 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.,* Lion's Mane 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 Lion's Mane 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 Lion's Mane 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 Lion's Mane 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 Lion's Mane mushroom β-glucans, when used as described, is safe and GRAS based on scientific procedures.

Signatures

[redacted]
Robert T. Morris, PhD

Nov. 30, 2022
Date

[redacted]

[redacted]

[redacted]

NOV. 30, 2022
Date

Part VII- SUPPORTING DOCUMENTS AND REFERENCES

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APPENDIX I

Analytical data from five non-consecutive manufacturing lots of Lion's Mane mushroom β-glucan

(Included separately)

CERTIFICATE OF ANALYSIS

Product Name: Lion's Mane (β-Glucan) Extract Powder
Manufacturing April 09, 2021
Lot Number: 20210409MBGSLM
Source of Origin: *Hericium erinaceus*

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 (%)	91.9	>90	By Difference (Calculation)
β-Glucan (%)	68.0	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 (%)	0.6	<1.0	AOAC 922.15
Moisture (%)	3.9	<5.0	AOAC 925.45A/V.O
Ash (%)	2.8	<3.0	AOAC 900.02
HEAVY METALS			
Lead	Negative	<0.1 ppm	ICP-MS
Arsenic	Negative	<0.1 ppm	ICP-MS, FDA EAM 4.7
Cadmium	Negative	<0.1 ppm	ICP-MS
Mercury	Negative	<0.05 ppm	ICP-OES
MICROBIOLOGICAL PARAMETERS			
Aerobic Plate Count (CFU/g)	Conformed to standard	<15,000	FDA BAM Chapter 3
Yeast and Mold (CFU/g)	Conformed to standard	<150 combined	FDA BAM Chapter 18; CMMEF APHA Chapter 21
Total *Coliforms* (MPN/g)	Negative	<10.0	AOAC 966.24
Staphylococcus aureus	Negative	N.D.	FDA BAM Chapter 12, AOAC 2003.08, 2003.11 and AOAC 2003.07
E. coli	Negative	N.D.	FDA BAM Chapter 4, AOAC 991.14
Salmonella sp.	Negative	N.D.	FDA BAM Chapter 4

Negative = not detected within the limit of detection (LOD), which is <10 CFU/gram for microbiological parameters and for all heavy metals the LOD are 10 ppb (parts per billion). CMMEF APHA = Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association

5 Holland 109, Irvine, CA 92618, Ph: 949-264-2888

QC: SS- 04/23/2021

CERTIFICATE OF ANALYSIS

Product Name: Lion's Mane (β-Glucan) Extract Powder
Manufacturing March 11, 2021
Lot Number: 20210311MBGSLM
Source of Origin: *Hericium erinaceus*

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 (%)	91.5	>90	By Difference (Calculation)
β-Glucan (%)	66.8	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 (%)	0.7	<1.0	AOAC 922.15
Moisture (%)	4.3	<5.0	AOAC 925.45A/V.O
Ash (%)	2.8	<3.0	AOAC 900.02
	HEAVY METALS		
Lead	Negative	<0.1 ppm	ICP-MS
Arsenic	Negative	<0.1 ppm	ICP-MS, FDA EAM 4.7
Cadmium	Negative	<0.1 ppm	ICP-MS
Mercury	Negative	<0.05 ppm	ICP-OES
	MICROBIOLOGICAL PARAMETERS		
Aerobic Plate Count (CFU/g)	Conformed to standard	<15,000	FDA BAM Chapter 3
Yeast and Mold (CFU/g)	Conformed to standard	<150 combined	FDA BAM Chapter 18; CMMEF APHA Chapter 21
Total *Coliforms* (MPN/g)	Negative	<10.0	AOAC 966.24
Staphylococcus aureus	Negative	N.D.	FDA BAM Chapter 12, AOAC 2003.08, 2003.11 and AOAC 2003.07
E. coli	Negative	N.D.	FDA BAM Chapter 4, AOAC 991.14
Salmonella sp.	Negative	N.D.	FDA BAM Chapter 4

Negative = not detected within the limit of detection (LOD), which is <10 CFU/gram for microbiological parameters and for all heavy metals the LOD are 10 ppb (parts per billion). CMMEF APHA = Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association

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QC: AY- 03/24/2021

CERTIFICATE OF ANALYSIS

Product Name: Lion's Mane (β-Glucan) Extract Powder
Manufacturing February 23, 2021
Lot Number: 20210223MBGSLM
Source of Origin: *Hericium erinaceus*

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 (%)	91.3	>90	By Difference (Calculation)
β-Glucan (%)	67.2	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 (%)	0.9	<1.0	AOAC 922.15
Moisture (%)	4.3	<5.0	AOAC 925.45A/V.O
Ash (%)	2.7	<3.0	AOAC 900.02
	HEAVY METALS		
Lead	Negative	<0.1 ppm	ICP-MS
Arsenic	Negative	<0.1 ppm	ICP-MS, FDA EAM 4.7
Cadmium	Negative	<0.1 ppm	ICP-MS
Mercury	Negative	<0.05 ppm	ICP-OES
	MICROBIOLOGICAL PARAMETERS		
Aerobic Plate Count (CFU/g)	Conformed to standard	<15,000	FDA BAM Chapter 3
Yeast and Mold (CFU/g)	Conformed to standard	<150 combined	FDA BAM Chapter 18; CMMEF APHA Chapter 21
Total *Coliforms* (MPN/g)	Negative	<10.0	AOAC 966.24
Staphylococcus aureus	Negative	N.D.	FDA BAM Chapter 12, AOAC 2003.08, 2003.11 and AOAC 2003.07
E. coli	Negative	N.D.	FDA BAM Chapter 4, AOAC 991.14
Salmonella sp.	Negative	N.D.	FDA BAM Chapter 4

Negative = not detected within the limit of detection (LOD), which is <10 CFU/gram for microbiological parameters and for all heavy metals the LOD are 10 ppb (parts per billion). CMMEF APHA = Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association

5 Holland 109, Irvine, CA 92618, Ph: 949-264-2888

QC: SS- 03/10/2021

CERTIFICATE OF ANALYSIS

Product Name: Lion's Mane (β-Glucan) Extract Powder
Manufacturing February 12, 2021
Lot Number: 20210212MBGSLM
Source of Origin: *Hericium erinaceus*

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)
β-Glucan (%)	66.2	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 (%)	4.2	<5.0	AOAC 925.45A/V.O
Ash (%)	2.7	<3.0	AOAC 900.02
	HEAVY METALS		
Lead	Negative	<0.1 ppm	ICP-MS
Arsenic	Negative	<0.1 ppm	ICP-MS, FDA EAM 4.7
Cadmium	Negative	<0.1 ppm	ICP-MS
Mercury	Negative	<0.05 ppm	ICP-OES
	MICROBIOLOGICAL PARAMETERS		
Aerobic Plate Count (CFU/g)	Conformed to standard	<15,000	FDA BAM Chapter 3
Yeast and Mold (CFU/g)	Conformed to standard	<150 combined	FDA BAM Chapter 18; CMMEF APHA Chapter 21
Total *Coliforms* (MPN/g)	Negative	<10.0	AOAC 966.24
Staphylococcus aureus	Negative	N.D.	FDA BAM Chapter 12, AOAC 2003.08, 2003.11 and AOAC 2003.07
E. coli	Negative	N.D.	FDA BAM Chapter 4, AOAC 991.14
Salmonella sp.	Negative	N.D.	FDA BAM Chapter 4

Negative = not detected within the limit of detection (LOD), which is <10 CFU/gram for microbiological parameters and for all heavy metals the LOD are 10 ppb (parts per billion). CMMEF APHA = Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association

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QC: AY- 02/25/2021

CERTIFICATE OF ANALYSIS

Product Name: Lion's Mane (β-Glucan) Extract Powder
Manufacturing April 26, 2021
Lot Number: 20210426MBGSLM
Source of Origin: *Hericium erinaceus*

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)
β-Glucan (%)	67.5	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 (%)	4.0	<5.0	AOAC 925.45A/V.O
Ash (%)	2.7	<3.0	AOAC 900.02
	HEAVY METALS		
Lead	Negative	<0.1 ppm	ICP-MS
Arsenic	Negative	<0.1 ppm	ICP-MS, FDA EAM 4.7
Cadmium	Negative	<0.1 ppm	ICP-MS
Mercury	Negative	<0.05 ppm	ICP-OES
	MICROBIOLOGICAL PARAMETERS		
Aerobic Plate Count (CFU/g)	Conformed to standard	<15,000	FDA BAM Chapter 3
Yeast and Mold (CFU/g)	Conformed to standard	<150 combined	FDA BAM Chapter 18; CMMEF APHA Chapter 21
Total *Coliforms* (MPN/g)	Negative	<10.0	AOAC 966.24
Staphylococcus aureus	Negative	N.D.	FDA BAM Chapter 12, AOAC 2003.08, 2003.11 and AOAC 2003.07
E. coli	Negative	N.D.	FDA BAM Chapter 4, AOAC 991.14
Salmonella sp.	Negative	N.D.	FDA BAM Chapter 4

Negative = not detected within the limit of detection (LOD), which is <10 CFU/gram for microbiological parameters and for all heavy metals the LOD are 10 ppb (parts per billion). CMMEF APHA = Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association

5 Holland 109, Irvine, CA 92618, Ph: 949-264-2888

QC: SS- 05/12/2021

From: Sherwin Chen
To: Hice, Stephanie
Subject: [EXTERNAL] Re: GRN 001124 - Questions for Notifier
Date: Friday, August 4, 2023 6:01:53 PM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png
GRN 1124- beta-glucan Hericium erinaceus GRAS- FDA Query Responses Final.pdf

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Dear Dr. Hice,

Attached please find the response to your email of July 24, 2023 regarding additional information and clarifications required for our GRAS notice (GRN 0001124). We are providing a point-by-point response to your queries.

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

Sincerely,

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

On Mon, Jul 24, 2023 at 10:15 AM Hice, Stephanie <Stephanie.Hice@fda.hhs.gov> wrote:

> Dear Mr. Chen,
>
> During our evaluation of GRAS Notice No. 001124, 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,

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?)

August 4, 2023

Dear Dr. Hice,

RE: β-Glucan from *Hericium erinaceus* GRAS Notice (GRN 1124)

This responds to your email of July 24, 2023 regarding additional information and clarifications required for our β-glucan from *Hericium erinaceus* GRAS notice (GRN 0001124). We are providing a point-by-point response to your queries along with some additional relevant discussion.

FDA Query 1: Please provide a detailed description of *Hericium erinaceus* strain BCRC 35669 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: *Hericium erinaceus* BCRC 35669 strain has not been investigated for genome sequencing or it's genotypic (e.g., pathogenicity) and phenotypic (e.g., production of antimicrobials, production of secondary metabolites and mycotoxins, antifungal resistance) characteristics, because the strain is commonly used in food production. Despite its nutritional and other uses, the current understanding of the molecular biology and genetics of *H. erinaceus* is limited. As such no genetic linkage map has been generated in *H. erinaceus*, thereby preventing the identification of genotype-phenotype associations (Gong et al., 2020). These investigators generated the first high-resolution genetic map of *H. erinaceus* using the genome-wide scale genotyping data of 127 SSIs. Based on available information from database searches, the genome assembly and annotation report for very few (three) strains of *H. erinaceus* are available[1] (Gong et al., 2020).

H. erinaceus BCRC 35669 was obtained from Bioresources Collection and Research Center (BCRC) (Hsinchu, Taiwan)[2]. The source of the strain is fruiting body and the strain is categorized as "Bio-safety 1". As mentioned in the GRAS notice, the strain has not been subjected to whole genome sequencing and bioinformatics analysis. However, based on the available information that includes traditional and current uses of *H. erinaceus* and available safety related information, this mushroom strain is unlikely to pose any safety concerns. The safety of final product, derived from this strain, has been extensively investigated and these studies support the safety in use of β-glucan derived from *H. erinaceus* and in turn the safety of source (strain) as well.

Lion's mane mushroom, scientifically known as *H. erinaceus*, is an edible fungus native to Europe, Central and North America, and Asia[3]. A key aspect of identifying *H. erinaceus* is that it's the only species that grows as a clump of dangling spines. Known for its unique appearance, *H. erinaceus* is considered an absolute delicacy. This species

[1] Accessible at: https://www.ncbi.nlm.nih.gov/genome/browse/#!/eukaryotes/82251/
[2] Accessible at: https://catalog.bcrc.firdi.org.tw/BcrcContent?bid=35669&rowid=1
[3] Accessible at: https://www.shroomer.com/lions-mane-mushrooms/

does not show branches or traditional-looking fruiting bodies. Its sweet aroma, soft texture, and lobster-like flavor make it a unique but versatile addition to any kitchen. This information also indicate that the strain used in the preparation of β-glucan is unlikely to pose safety concerns.

FDA Query 2: On pages 25-28, the notifier summarizes the results from published studies investigating the toxigenicity of *H. erinaceus* and states that the production strain, *H. erinaceus* strain BCRC 35669 is non-toxigenic. However, on page 25, when describing an acute toxicity study, the notifier states, "The findings suggest that aqueous extract of *Hericium erinaceus* is relatively non-toxic." For the administrative record, please clarify this statement.

Response: We are sorry for the interpretation presented on page 25 related to findings from an acute toxicity study. The result from the acute toxicity study showed that aqueous extract of *H. erinaceus* was well tolerated by experimental rats and safe even at high dose level of 5 g/kg bw (Wong et al., 2013).

FDA Query 3: On page 8, notifier states, "The *H. erinaceus* strain used in the production of Lion's Mane mushroom β-glucans was obtained from Bioresources Collection and Research Center (BCRC) in the Food Industry Research and Development Institute (Hsinchu, Taiwan)." Please state where the strain was isolated from.

Response: As indicated above, the strain was obtained from the depository BCRC (Taiwan). The depository website reports, the strain as fruiting body and location of its isolation as Nantou, Taiwan. It is not mentioned, where the strain was isolated from. We are requesting the strain provider depositor (BCRC) for additional information.

FDA Query 4: On page 7, the notifier states, "The Audubon Society Field Guide to North American Mushrooms lists four species of Hericium mushrooms including *H. erinaceus* (Fr.) Pers.; the edibility of all four Hericium species is characterized as "edible and very good"," and describes *H. erinaceus* as a "… commonly consumed mushroom" (pages 7-8). On page 36 the notifier states, "Lion's Mane has been consumed widely for hundreds of years." Despite these statements, the notifier does not provide references for the latter two statements, nor do they elaborate on the edibility of *H. erinaceus* in further detail. For the administrative record, please briefly elaborate on these statements, and provide relevant references, as applicable.

Response: Sorry for our oversight. We apologize that we did not cite the exact references above statements. Apparently, the above described statements in the GRAS notice are based on internet searches for general information on mushroom, including *H. erinaceus*. We checked the first edition of Audubon Society Field Guide (Lincoff, 1981), in this reference the edibility of *H. erinaceus*, is described as "choice", which can be interpreted as edible and very good. We also note from internet searches that subsequent to the first

edition, there have been several editions of Audubon guides that also describe the *H. erinaceus*. The available general information from internet searches indicate that *H. erinaceus* is consumed for long.

H. erinaceus is widely consumed in Asian countries for its nutritional and health benefits (Friedman, 2015). It is widely cultivated, mainly in Asia (China, Japan, Malaysia and others) (Gry and Andersson, 2014). Since ancient times, *H. erinaceus* has been a popular species due to its nutritional value and traditional therapeutic benefits in China (Khan et al. 2013). Traditionally used for more than 1,000 years, *Hericium* was found to have various pharmacological effects. Historically, the first strains of *Hericium* were cultivated in China and belonged to the species *H. erinaceus*. Later this species became the commercial *Hericium* strain for cultivation (Gonkhom et al., 2021). *Hericium* is economically important, since the mushrooms are valuable resources for agricultural, food, and traditional therapeutic applications (Gonkhom et al., 2021). There are no intoxications reported after consumption of Bearded Tooth mushroom (*H. erinaceus*) (Gry and Anderson, 2014).

In several review articles, historical uses, bioactive components, nutritional value, and application in functional food of *H. erinaceus* are extensively discussed (He et al., 2017; Kumar, 2022; Gravina et al., 2023; Łysakowska et al., 2023). Kumar (2022) reported that *H. erinaceus* is an important edible mushroom widely distributed throughout North America, Europe, China and Japan with good dietetic and pharmacological activities without harmful effects. This mushroom is being used in traditional Chinese medicine and people in Asia use it for both culinary and medicinal purposes. It has a mild pleasant taste and can be used for salads, soups, or as a delicious side dish. Gravina et al. (2023) analyzed available evidence on the digestive therapeutic potential of *H. erinaceus* mushroom as well as the possible underlying molecular mechanisms.

FDA Query 5: On page 11, the notifier states, "The purity of the production strain is ensured by conducting an identification of the subcultured strain … The identity of the strain is validated throughout the production." Please briefly explain what "an identification" refers to in this context.

Response: The validation process was to compare the production strain with the original obtained strain inoculated on a MEA (Malt Extract Agar) and to ensure there is no contamination.

FDA Query 6: In Table 3 (page 9), the notifier lists the method used to detect Salmonella serovars as the FDA Bacteriological Analytical Manual (BAM), chapter 4, which corresponds to "Enumeration of Escherichia coli and the Coliform Bacteria." Please clarify whether the intended reference should be "FDA BAM, chapter 5, Salmonella."

Response: Sorry for the confusion, the intended reference should have been FDA BAM, Chapter 5, Salmonella.

FDA Query 7: On page 9, the notifier states, “Erinacine A, a type of diterpenoids (triterpenes/triterpenoids), found in Lion's Mane mushroom is not present in the Lion's Mane β-glucan preparation, the subject of this present GRAS.” Please provide analytical data to support this statement. In addition, we note that erinacine A is a type of diterpenoid, not triterpene or triterpenoid.

Response: We agree that erinacine A is a diterpenoid. In the GRAS notice, we inadvertently included (triterpenes/triterpenoids) after diterpenoid. The analytical data from one batch for Erinacine A is provided in Appendix I (below given pdf file).

FDA Query 8: The notifier provides a safety narrative in Part 6 of the GRAS notice (page 21).

a. Please provide an updated literature search including the date (month and year) the literature search was performed and discuss the identity and safety of *H. erinaceus*.

Response: The updated literature search was performed on July 26, 2023. As such no significant new publications were found on *H. erinaceus* for its safety or identity. Majority of the articles were related to the efficacy of *H. erinaceus* or ingredients derived from it. Some of the articles are described below.

Roda et al. (2022) reported that *H. erinaceus* has been demonstrated to display a variety of physiological effects and represents an attractive natural source for developing novel therapeutics and functional foods, based on the identification of its active ingredients and metabolites.

In a review article, Friedman (2015) extensively described the chemistry, nutrition, and health-promoting properties of *H. erinaceus* mushroom fruiting bodies and mycelia and their bioactive compounds. The authors noted, because this culinary-medicinal mushroom species, widely consumed in Asian countries but apparently not in the United States, seems to lack toxicity in rodents and humans, mushroom growers, nutritionists, and physicians should consider recommending these mushrooms to their clients and patients. The apparent exceptional beneficial properties will, hopefully, induce mushroom growers in the United States to produce *H. erinaceus* mushrooms for marketing in stores and restaurants. Some of the publications found in the recent search are also described earlier in response to FDA Query 4.

b. When discussing previous, related GRAS notices evaluated by FDA that received “no questions” response letters, the notifier did not include GRNs 000437 and 000544, the subjects of which were both β-glucans from oat bran as a source of fiber in various foods. For the administrative record, please briefly discuss whether these two GRAS notices affect the notifier’s GRAS conclusion.

Response: Thank you for bringing this to our attention. Please see below a brief description of the two GRAS notices on beta-glucan from oat (GRNs 000437 and 000544):

GRN 544. β-Glucans from Oat Brans[4]:

In this GRAS notice, submitted to FDA in 2014, Tate and Lyle informed FDA that oat β-glucan is GRAS, through scientific procedures, for use as a source of fiber in food in general, at a level not to exceed current good manufacturing practices (cGMP). In this GRAS notice, the notifier discussed the safety assessment of oat derived β-glucan, based on the history of oat consumption as well as the similarity of oat β-glucan with barley β-glucan. In this GRAS notice, published studies on the similar metabolic fate of oat, barley, and wheat β-glucan in animals and humans are discussed. The notifier explained that safety studies conducted with barley β-glucan can be used to corroborate the safety of oat β-glucan because: (a) the chemical structures and physiological properties of β-glucan from oats and barley are similar, and (b) they are metabolized similarly in the gastrointestinal tract. The notifier discussed the published animals and human studies with barley β-glucan that include three 28-day oral toxicity studies in mice and rats and a 42-day oral toxicity study in male rats. The investigators of the barley β-glucan oral toxicity studies in rats and mice concluded that it was well tolerated and no adverse effects were observed at the highest levels of barley β-glucan consumed (i.e., 19.0 g/kg bw/day in male and 23.6 g/kg bw/day in female mice; and 5.8 g/kg bw/day in both male and female rats). In this GRAS notice, the findings from several published human studies evaluating the potential effects of β-glucan products, were also described. In these studies, there were no significant adverse effects and only mild, transient GI effects were noted following a shift from a low to high fiber diet (≥ 6g β-glucan/day). The notifier also discussed the possible effects of oat β-glucan on mineral absorption and noted that there were no safety concerns. Based on its consideration of all these studies, the notifier concluded that oat β-glucan is GRAS for its intended use. The FDA reviewed the notice and did not question the notifier's conclusion that oat β-glucans is GRAS under the intended conditions of use.

GRN 437. β-Glucans from Oat Brans[5]:

In 2012, Garuda International submitted a GRAS notice on oat β-glucans to FDA. In this notice, oat β-glucan was determined as GRAS, based scientific procedures, for use as a source of fiber in a variety of foods at levels resulting in 0.75 to 3.0 g of β-glucans per serving. In this GRAS notice, findings that were based on the identity and source of oat bran β-glucans, the similarities of oat bran β-glucans to those from other edible cereals and fungi, the absence of adverse effects in published safety studies conducted on oat-derived β-glucans, and the history and current regulatory status of β-glucans in food, were summarized. The notifier discussed the safety assessment of oat bran β-glucans, based on the history of oat consumption as well as on the similarity of β-glucans from oat bran to those from barley and yeast. Garuda claimed that as the chemical structures and physiological properties of β-glucans from barley,

[4] Accessible at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=544
[5] Accessible at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=437

yeast, and oat are similar, and as they are handled similarly by the gastrointestinal tract, safety studies conducted with barley and yeast β-glucans can be used to corroborate the safety of oat bran β-glucans. In this GRAS notice, findings from published studies conducted using barley and yeast β-glucans were discussed. These studies with barley β-glucans included 28-day toxicity studies in mice and in rats, and the published yeast β-glucans 90-day toxicity study in rats. No adverse effects were noted in the short-term as well as subchronic toxicity study (0.1 g/kg bw/day) at the highest dose tested. Garuda concluded that the available studies on barley and yeast β-glucans support the safety of oat bran β-glucans for the intended use. The FDA reviewed the notice and did not question the Garuda's conclusion that oat β-glucans is GRAS under the intended conditions of use.

FDA Query 9: Comment for the administrative record; response not requested. On page 34, the notifier states, "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." For the administrative record, we note that per the Food Allergy Safety, Treatment, Education, and Research Act, sesame has been added as one of the major food allergens.

Response: Thank you for bringing this to our attention. Sorry for our oversight.

FDA Query 10: In Appendix I, the batch results for trans fat are reported as "ND." Please provide a limit of detection (LOD) for the analytical method used to determine trans fat levels in the ingredient.

Response: The limit of detection (LOD) for trans-fat by Gas Chromatography is 0.01% (1 ppm).

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

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Appendix I- Analytical Report for Erinacine A

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Erinacine A residue analytical report

The testing of the powder sample received on May 26, 2021 has been completed (see below).

Sample Identification	Results Erinacine A
RM20210426MBGSLM *H. erinaceus* (Lion's Mane) β-Glucan powder	BLD*

*BLD represents below the lower limit of detection. Amounts below the lower limit of detection (LOD) at 0.5 ng/mL cannot be reliably detected.

Test Method Description

The *H. erinaceus* (Lion's Mane) β-glucan extract powder (500 grams) was processed using 95% ethanol. The resulting solution underwent concentration and fractionation through solvent partition between ethyl acetate (EtOAc) and water, yielding distinct H2O and EtOAc layers. Subsequently, the EtOAc layer was subjected to silica gel column chromatography, following established methodologies[1, 2]. Modifications were applied to the HPLC analysis of erinacine A. The analytical column used was a COSMOSIL 5C18-AR-II (250 x 4.6 mm; particle size 5 µm, Nacalai USA, Inc., Kyoto, Japan). Both HPLC and LC-MS analyses were performed on the *H. erinaceus* (Lion's Mane) β-glucan extract powder. The peak's retention time was measured at 7.5 minutes (UV detection at 340 nm), with lower limit of quantitation (LOQ) at 0.5 ng/mL.[3]

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