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SENT *VIA* FEDEX

January 11, 2008

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notice for Yeast Beta-Glucan

Dear Dr. Martin:

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting in triplicate, as the notifier [Biothera, Inc., 3388 Mike Collins Drive, Eagan, MN, 55121], a Notice of the determination, on the basis of scientific procedures, that yeast beta-glucan, as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act.* Information setting forth the basis for the GRAS determination, which includes a comprehensive summary of the data available and reviewed by an independent panel of experts (the Expert Panel) in support of the safety of yeast beta-glucan under the intended conditions of use, as well as *curricula vitae* evidencing the qualifications of the members of the Expert Panel for evaluating the safety of food ingredients, also is enclosed for review by the agency.

I trust that the enclosed Notice is acceptable. Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

Dr. Donald J. Cox
Vice President of R&D and Business Development
Healthcare Group
Biothera, Inc.

Enclosures

3388 Mike Collins Drive
Eagan - Minnesota 55121

www.biotherapharma.com

I GRAS Exemption Claim

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

Yeast beta-glucan, as defined in the report in Appendix I entitled, "**EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF YEAST BETA-GLUCAN FOR USE AS A FOOD INGREDIENT**", dated August 29, 2007, has been determined by Biothera, Inc (Biothera) to be Generally Recognized As Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act* This determination is based on scientific procedures, as described in the following sections, and on the consensus opinion of an independent panel of experts qualified by scientific training and expertise to evaluate the safety of yeast beta-glucan under the intended conditions of use. Therefore, the use of yeast beta-glucan as a food ingredient, as described below is exempt from the requirement of premarket approval

Signed,

11JAN08

Dr Donald J Cox
Vice President of R&D and Business Development
Healthcare Group
Biothera, Inc

Date

RECEIVED
JAN 16 2008
BY:

B. Name and Address of Notifier

Donald J Cox, Ph.D
Vice President of R&D and Business Development
Healthcare Group
Biothera, Inc
3388 Mike Collins Drive
Eagan, MN 55121

C. Common Name of the Notified Substance

Yeast beta-glucan

D. Conditions of Intended Use in Food

Biothera intends to market yeast beta-glucan, derived from food-grade bakers yeast (*Saccharomyces cerevisiae*), as a food ingredient in the United States in a variety of food products including baked goods and baking mixes, beverages and beverage bases, cereal and

cereal products, dairy product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, soft candy, and soup and soup mixes at a level of up to 200 mg/serving (see Table 2 of Appendix I) Yeast beta-glucan is not intended for use in meat or meat-containing products.

The consumption of yeast beta-glucan from all proposed food uses was estimated using the United States Department of Agriculture 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996) survey (USDA, 2000). Under the intended conditions of use, the total population all-user mean intake of yeast beta-glucan was estimated to be 413.02 mg/person/day (8.90 mg/kg body weight/day), with an estimated heavy consumer (90th percentile) all-user intake of 827.32 mg/person/day (20 66 mg/kg body weight/day). On an absolute basis, the greatest mean and 90th percentile all-user intakes of yeast beta-glucan were estimated to occur in male teenagers, at 580 95 and 1,186.49 mg/ person/day, respectively (see Table 3 of Appendix I) On a body weight basis, the greatest mean and 90th percentile all-user intakes of yeast beta-glucan were highest in infants, with intakes of 27.88 and 53.96 mg/kg body weight/day, respectively (see Table 4 of Appendix I); however, it should be noted that the intended food uses will not be marketed directly for use in infant products (*i e* , yeast beta-glucan is not intended to be incorporated in infant formula/foods or toddler foods), and hence the actual infant and toddler consumption of yeast beta-glucan is expected to be limited

E. Basis for the GRAS Determination

Pursuant to 21 CFR §170.30, yeast beta-glucan has been determined by Biothera to be GRAS on the basis of scientific procedures (U S FDA, 2007a). This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of yeast beta-glucan as a component of food The safety of yeast beta-glucan is based on data generally available in the public domain on the ingredient itself and other yeast-derived beta-glucan ingredients, as discussed herein and in the accompanying documents [see Appendix I, **EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF YEAST BETA-GLUCAN FOR USE AS A FOOD INGREDIENT**].

F. Availability of Information

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

Biothera, Inc.
3388 Mike Collins Drive
Eagan, MN
55121

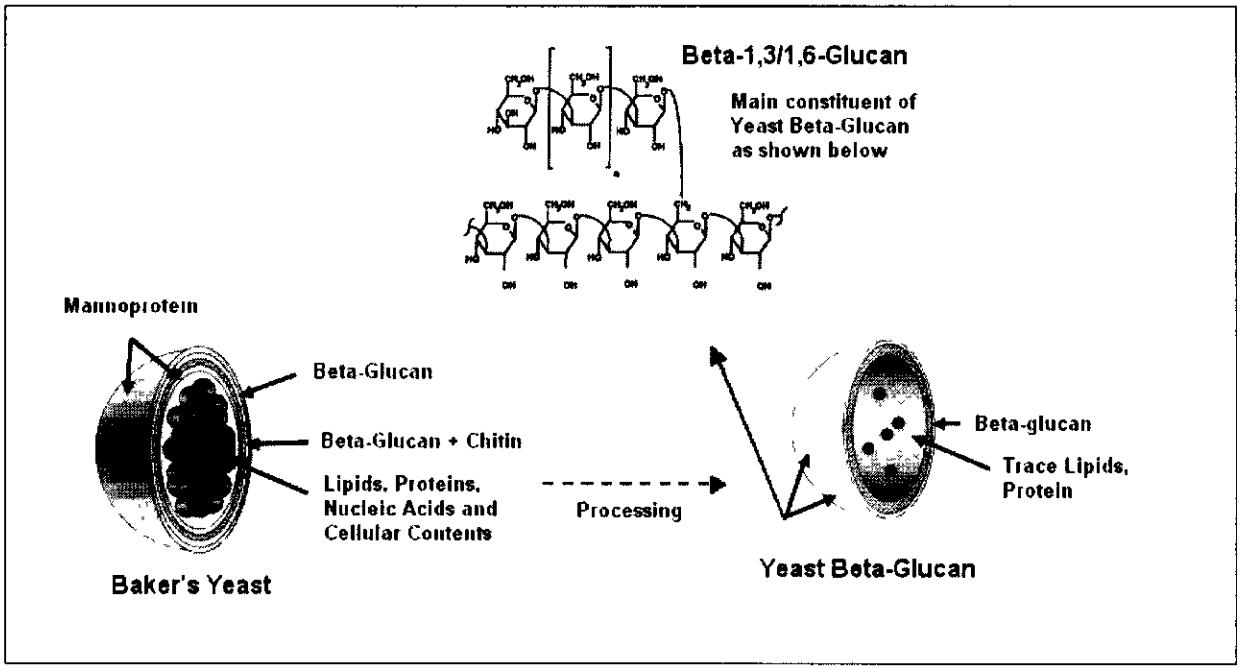
Should the FDA have any questions or additional information requests regarding this Notice, Biothera also will supply these data and information.

II. Detailed Information About the Identity of the Substance

A. Identity

The common or usual name of this product is yeast beta-glucan. Biothera's yeast beta-glucan is a dried spray-dried yeast extract powder with fine beige to tan coloration. The ingredient contains at least 70% insoluble beta-1,3/1,6-glucan, and comprises a physical make-up that is essentially represented as a mixture of hollow spheres or "ghost" cells as depicted in Figure 1 In addition to trace amounts of protein and lipids, small amounts of beta-1,6-glucan and chitin also are expected to be present in the final product

Figure 1 Pictorial Representation of Yeast Beta-Glucan



A typical preparation of Biothera's yeast beta-glucan consists of "hollow" yeast cell wall spheres approximately 2 to 4 microns in diameter

Common or Usual Name: Yeast beta-glucan
Chemical Name: (1-3),(1-6)-β-D-glucan, Poly-(1-6)-β-D-glucopyranosyl-(1,3)-β-D-glucopyranose

Chemical Abstracts Service (CAS) Number: Not available
Empirical Formula and Formula Weight: Not available

Structural Formula: See Figure 1.

B. Manufacturing

Yeast beta-glucan is manufactured according to current Good Manufacturing Practices (cGMP) using appropriate food grade reagents and ingredients The S. cerevisiae used in the production of yeast beta-glucan is standard food-grade Baker's yeast, which is grown at Biothera's production plant under controlled conditions in stainless steel fermentation vessels The yeast cells are lysed to extract the beta-glucan component of the cell wall, which is purified to produce the final ingredient. Autolysis of the fermentation mixture occurs in the fermentation vessel where the cells are lysed using a thermal process that takes place over several days. The yeast extract is then separated from the cell wall using centrifugal separation Following centrifugation, the total solids content of the cell wall is 10 to 12% of the original mass. The cell wall isolate then undergoes a caustic treatment to strip the mannosylated cell wall proteins, which are linked to the cell wall by alkali sensitive bonds. This step also removes any residual cellular lipids trapped within the cell wall that become soluble under alkaline pH Subsequently, the ingredient undergoes an acid treatment, which results in the depolymerization and deacetylation of chitin to form free glucosamine hydrochloride, and essentially results in the removal of most of the chitin. Next, the yeast wall slurry undergoes subsequent flash sterilization and pH adjustment steps resulting in a sterile solution with an approximate pH range 5-6. The resulting mixture contains between 6 to 12% of the original solids and is essentially pure beta-1,3/1,6-glucan. The mixture is then spray-dried, and once dry, the powder is sieved through a mesh sifter. The final beta-1,3/1,6-glucan product is then packaged into cardboard boxes with 10 kg double inner bags made out of polyethylene

C. Specifications for Food Grade Material

In order to ensure consistent product, Biothera has established specifications for the final ingredient (see Table 1), which are similar to those described for bakers yeast glycan (U.S. FDA, 2007b). Representative lots are routinely assayed to ensure compliance with final product chemical, physical, and microbiological specifications. The ingredient comprises mainly carbohydrates (at least 80%), with minor amounts of protein and fat, all of which already are present in the human diet *via* the consumption of bakers yeast and ingredients derived thereof. Biothera's yeast beta-glucan meets appropriate food-grade specifications and all processing aids used in the manufacture of the ingredient (*i e.,* pH-adjusting agents) are appropriate for food use. The results of studies of the stability of yeast beta-glucan indicated that the ingredient is very stable under normal storage conditions (*i.e.*, up to 5 years).

Table 1 Product Specifications for Biothera's Yeast Beta-Glucan

Specification Parameter	Specification	Method of Analysis
Physical Specifications		
Description	Fine beige/tan powder	Visual inspection
Taste	Bland	Taste
Odor	Faint/mild	Olfactory
Chemical Specifications		
Total Carbohydrate (%)	At least 80	By difference
Beta-glucan (1,3/1,6) (%)	At least 70[a,b]	Internal Method
Protein (%)	<10	AOAC Method 990 03
Fat (%)	<20	AOAC Method 989 05
Ash (%)	<5	AOAC Method 942 05
Moisture (%)	<8	AOAC Method 930.15
Lead (ppm)	<0 5	AOAC Method 984 27
Microbiological Specifications		
Aerobic Plate Count (CFU/g)	<20,000	AOAC Method 966 23
Yeast and Mold (CFU/g)	≤25	U S. FDA BAM, 7th ed.
Coliforms (CFU/g)	<10	AOAC Method 966 24
Escherichia coli	Negative	AOAC Method 966 08
Salmonella sp	Negative	AOAC Method 966 24

AOAC = Association of Analytical Communities; CFU = colony-forming units; U.S FDA BAM = U.S. Food and Drug Administration standard bacterial analytical manual methodologies (U.S. FDA, 1998)
[a] For commercial purposes, the product will be designated WGP® 3-6 if it contains a beta-glucan content of at least 75%, and otherwise will be designated BetaRight® 3-6.
[b] Small amounts of beta-1,6-glucan and chitin also are expected to be present in the final product

The final yeast beta-glucan ingredient is a fine beige to tan powder containing at least 70% insoluble beta-1,3/1,6-glucan and is sold as BetaRight® 3-6 (at least 70% beta-glucan) or WGP® 3-6 (at least 75% beta-glucan). Although BetaRight® 3-6 and WGP® 3-6 exist as separate commercial products, it should be noted that only one cGMP manufacturing process is used in the manufacture of Biothera's yeast beta-glucan ingredient(s), and the final ingredient is designated as either BetaRight® 3-6 or WGP® 3-6 based on the beta-glucan content of the final product.

Volatile organic compounds (VOCs) were identified as a by-product of the production process used to make the yeast beta-glucan ingredient (*i.e.*, small amounts of methanol, ethanol, isopropanol, acetone, and hexane can be detected in the final product), even though no organic solvents are used at any stage of the manufacturing process. Biothera determined that the VOCs are produced during the manufacturing process *via* fatty acid decomposition reactions as a result of removal of the lipids using an alkali wash procedure, and that the presence of trace VOCs are common to many yeast food ingredients and beta-glucan dietary supplements. The

levels of these compounds are present in a highly consistent manner from batch-to-batch, and the maximum level of VOC production will be restricted by quality control procedures that limit the levels of primary reactant (the lipid content) required for VOC formation in the final product to no more than 7%, which is well below the specification for this parameter Based on the levels of VOCs in the final product and the fact that the estimated intakes of these compounds will be below upper limits established by the U.S. Environmental Protection Agency and relevant pre-clinical toxicological information, the exposure to VOCs from the consumption of yeast beta-glucan from the estimated intake is not expected to produce adverse effects on human health.

III. Self-Limiting Levels of Use

The levels of use of yeast beta-glucan will be limited due to food formulation issues, as the ingredient will impart undesirable characteristics to the food regarding taste, flavor, and viscosity Specific thresholds for these effects have not been determined.

IV. Basis for GRAS Determination

Pursuant to 21 CFR §170 30, yeast beta-glucan, under the intended conditions of use specified herein and defined in Appendix I **[EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF YEAST BETA-GLUCAN FOR USE AS A FOOD INGREDIENT]** has been determined by Biothera to be GRAS on the basis of scientific procedures (U.S FDA, 2007a) This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of yeast glucan as a component of food.

The safety of yeast beta-glucan is based on data generally available in the public domain on the ingredient itself and other yeast-derived beta-glucan ingredients. These data include a published subchronic oral toxicity study of WGP® 3-6 in rats in which no compound-related toxicity was observed at the highest permissible dose (Babíček *et al* , 2007) and pre-clinical studies of other beta-1,3-glucan test articles, as well as other scientific data pertaining to the safety of yeast beta-glucan, and in particular, published clinical trials of beta-glucan ingredients. The safety of yeast beta-glucan is supported by the compositional similarity of the ingredient to bakers yeast glycan, which is approved by the FDA for direct addition as a multi-purpose food additive to various food products including salad dressing, frozen desserts, sour cream and cheese spread analogs, and flavored snack dips (21 CFR §172.898) (U.S. FDA, 2007b), as well as the background consumption of bakers yeast and ingredients derived thereof Additionally, the safety of Biothera's yeast beta-glucan is supported by unpublished human trials of the ingredient. Moreover, bakers yeast, has been used for over a thousand years as a food ingredient, and its most common present day and historical uses are in the production of bread and in the fermentation process of wine and beer (Mortimer, 2000), and significant levels of beta-1,3-glucans also occur naturally in a number of foods, such as edible mushrooms, celery,

chi-chian leaves, carrot, radish, and soybeans (Ko and Lin, 2004) Therefore, the consumption of Biothera's yeast beta-glucan will not result in exposure to any substance that is not already present in the human diet as a result of the inherent composition and use of bakers yeast glycan and its source, *S. cerevisiae*, as well as other natural foods In addition, all of the components of yeast beta-glucan (*i.e.*, carbohydrates, proteins, and fats) are expected to be subject to known metabolic processes. Using these data to support the safety of the ingredient, yeast beta-glucan is GRAS based on scientific procedures. A summary of this information is provided in Appendix I **[EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF YEAST BETA-GLUCAN FOR USE AS A FOOD INGREDIENT]**.

V. References

Babiček, K., Čechová, I.; Simon, R R., Harwood, M., Cox, D.J. 2007. Toxicological assessment of a particulate yeast (1,3)-β-D glucan in rats. Food Chem Toxicol 45(9)·1719-1730.

Ko, Y.-T ; Lin, Y.-L. 2004. 1,3-β-glucan quantification by a fluorescence microassay and analysis of its distribution in foods J Agric Food Chem 52(11):3313-3318.

Mortimer, R K 2000. Evolution and variation of the yeast (*Saccharomyces*) genome Genome Res 10(4)·403-409 & [Erratum, 10(6)891].

USDA 2000. 1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS) (On CD-ROM). U.S. Department of Agriculture (USDA); Riverdale, Maryland. [PB2000-500027].

U S FDA. 1998. Bacteriological Analytical Manual [BAM] (8th Ed.). U.S. Food and Drug Administration (U.S FDA), Center for Food Safety and Applied Nutrition (CFSAN), College Park, Maryland. Available from· http·//www.cfsan.fda.gov/~ebam/bam-toc html.

U.S. FDA. 2007a. Part 170—Food labeling Section § 170 30— Eligibility for classification as generally recognized as safe (GRAS). In: U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (U.S. Food and Drug Administration). U S Government Printing Office (GPO); Washington, DC, pp 13-15

U.S. FDA 2007b. Part 172—Food additives permitted for direct addition to food for human consumption. §172.898—Bakers yeast glycan. In. U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (U S Food and Drug Administration). U.S. Government Printing Office (GPO), Washington, DC, p 120.

APPENDIX I

EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF YEAST BETA-GLUCAN FOR USE IN AS A FOOD INGREDIENT

Appendix 1

EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF YEAST BETA-GLUCAN FOR USE AS A FOOD INGREDIENT

INTRODUCTION

At the request of Biothera Inc. (Biothera), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information on yeast beta-glucan, and determine whether, under the conditions of intended use as a food ingredient, yeast beta-glucan, derived from bakers yeast, *Saccharomyces cerevisiae*, would be safe and Generally Recognized as Safe (GRAS) based on scientific procedures. In the United States, bakers yeast extract is affirmed as GRAS for direct addition to food, and bakers yeast protein and glycan are permitted direct food additives. The Panel consisted of the below-signed qualified scientific experts. Prof Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University, Medical College of Virginia), Prof. Robert J. Nicolosi, Ph.D (University of Massachusetts Lowell), and Prof Gary M Williams, M.D (New York Medical College) *Curricula vitae* evidencing the Panel members' qualifications for evaluating the safety of food ingredients are provided in Attachment 1

The Panel, independently and collectively, critically examined a comprehensive package of data provided by Biothera and compiled by Cantox Health Sciences International (Cantox). In addition, the Panel evaluated other information deemed appropriate or necessary, including scientific data obtained from the literature and other published sources through September 2006 by Cantox. The information evaluated by the Panel included data pertaining to the method of manufacture and product specifications of the yeast beta-glucan ingredient, supporting analytical data, information establishing the compositional similarity of Biothera's yeast beta-glucan to bakers yeast glycan, the intended use levels of yeast beta-glucan in specified food products, consumption estimates for all intended uses, background dietary consumption of bakers yeast and ingredients derived thereof, and a comprehensive assessment of the available scientific literature pertaining to the safety of yeast and other beta-glucan ingredients.

Following independent critical evaluation of such data and information, the Panel convened on 27 November 2006 and unanimously concluded that the proposed uses in traditional foods described herein for yeast beta-glucan derived from *S. cerevisiae*, meeting appropriate food-grade specifications and manufactured according to current Good Manufacturing Practice (cGMP), are safe. The Panel further concluded that these uses are GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below

MANUFACTURING AND COMPOSITION

The *S. cerevisiae* used in the production of yeast beta-glucan is standard food-grade bakers yeast, which is grown at Biothera's production plant under controlled conditions in stainless steel fermentation vessels. The yeast cells are lysed to extract the beta-glucan component of the cell wall, which is purified to produce the final ingredient Yeast beta-glucan is manufactured according to cGMP and comprises mainly carbohydrates (at least 80%), with minor amounts of protein and fat, all of which already are present in the human diet *via* the consumption of bakers yeast and ingredients derived thereof The final yeast beta-glucan ingredient is a fine beige to tan powder containing at least 70% insoluble beta-1,3/1,6-glucan and is sold as BetaRight® 3-6 (at least 70% beta-glucan) or WGP® 3-6 (at least 75% beta-glucan). Although BetaRight® 3-6 and WGP® 3-6 exist as separate trade products, it should be noted that only one cGMP manufacturing process is used in the manufacture of Biothera's yeast beta-glucan ingredient(s), and the final ingredient is designated as either BetaRight® 3-6 or WGP® 3-6 based on the beta-glucan content of the final product

In order to ensure consistent product(s), Biothera has established specifications for the final ingredient(s) (see Table 1), which are similar to those described for bakers yeast glycan (U S FDA, 2007a). Cantox reviewed batch analysis data submitted by Biothera and concluded that a consistent product can be produced. Representative lots are routinely assayed to ensure compliance with final product chemical, physical, and microbiological specifications Biothera's yeast beta-glucan meets appropriate food-grade specifications and all processing aids used in the manufacture of the ingredient (*i.e* , pH-adjusting agents) are appropriate for food use. The results of studies of the stability of yeast beta-glucan indicated that the ingredient is very stable under normal storage conditions (*i.e* , up to 5 years)

Table 1 Product Specifications for Biothera's Yeast Beta-Glucan

Specification Parameter	Specification	Method of Analysis
Physical Specifications		
Description	Fine beige/tan powder	Visual inspection
Taste	Bland	Taste
Odor	Faint/mild	Olfactory
Chemical Specifications		
Total Carbohydrate (%)	At least 80	By difference
Beta-glucan (1,3/1,6) (%)	At least 70[a]	Internal Method
Protein (%)	<10	AOAC Method 990 03
Fat (%)	<20	AOAC Method 989 05
Ash (%)	<5	AOAC Method 942 05
Moisture (%)	<8	AOAC Method 930 15
Lead (ppm)	<0 5	AOAC Method 984 27

Table 1 Product Specifications for Biothera's Yeast Beta-Glucan		
Specification Parameter	**Specification**	**Method of Analysis**
Microbiological Specifications		
Aerobic Plate Count (CFU/g)	<20,000	AOAC Method 966 23
Yeast and Mold (CFU/g)	≤25	U S FDA BAM, 7th ed
Coliforms (CFU/g)	<10	AOAC Method 966 24
Escherichia coli	Negative	AOAC Method 966 08
Salmonella sp	Negative	AOAC Method 966 24

AOAC = Association of Analytical Communities, BAM = standard bacterial analytical manual methodologies (U S FDA, 1998), CFU = colony-forming units, U S EPA = U S Environmental Protection Agency, U S FDA = U S Food and Drug Administration

[a] For trade purposes, the product will be designated WGP® 3-6 if it contains a beta-glucan content of at least 75%, and otherwise will be designated BetaRight® 3-6

Volatile organic compounds (VOCs) were identified as a by-product of the production process used to make the yeast beta-glucan ingredient, and small amounts of methanol, ethanol, isopropanol, acetone, and hexane can be detected in the final product (<744 ± 70 ppm, on average), even though no organic solvents are used at any stage of the manufacturing process Biothera determined that the VOCs are produced during the manufacture of the yeast beta-glucan ingredient *via* fatty acid decomposition reactions as a result of removal of the lipids using an alkali wash procedure. Biothera also determined that the presence of trace VOCs are common to many yeast food ingredients and beta-glucan dietary supplements. As the VOC are produced during a GMP production process, the levels of these compounds are therefore present in a highly consistent manner from batch-to-batch. In addition, the maximum level of VOC production will be restricted by quality control procedures that limit the levels of primary reactant (the lipid content) required for VOC formation in the final product to no more than 7%, which is well below the specification for this parameter Based on the level of VOCs in the final product and the fact that the estimated intakes of these compounds will be below upper limits established by the U S Environmental Protection Agency (EPA) and relevant pre-clinical toxicological information, the exposure to VOCs from the consumption of yeast beta-glucan from the estimated intake is not expected to produce adverse effects on human health.

INTENDED USE AND ESTIMATED EXPOSURE OF YEAST BETA-GLUCAN

Biothera intends to market yeast beta-glucan as a food ingredient in the United States in a variety of food products including baked goods and baking mixes, beverages and beverage bases, cereal and cereal products, dairy product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, soft candy, and soup and soup mixes at a level of 200 mg/serving. The individual proposed food-uses and use-levels are summarized below in Table 2 The use of yeast beta-glucan in food and beverage products is self-limiting due to certain food formulation issues, such as taste, flavor, and viscosity.

Table 2 Proposed Food Uses and Use Levels for Yeast Beta-Glucan				
Food Category	**Proposed Food-Use**	**RACC[a] (g or mL)**	**Yeast Beta-Glucan Level[b]**	
			(mg/serving)	**(%)**
Baked Goods and Baking Mixes	Cookies	30 to 40	200	0 5 to 0 667
Beverages and Beverage Bases	Meal Replacement Beverages (not Milk-Based)	240	200	0 083
Cereal and Cereal Products	Nutritional Bars (Breakfast, Granola, Protein)	40	200	0 500
Dairy Product Analogs	Soy Milk	240	200	0 083
Milk and Milk Products	Meal Replacement Beverages	240	200	0 083
	Probiotic Beverages	240	200	0 083
	Yogurt	225	200	0 089
	Yogurt Beverages	240	200	0 083
Plant Protein Products	Soy Protein Bars	40	200	0 500
Processed Fruits and Fruit Juices	Fruit Beverages (Drinks, Juices, Smoothies)	240	200	0 083
Soft Candy	Chocolate Confections	40	200	0 500
Soup and Soup Mixes	Soups	245	200	0 082

[a] RACC = Reference Amounts Customarily Consumed Per Eating Occasion [21 CFR §101 12 (U S FDA, 2007b)] When a range of values is reported for a proposed food use, particular foods within that food use may differ with respect to their RACC

[b] The intended use levels of up to 200 mg/serving comprise the maximum use level for each trade ingredient separately (*i e* , BetaRight® 3-6 or WGP® 3-6) or for both ingredients combined (*i e* , up to 200 mg BetaRight® 3-6 and WGP® 3-6 combined), therefore, for the purpose of this report, the intended uses of the ingredient(s) are stated simply as yeast beta-glucan

Using the United States Department of Agriculture (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996) survey (USDA, 2000), under the conditions of intended use, the total population all-user mean intake of yeast beta-glucan was estimated to be 413.02 mg/person/day (8 90 mg/kg body weight/day), with an estimated heavy consumer (90th percentile) all-user intake of 827 32 mg/person/day (20 66 mg/kg body weight/day). On an absolute basis, the greatest mean and 90th percentile all-user intakes of yeast beta-glucan were estimated to occur in male teenagers, at 580 95 and 1,186.49 mg/person/day, respectively (see Table 3). On a body weight basis, the greatest mean and 90th percentile all-user intakes of yeast beta-glucan were highest in infants, with intakes of 27 88 and 53 96 mg/kg body weight/day, respectively (see Table 4), however, it should be noted that the intended food uses will not be marketed directly for use in infant products (*i e* , yeast beta-glucan is not intended to be incorporated in infant formula/foods or toddler foods), and hence the actual infant and toddler consumption of yeast beta-glucan is expected to be limited.

Table 3	Summary of the Estimated Daily Intake of Yeast Beta-Glucan from All Proposed Food Categories in the U.S. by Population Group (1994-1996, 1998 USDA CSFII Data)						
Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (mg)	90th Percentile (mg)	Mean (mg)	90th Percentile (mg)
Infant	0 to 2	71 6	2,564	273 25	636 11	350 34	693 21
Child	3 to 11	98 4	6,205	475 32	869 07	484 94	870 62
Female Teenager	12 to 19	91 5	642	403 57	856 81	441 20	886 05
Male Teenager	12 to 19	91 7	638	531 93	1,134 57	580 95	1,186 49
Female Adult	20 and Up	85 0	3,887	291 07	642 50	339 21	673 10
Male Adult	20 and Up	81 6	3,876	354 34	827 75	433 08	906 93
Total Population	All Ages	86 4	17,812	356 92	786 22	413 02	827 32

Table 4	Summary of the Estimated Daily Per Kilogram Body Weight Intake of Yeast Beta-Glucan from All Proposed Food Categories in the U.S. by Population Group (1994-1996, 1998 USDA CSFII Data)						
Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Infant	0 to 2	71 6	2,564	21 70	50 50	27 88	53 96
Child	3 to 11	98 4	6,205	19 69	37 87	20 10	38 02
Female Teenager	12 to 19	91 5	642	7 49	17 47	8 21	17 72
Male Teenager	12 to 19	91 7	638	8 72	18 43	9 54	19 91
Female Adult	20 and Up	85 0	3,887	4 54	10 27	5 29	10 83
Male Adult	20 and Up	81 6	3,876	4 42	10 51	5 40	11 53
Total Population	All Ages	86 4	17,812	7 68	18 77	8 90	20 66

S cerevisiae, more commonly referred to as bakers yeast, has been used for over a thousand years as a food ingredient, and its most common present day and historical uses are in the production of bread and in the fermentation process of wine and beer (Mortimer, 2000). As mentioned, bakers yeast and various ingredients derived from bakers yeast are permitted for use as direct ingredients in certain food products for sale in the U S (U S FDA, 2007a)[1], and significant levels of beta-1,3-glucans also occur naturally in a number of foods, such as edible mushrooms, specifically the Shiitake (*Lentinus edodes*), Maitake (*Grifola frondosa*), Wood

[1] According to a survey of industrial food additive use in the United States, the use of bakers yeast in foods was reported to be 58,900 pounds/year, which on a *per capita* basis would correspond to 3.07 mg/person/day (0 04 mg/kg body weight/day) (Lucas *et al* , 1999)

Cauliflower (*Sparassis crispa Fr*), and snow mushroom (*Tremella fuciformis*) varieties (Ko and Lin, 2004) Several non-fungus-derived food sources, including celery, chi-chian leaves, carrot, and radish, contain nearly 20% beta-1,3-D-glucan in their total carbohydrate fraction, and soybeans contain up to 0.8% beta-1,3-D-glucan (dry weight) (Ko and Lin, 2004). Moreover, Biothera markets their WGP 3-6® ingredient (as Imucell WGP® 3-6) as a dietary ingredient for use in dietary supplement products, and various other yeast beta-glucan supplements are marketed in the U.S. Therefore, all of the components present in Biothera's yeast beta-glucan are already present in the diet

BASIS FOR GRAS STATUS OF YEAST BETA-GLUCAN

The safety of yeast beta-glucan is based on data generally available in the public domain on the ingredient itself and other yeast-derived beta-glucan ingredients The safety of yeast beta-glucan is supported by the compositional similarity of the ingredient to bakers yeast glycan, which is approved by the U.S. Food and Drug Administration (FDA) for direct addition as a multi-purpose food additive (21 CFR §172 898) to various food products including salad dressing, frozen desserts, sour cream and cheese spread analogs, and flavored snack dips (U.S. FDA, 2007a), the background consumption of bakers yeast and ingredients derived thereof; and other scientific data pertaining to the safety of yeast beta-glucan, including unpublished human trials of the ingredient. Moreover, the consumption of Biothera's yeast beta-glucan will not result in exposure to any substance that is not present in bakers yeast glycan or its source, *S. cerevisiae*, which are already present in the human diet, and all of the components of yeast beta-glucan (*i.e.*, carbohydrates, proteins, and fats) are expected to be subject to known metabolic processes Using these data to support the safety of the ingredient, yeast beta-glucan is GRAS based on scientific procedures. A summary of this information is provided below.

Compositional Similarity of Yeast Beta-Glucan to Bakers Yeast Glycan

As stated in the *Code of Federal Regulations* (CFR), "the FDA believes that in certain instances the concept of equivalence may have applicability to the technical element of a GRAS determination" (U S. FDA, 1997). Substantial equivalence is referred to in the joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) report as embodying "the concept that if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety (*i.e* , the food or food component can be concluded to be as safe as the conventional food or food component" (FAO/WHO, 1996; U.S. FDA, 1997).

In establishing the equivalence of a product to an existing food product it is important to determine that any differences in the new food product do not outweigh the similarities between the two products. Thus, the FDA has stated that a critical factor that must be determined when

applying the concept of equivalence is any difference in composition between the substances being compared (U.S. FDA, 1997)

Biothera's yeast beta-glucan and bakers yeast glycan can both be defined as the dried cell wall of the yeast, *S cerevisiae* The cell wall of *S cerevisiae* is predominantly composed of linear helical chains of beta-D glucose molecules bonded through 1,3 linkages, and characteristic of *S cerevisiae*, these glucan chains also contain a number of 1,6 branch points. Both food ingredients are composed almost entirely of carbohydrates, as both products are approximately 80% pure carbohydrate on a dry weight basis, and the ingredients' product specifications include similar limits for chemical and microbiological parameters. A comparison of the specifications for yeast beta-glucan and bakers yeast glycan is presented in Table 5.

Table 5 Comparison of Biothera's Yeast Beta-Glucan with Bakers Yeast Glycan

Specification	Yeast Beta-Glucan	Bakers Yeast Glycan*
Description	Fine beige/tan powder	Comminuted, washed, pasteurized, and dried cell walls of the yeast, *Saccharomyces cerevisiae*
Organism	*S cerevisiae*	*S cerevisiae*
Total Carbohydrate (%)	At least 80	At least 85
Beta-glucan (%)	At least 70	At least 57 [a]
Protein (%)	<10	na
Fat (%)	<20	na
Ash (%)	<5	na
Moisture (%)	<8	na
Lead (ppm)	<0 5	<0 2
Mercury (ppm)	<0 05	<0 05
Arsenic (ppm)	<0 1 [b]	<0 4
Selenium (ppm)	N/A	<0 09
Cadmium (ppm)	<1 [b]	<13
Zinc (ppm)	N/A	<10
Aerobic Plate Count (CFU/g)	<20,000	<10,000
Yeast and Mold (CFU/g)	≤25	<10
Coliforms (CFU/g)	<10	na
Escherichia coli	Negative	Negative
Salmonella sp	Negative	Negative
Coagulase positive *Staphylococci*, *Clostridium perfringens*, *Clostridium botulinum*, or any other recognized pathogen or harmful microbial toxin	N/A	Negative

CFU = colony forming units, N/A = not analyzed for by Biothera, na = not available per 21 CFR §172.898
*All parameters specified under 21 CFR §172 898 for bakers yeast glycan Additional details were not available
[a] The total carbohydrate fraction comprises beta-glucan and mannose units in approximately a 2 1 ratio
[b] Derived from certificates of analysis for various batches of Biothera's yeast beta-glucan

The primary difference between the two products pertains to the carbohydrate composition Bakers yeast glycan contains mannose at a ratio of 1:2 to beta-glucan, whereas mannose is not expected to be present in Biothera's yeast beta-glucan. Given that the total carbohydrate level present in bakers yeast glycan is at least 85%, and using a ratio of 1·2 of mannose to beta-glucan, the level of beta-glucan present in bakers yeast glycan is expected to be at least 56%, which is only slightly less than the beta-glucan level of Biothera's yeast beta-glucan ingredient (*i.e.*, at least 70%)

Pursuant to the CFR, bakers yeast glycan is permitted for use in salad dressing at a level not to exceed 5% of the finished product and also is permitted for use in frozen desserts, sour cream analogs, cheese spread analogs, and cheese flavored and sour cream flavored snack dips at levels that are not to exceed good manufacturing practice. These levels are at least 10-fold higher than the proposed levels of use of yeast beta-glucan in any one food category, and thus the intended conditions of use of yeast-beta glucan are not anticipated to represent an exposure that would be substantially different than exposure from the permitted uses of bakers yeast glycan.

In conclusion, based on the fact that both ingredients are derived from the cell wall of *S. cerevisiae* and analytically are similar in composition, and that the consumption of Biothera's yeast beta-glucan would result in comparable exposure to beta glucan and other constituents present in bakers yeast glycan (minus mannose), the compositional similarity of Biothera's yeast beta-glucan to bakers yeast glycan as permitted for use by the FDA and regulated under 21 CFR §172.898 (U S FDA, 2007a) supports the safe use of the ingredient in food.

Absorption, Distribution, Metabolism, and Excretion of Yeast Beta-Glucan

Biothera's ingredient is an insoluble yeast beta-glucan cell wall particulate carbohydrate. Humans are unable to digest carbohydrate polymers with beta-glucosidic linkages (Wisker *et al.*, 1985), and therefore absorption by the intestinal epithelium and significant systemic exposure to particulate yeast glucans will not occur (Lehne *et al*, 2005, Rice *et al*, 2005) Some systemic exposure following oral administration does occur, however, and is likely a rate-limited process[2] that is regulated by processing within the Peyer's patches of the small intestine and transport to the reticuloendothelial system *via* macrophages (Beier and Gebert, 1998; Hong *et al.*, 2004). Oxidative degradation of the glucan particle then occurs over several days (Hong *et al*, 2004). This is in contrast to exposure to yeast beta-glucans *via* intraperitoneal or intravenous routes, which results in significant accumulation of the carbohydrate within the organs of the reticuloendothelial system, however, due to minimal (if any) bioavailability of

[2] Although there is no direct empirical evidence to substantiate this, there is no reason to believe these compounds would be handled any differently than the consumption of Bakers yeast In addition, it should also be emphasized that the Peyer's patches are sensory organs and do not display dietary uptake functions

orally-administered yeast beta-glucan, parenteral routes of exposure are inappropriate for the safety evaluation of food ingredients that are ingested and not absorbed.

Toxicological Studies

A critical evaluation of the available acute, sub-chronic, and chronic oral toxicity data on yeast beta-glucans indicated that insoluble particulate yeast beta-1,3-glucans are of low toxicity. In addition, available information on *S cerevisiae*-derived particulate beta-1,3-glucans was evaluated for the purpose of supporting the safety of Biothera's yeast beta-glucan These studies are summarized below. Yeast beta-glucan cannot be detected in the plasma following oral administration (Lehne *et al*, 2005, Rice *et al.*, 2005) due to minimal or no absorption from the gastrointestinal tract. Studies involving parenteral exposure to yeast beta-glucans were identified and reviewed for the purpose of this report, but were not considered in the safety assessment of yeast beta-glucan under the conditions of use in food

Acute Toxicity Studies

The administration of a single oral dose of 2,000 mg yeast beta-glucan (as WGP® 3-6)/kg body weight was well tolerated in BrlHan WIST@Jcl rats, and no clinical or histopathological evidence of toxicity was observed (OECD, 2001; Babíček *et al*, 2007), which is consistent with the fact that orally administered yeast beta-glucans are not digested or absorbed to any significant degree

Studies of acute parenteral exposure to soluble beta-glucan also indicated that yeast-derived beta-glucan[3] was well tolerated in mice and rats following single intravenous doses of up to 1,000 and 500 mg/kg body, respectively, and following intraperitoneal administration of 250 mg yeast beta-glucan/kg body weight for a period of 7 days in mice, although significantly reduced weight gain was observed in guinea pigs under the same study conditions (Williams *et al*, 1988).

Sub-Chronic/Chronic Toxicity Studies

A sub-chronic oral toxicity study of yeast beta-glucan (as WGP® 3-6) administered by gavage was conducted in Fisher CDF(F-344)/CrlBR rats The animals (10/sex/group) were administered 0, 2, 33 3, or 100 mg/kg body weight/day of WGP® 3-6 for 91 consecutive days (OECD, 1998, Babíček *et al.*, 2007). Separate control and treatment (100 mg/kg body weight/day) recovery groups for each sex also were included, and were maintained for an additional 14 days without dosing following the treatment period This study was conducted consistent with Organisation for Economic Co-operation and Development (OECD) Guideline 408, which states that the maximum volume to be administered by gavage is not to exceed 10

[3] Based on references in the document, it appears that the glucan used in the experiment was a soluble beta-glucan preparation derived from *S cerevisiae*, however, detailed descriptions of the test article were not reported

mL/kg body weight Based on maximum flowable concentration , the authors stated that 100 mg/kg body weight was the highest permissible dose that could be administered by gavage Although no information was presented by the authors regarding the use of gavage *versus* inclusion of yeast beta-glucan in the food, it is known that yeast beta-glucan is bitter tasting at high doses and thus palatability issues may have prevented administration of the test article in the diet.

WGP® 3-6 was well tolerated throughout the treatment period, and no mortality, signs of morbidity, or significant differences in weight gain or food consumption were observed. A few sporadic, statistically significant, increases in red blood cell (RBC) count, mean corpuscular volume (MCV), and hematocrit (Hct) occurred in males in the highest dose group (100 mg/kg body weight/day) compared to controls; however, the differences were less than 10% and did not exceed the maximums of physiological and historical control data ranges, and the same effect was not observed in the corresponding recovery group Similarly, a dose-dependent difference in clotting times observed in male animals compared to controls that was significant at the 2 highest doses (33 3 and 100 mg/kg body weight/day; decreased by 33 and 44%, respectively) was within the historical control range, was not present in both sexes, and returned to baseline levels following withdrawal of dosing. Moreover, the authors reported that the clotting times were unexpectedly elevated in the control group; therefore, the effect was considered to be clinically non-significant. Similar to the males, a statistically significant increase in MCV was observed in females, however, the effect was modest (6%) and occurred equally in all dose groups. Isolated statistically significant changes were observed for some clinical chemistry parameters (bilirubin, glucose, sodium, potassium, cholesterol, low-density lipoprotein cholesterol, urea, and total protein); however, since the changes were within historical control values, were not dose responsive, and did not occur in both sexes, the effects were considered to be of no toxicological significance. Infrequent non-dose-related findings of very fine nodular adhesions were detected on the spleen capsule of 4 treatment animals, and 1 male in the control group developed a malignant lymphoma. No pathological findings were present in any animals subjected to necropsy after termination of the recovery period. There were some slight (≤10%) but statistically significant changes in the absolute weights and relative organ weights in treatment *versus* control groups; however, these effects were sporadic, the differences in organ weights were not connected with any pathologic processes (*i e.*, the incidence of histopathological findings for controls and high-dose animals were similar), and no connection with potential effects of WGP® 3-6 was observed. Moreover, all mean values of absolute weights were within the limits of historical variability The findings occurring more frequently (*i e* , calcifications in the female renal tubules, small hyaline casts in the male renal tubules, small foci of fibrous adhesions on the spleen capsule) were observed randomly at similar frequencies in animals of the control groups and animals treated with the test item. The authors also stated that neither proliferative, hyperplastic, nor hypertrophic alterations were observed in lymphoid organs. Based on clinical, pathological, and statistical evaluations, the authors concluded that no compound-related toxicity was observed over the 91-day

administration period, and the no observed adverse effect level (NOAEL) of WGP® 3-6 was reported to be 100 mg/kg body weight/day, the highest permissible dose tested

Feletti *et al.* (1992) investigated the effects of chronic (52 weeks) oral administration of an insoluble yeast beta-glucan granulate (99 4% purity) derived from *Candida albicans* in Sprague-Dawley rats. Animals (20/sex/group) were administered the test material by gavage over a period of 52 weeks at doses of 0, 50, 100, or 200 mg total beta-glucan/kg body weight/day. Separate recovery control groups also were included in the study No compound-related abnormalities in the clinical measures or deaths were observed over the experimental period, nor were significant differences in body weight, food consumption, or water intake observed between the control and treatment groups. There also were no significant differences in the hematology, blood chemistry, urinalysis, or organ weight measurements. The only finding attributed to beta-glucan administration was soft stools or diarrhea and cecal enlargement with variable hyperplasia of the colon mucosa in the high-dose group (200 mg/kg body weight/day), an effect that returned to normal after the cessation of treatment Based on observations of cecal enlargement at the highest dose, the authors determined the no observed effect level (NOEL) to be 100 mg/kg body weight/day. However, cecal enlargement is a well established response in rodents consuming large quantities of polyols (Newberne *et al.*, 1988), and is not considered toxicologically significant and not relevant to humans (WHO, 1987). A no observed adverse effect level (NOAEL) of 200 mg/kg body weight/day, the highest dose tested, is more appropriate

Williams *et al.* (1988) reported on a series of sub-chronic parenteral toxicity studies of soluble beta-glucan in ICR/HSD mice Bi-weekly intravenous administration of yeast-derived beta-glucan at doses of 0, 40, 200, or 1,000 mg/kg body weight to males (5/group) in 2 separate 30- and 60-day experiments did not significantly alter body weights or liver, lung, or kidney weights in mice, although, following 30 days of treatment, a non-dose-related significant increase in spleen weight was reported in mice receiving 40 or 1,000 mg/kg body weight/day compared to the control group A similar effect was observed during the 60-day study, with spleen weights increasing in a dose-dependent manner and achieving statistical significance at the highest dose (1,000 mg/kg body weight/day). No deaths occurred and there were no behavioral or appearance changes observed in any group for either experiment. The 30- and 60-day 1,000 mg/kg body weight/day dose groups showed significant histological changes in the liver, with an observation of granuloma formation and mononuclear infiltrate consisting of monocytes and an unidentified cell type the authors speculated might have been of lymphocyte origin. There were no significant histopathological changes in the lung, kidney, or spleen at any dose level for either the 30- or 60-day animals These studies are not relevant to the safety assessment of yeast beta-glucan under the intended conditions of use in food.

Mutagenicity/Genotoxicity Studies

No mutagenicity or genotoxicity studies for yeast beta-glucan were identified in the literature, although two studies indicated that yeast-derived beta-glucans display anti-mutagenic properties *in vitro* (Zhang and Ohta, 1993; Miadoková *et al.*, 2005) In addition, numerous studies document the antioxidant, chemoprotectant, and anti-cancer properties of various yeast beta-glucans *in vitro* and in animal models (Chorvatovičová and Navarová, 1992; Gomaa *et al.*, 1992, Cheung *et al* , 2002; Križková *et al* , 2003; Slameňová *et al.*, 2003, Hong *et al* , 2004; Allendorf *et al.*, 2005, Gu *et al.*, 2005), and the mushroom beta-glucan, lentinan, has been used in Japan for the treatment of cancer for a number of years (Matsuoka *et al.*, 1997; Nakano *et al* , 1999; Yoshino *et al.*, 2000).

Reproductive and Developmental Toxicity Studies

No reproductive or developmental toxicity studies of orally administered yeast beta glucans were identified in the literature Studies in CrL COBS CD (SD) BR rats investigating the potential reproductive and developmental toxicity of intravenous lentinan administration were reported by Cozens *et al.* (1981a,b,c,d). These studies were designed to assess the effects of lentinan administration in males and females prior to mating, and/or to assess the effects of lentinan *in utero* and post-natally by administration of lentinan to females during gestation and post-partum. Significant gonadal damage and impaired reproductive capacity was observed in male rats administered lentinan at doses equal to or greater than 0 10 mg/kg body weight/day, while a dose of 0 01 mg/kg body weight/day in male rats resulted in some morphological evidence of gonadal damage without resulting reproductive capacity. No significant differences in reproductive capacity of the females or in parameters of fetal development were observed. In rabbits, intravenous administration of up to 1 0 mg lentinan/kg body weight/day to dams during gestation did not result in any significant compound-related effects on developmental parameters (Cozens *et al.*, 1981d). Similar to observations in sub-chronic dog and monkey studies (Chesterman *et al.*, 1981, Sortwell *et al.*, 1981), significant dose-dependent increases in spleen weights were reported in both males and females receiving lentinan at intravenous doses of 0 10 mg/kg body weight/day and higher (Cozens *et al.*, 1981a,b,c), and lentinan at doses above 0 5 mg/kg body weight/day resulted in reticuloendothelial toxicity. Similarly, significantly increased spleen weights were reported in rabbits receiving 30 mg/kg body weight/day of lentinan (Cozens *et al.*, 1981d) Although lentinan is a 1,3/1,6-beta glucan, the compound is not yeast-derived and is at least an order of magnitude higher in molecular weight than *S cerevisiae*-derived beta-glucans, and the product used in the identified systemic reproductive studies was partially soluble, compared to the insoluble yeast beta-glucan particulate produced by Biothera Therefore, although some toxic effects were observed in association with systemic lentinan exposure, the studies are not considered in the safety assessment of yeast beta-glucans under the intended conditions of use in foods because the test compounds are different and the route of exposure for lentinan is inappropriate

Studies in Humans

In a double blind, placebo-controlled clinical trial with 20 healthy volunteers, treatment subjects consumed a single capsule providing 250 mg WGP® 3-6/day for 10 days (BEI, 2005a). Blood collection was performed on Day 0 prior to treatment and on Day 10, and the subjects were monitored for 30 days for clinical observations In addition to standard clinical chemistry and hematology, a number of immunological parameters also were determined. No significant differences in white blood cell differential count, whole blood phenotyping, or natural killer (NK) cell activity were observed Assessed by flow cytometry, 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; glucose increased in one subject and decreased in another, and calcium levels were increased in 1 patient. Overall, WGP® 3-6 at a dose of 250 mg/person/day for 10 days was safe and well tolerated and blood biochemistry parameters were essentially unaffected by beta-glucan treatment

A second placebo-controlled double blind study was performed with 62 subjects to assess the safety and impact of WGP® 3-6 on immune biomarkers in subjects exposed to rhinovirus (common cold) (BEI, 2005b). All volunteers were pre-screened to exclude subjects that exhibited levels of rhinovirus antibodies, and each participant consumed 2 daily doses of 250 mg of WGP® 3-6 per day for 10 days. Standard safety endpoints and a number of immune-related hematological biomarkers were measured. 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) reported on a clinical study that investigated the safety and potential cholesterol-lowering effect of an oral insoluble particulate yeast beta-glucan at a dose of 15 g/person/day (approximately 150 mg/kg body weight/day) in 15 free-living, obese, hypercholesterolemic men over a period of 8 weeks The beta-glucan test article was not well characterized in the published study article, however, the authors indicated that the product was 85 to 90% pure beta-glucan, consisting of beta-1,3 linkages and forming distinct hollow microsphere structures that were readily miscible in water and communication with Biothera confirmed that the yeast beta-glucan preparation used in the study was identical to WGP® 3-6. Compared to average baseline values, the consumption of yeast beta-glucan decreased plasma total cholesterol levels by 8% at Week 7 and by 6% at Week 8. Following discontinuation of beta-glucan treatment, total cholesterol levels returned to baseline values, however, a significant increase (16%) in high-density lipoprotein (HDL)-cholesterol was reported at week 12. No significant change in low-density lipoprotein cholesterol was observed throughout any of the time-points. The mean ratio of total cholesterol to HDL cholesterol declined significantly

from baseline on Weeks 7 and 8, but returned to baseline levels by Week 12. Side 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 high doses of yeast beta-glucan (15 g/person/day) were well tolerated in adults.

The safety of oral supplementation with a "soluble" branched beta-1,3-glucan (SBG)[4] derived from *S. cerevisiae* was investigated by Lehne *et al.* (2005) in an open dose-escalation study in 18 healthy volunteers Subjects (6/group) were randomized to receive 100, 200, or 400 mg SBG/person for a period of 4 consecutive days. Data collected included routine hematological data, clinical chemistry data, urinalysis, blood and saliva levels of total immunoglobulin (Ig) A, IgG, IL-6 and TNF-alpha, saliva levels of IL-1β, vital signs, and oral cavity observations. On Days 1 (before and 1 hour after administration), 2, 5, and 8, plasma concentrations of beta 1,3-D-glucan were measured Plasma concentrations of beta-1,3-D-glucan did not differ between the pre-study values and the values recorded on Days 5 and 8, demonstrating that there was no systemic absorption of beta-1,3-D-glucan following oral supplementation with SBG. No abnormalities in vital signs were observed and no adverse event was considered related to SBG administration. Minor mucosal lesions of the oral cavity were observed in 7 subjects but were considered normal physiological variations. Increased C-reactive protein, fibrinogen, and abnormal differential counts of leucocytes were observed in 5 subjects with pre-existing respiratory infections, including 1 with herpes labialis. All other hematological and biochemical parameters were within normal physiological ranges throughout the study There was a significant increase in the saliva IgA value on Day 5 compared to Day 1 in the 400 mg SBG/day 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-α between treatment groups.

Other Considerations

Potential Hypersensitivity/Allergenicity of Yeast Beta-Glucan

In a study conducted by Instanes *et al.* (2004), injection (into the fat pad of the hind foot) of a beta-1,3-glucan extract derived from *S. cerevisiae* was reported to increase the allergic response to ovalbumin (OVA) immunization in female balb/c mice, although no IgE or IgG1 anti-OVA antibodies were observed for animals receiving beta-glucan in the absence of OVA. Similar yet more modest delayed hypersensitivity reactions were reported by Tokunaka *et al.* (2000), where a significant increase in IgG production was observed in ICR mice following the intraperitoneal administration of a soluble beta-D glucan (from *Candida* and *Sclerotina* yeasts)

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[4] The SBG was formulated as a water suspension that subjects were instructed to use as a mouthwash for 2 minutes before swallowing Although the beta-glucan was stated to be soluble, the mouthwash solution was characterized as "hazy" by the authors and suggests that the glucan test material was actually poorly soluble in water, and a significant percentage of the glucan would therefore have been in a particulate form

used as an adjuvant with bovine serum albumin antigen. Delayed hypersensitivity reactions also were observed by Perez *et al* (1984) in C3H/He mice administered insoluble beta-glucan (derived from *S cerevisiae*) *via* intraperitoneal or subcutaneous routes before and in conjunction with immunizing doses of sheep RBC.

The effect of *S. cerevisiae*–derived beta-glucan on the immune response to OVA also was assessed by Li *et al.* (2005) in piglets. Upon weaning, 24 pigs were randomized to receive 1 of 2 diets supplemented with beta-glucan at 0 or 50 mg/kg feed (providing approximately 60 mg/kg body weight/day) for a period of 31 days. On Day 14, animals were injected with OVA and the antibody response was determined on Days 21 and 28. An increase in OVA response (20%) was observed on Day 21 for beta-glucan-treated animals, although the effect was transient and OVA antibody levels returned to baseline by Day 28. In a second study by Li *et al.* (2006), yeast beta-glucan (*S cerevisiae*-derived) was administered once again to weaned pigs at concentrations up to 200 ppm in the diet for a period of 35 days. The glucan diet was well tolerated at all feed supplementation levels, as evidenced by no compound-related changes in body weight, and had no effect on *ex vivo* lymphocyte proliferation relative to controls. In weanling crossbred male pigs receiving *S. cerevisiae* beta-glucan (312 mg/kg body weight/day) in the diet, improved growth and decreased stress response to endotoxin challenge was observed relative to control animals not receiving beta-glucan supplementation (Eicher *et al* , 2006)

In humans, positive skin prick tests to *S. cerevisiae* have been reported in as high a proportion as 70 to 94% of patients with atopic dermatitis Moreover, delayed hypersensitivity to intravenous beta glucan was observed in surgical trauma patients, however, the increased sensitivization effect resulted in a beneficial effect in trauma patients, as a significant decrease in mortality and overall septic morbidity was observed (Browder *et al* , 1990)

Although direct allergy to *S. cerevisiae* occurring from food consumption is rare, confirmed cases of food allergy to bakers yeast have been reported in the literature (Pajno *et al.*, 2005) The effects were not life threatening, however, and mechanistically, yeast allergy is typically a function of hypersensitivity to the enolase and/or mannan in the yeast, not the beta-glucan fraction (Kortekangas-Savolainen *et al* , 1993; Nittner-Marszalska *et al.*, 2001), and therefore, allergenicity and/or hypersensitivity to yeast beta-glucan is not anticipated to occur. Since the ingredient will be labeled as yeast beta-glucan, it is anticipated that individuals with yeast or beta-glucan allergy or sensitivity will avoid ingestion of this product

Mineral Binding

Although the consumption of dietary fiber has been reported to reduce the bioavailability of various minerals, the effect is predominantly a function of the phytate content of the food, and mechanistic evidence to support a direct role of indigestible fiber in the sequestering of minerals is conflicting at best (Greger, 1999). In fact, most studies suggest that various pectins, gums,

and indigestible sugars improve mineral bioavailability (Greger, 1999). For example, in a study by Mai *et al* (2002) designed to investigate the potential effects of brewer's yeast and 2 of its components (glucan and mannan) on the absorption of radiolabeled iron in anemic rats, 5 groups of anemic male 4-week-old Sprague-Dawley rats (5 to 6 animals/group) were provided single gavage doses of a control mixture, a glucan mixture (14 mg/rat), a yeast cell wall mixture (50 mg/rat), a mixture of glucan and mannan (14 and 7 mg/rat), or a yeast cytoplasm mixture (50 mg/rat). Glucan treatment significantly increased the blood iron levels relative to controls and also significantly increased kidney and small intestine levels of radiolabeled iron. The authors reported that the glucan and yeast cell wall treatments stimulated the incorporation of dietary iron into iron stores, and that beta-glucan was responsible for this effect. Increased bioavailability similar to the effects reported by Mai *et al.* (2002) has been reported by others (*e g* , Greger, 1999) It is unlikely that the consumption of yeast beta-glucans would impair mineral bioavailability under their intended conditions of use in food

Plasma Cholesterol, Bile Acid Secretion and Short Chain Fatty Acid Production

A study designed to investigate the effect of various crude and refined soluble and insoluble yeast (*Kluyveromyces marxianus*) cell wall preparations on plasma cholesterol, fecal sterol excretion, and short chain fatty acid production was performed by Yoshida *et al.* (2005) in which 5-week-old male Wistar (8/group) rats fed a high-cholesterol diet supplemented with 50 g/kg feed [approximately 5,000 mg/kg body weight/day (U S. FDA, 1993)] of the various test articles over a period of 14 days were reported not to exhibit any significant differences in weight gain, however, total cholesterol was significantly decreased with both insoluble yeast cell wall preparations, without any significant differences in HDL-cholesterol, triglyceride, or phospholipid levels Fecal sterol excretion was increased by the insoluble yeast cell wall supplementation, as were total neutral sterols, bile acids, and total sterols relative to controls. Total fecal short chain fatty acid (SCFA) excretion also was increased from insoluble yeast cell wall supplementation. No adverse effects on gastrointestinal function were observed, and the changes observed were overall of a beneficial nature.

In an experiment by Nakamura *et al.* (2001a), brewer's yeast cell wall beta-glucan derived from *S cerevisiae* provided *via* the diet at levels of 0, 2, 3, or 5% [0, 2,000, 3,000 and 5,000 mg/kg body weight/day, respectively (U S FDA, 1993)] was reported to positively affect experimentally-induced constipated rats, as significant increases in number of feces, fecal dry weight, and fecal water content were observed relative to a no-fiber diet, and in most cases, relative to cellulose positive controls Similar to the experiment by Yoshida *et al.* (2005), generalized increases of volatile fatty acids were observed, an effect characterized by significant increases in butyrate, propionate, and acetate production relative to both positive and normal controls

In a similar study, Nakamura *et al.* (2001b) assessed the effect of yogurt supplemented with 0, 1, 3, or 5% brewer's yeast cell wall [approximately 0, 1,000, 3,000 or 5,000 mg/kg body

weight/day (U.S. FDA, 1993)] on experimentally-induced constipation and intestinal microflora in rats (8 to 10 animals/group). The yeast cell wall administration increased fecal frequency in non-constipated rats and resulted in a dose-responsive increase in fecal frequency and fecal volume in constipated rats at the highest dose without effects on fecal water content, and increased fecal SCFA (*i.e.*, acetic, proprionic, and butyric acids) production was observed in a dose-dependent manner. In addition, treatment significantly increased the number of *Lactobacilli*, *Eubacteria*, and total anaerobes in the gastrointestinal tract relative to cellulose controls without effects on the microbial populations of *Bifidobacterium* or *Bacterioideceae*. The authors concluded that the yeast cell wall was fermented by intestinal bacteria, and that the effect resulted in the alleviation of loperimide-induced constipation and an improved bowel environment. Similar results were reported by McBurney (1991) in an *in vitro* experiment in which various fiber sources were fermented with human fecal microbiota, and the highest level of SCFA was observed in the beta-glucan group (compared to a mixed fiber and psyllium group) In contrast, yeast glucan was the least effective fiber in terms of water holding capacity.

The results from these studies demonstrate that yeast beta-glucans were substrates of bacterial metabolism. The metabolic products were characterized primarily as acetate, propionate, and butyrate, with lesser amounts of other endogenous SCFA. These metabolic compounds are not toxic, and humans are routinely exposed to these SCFA through the routine consumption of food products containing indigestible fibers such as cellulose An additional gastrointestinal effect reported was increased laxation, which was probably mediated by osmotic effects resulting in fecal overloading. This effect is not considered adverse.

SUMMARY

Yeast beta-glucan is isolated from the cell wall of bakers yeast (*S cerevisiae*) and is composed primarily of beta-1,3-glucan (at least 70%). The ingredient, which will be marketed as BetaRight® 3-6 (containing at least 70% beta-glucan) or WGP® 3-6 (containing at least 75% beta-glucan), is intended for use in a number of food products, including cookies, meal replacement beverages, breakfast, granola and protein nutritional bars, soy milk, probiotic beverages, yogurt, and yogurt beverages, fruit beverages, drinks, juices, and smoothies, soft candy, and soups at a level of 200 mg per serving. Under the conditions of intended use, total population all-user mean and 90th percentile daily intakes of yeast beta-glucan are estimated to be 413 02 and 827 32 mg/person/day, respectively (corresponding to 8.90 and 20.66 mg/kg body weight/day). Yeast beta-glucan is produced in accordance with cGMP, is highly stable under appropriate storage conditions, and the final product meets appropriate food-grade specifications Small amounts of VOC are produced during the manufacturing process of Biothera's yeast-derived beta-glucans *via* the degradation of fatty acids, despite the fact that no VOC are used in the production of the ingredient. However, this is not uncommon in yeast beta-glucan products and Biothera demonstrated that a number of currently-marketed yeast glucan food and dietary ingredients contain VOC at levels that are comparable to Biothera's yeast beta-

glucan. Based on established limits and available safety data for each VOC, the levels present in yeast beta-glucan are not expected to be of concern under the intended conditions of use of the ingredient in food.

The proposed uses of yeast beta-glucan are safe and also are GRAS based on scientific procedures The safety of yeast beta-glucan is based on pre-clinical and clinical safety data. Yeast beta-glucan, like many other dietary fibers, is not absorbed following consumption (Hong *et al.*, 2004; Lehne *et al* , 2005), and there is little risk of systemic toxicity. Any effects would be generally limited to those on the structure and function of the gastrointestinal tract, which would be effects that are typical of indigestible fiber Degradation of yeast beta-glucan occurs primarily in the large intestine where microflora fermentation occurs, resulting in the generation of short-chain fatty acids, which are subsequently absorbed and utilized in well-characterized biochemical pathways Small amounts of yeast beta-glucan may enter the systemic circulation *via* transport by macrophage cells to the bone marrow, spleen, and lymph nodes, where the beta-glucan is completely degraded.

A single oral dose of 2,000 mg WGP® 3-6/kg body weight to rats was well tolerated, and daily oral gavage dosing of WGP® 3-6 for 91 days in rats was without evidence of systemic or local (gastrointestinal) toxicity at the highest possible dose of 100 mg/kg body weight/day, which was determined by the authors to be the NOAEL under the conditions of this study (Babíček *et al.*, 2007) Feletti *et al.* (1992) reported on a 52-week study in rats using an oral particulate glucan (derived from *C albicans*) and no systemic toxicity was observed at the highest oral dose tested, 200 mg/kg body weight/day, which is the NOAEL

No adverse clinical effects or changes in biochemistry or hematology parameters were reported following WGP® 3-6 supplementation at quantities up to 500 mg/person/day for a period of 10 days to healthy human volunteers (BEI, 2005a,b) Higher doses of WGP® 3-6 were well tolerated in humans in a study by Nicolosi *et al.* (1999) in which daily oral supplementation with 15 g of WGP® 3-6 over a period of 8 weeks in 15 free-living obese hypercholesterolemic men was without any adverse effects typical of fiber consumption (*e.g* , diarrhea, nausea, abdominal discomfort and or distension, and flatulence)

Although WGP 3-6 has been shown to be taken up by immune cells, and to display immune modulation effects in rodents, the observations are difficult to extrapolate to humans, and overall, there is no evidence to suggest that Biothera's yeast glucans would adversely affect the immune system in humans The administration of WGP® 3-6 (250 mg/person) in healthy volunteers had no effect on total and differential white blood cell count, and no effects on whole blood phenotyping or NK cell activity The only effect observed during this study was an enhanced phagocytic capacity, suggesting enhanced macrophage activity and/or function. In healthy subjects exposed to rhinovirus, WGP® 3-6 at a level of 500 mg/person had no effects on NK cell numbers relative to placebo, and no significant effects on T cell number or cytokine levels were observed. Increased hypersensitivity effects have been reported in both humans

and animals (Perez *et al.*, 1984, Browder *et al.*, 1990; Instanes *et al.*, 2004), however, the effects are limited to the use of soluble yeast glucans and administration *via* parenteral routes. Allergenicity to bakers yeast has been reported in the literature (Pajno *et al.*, 2005), although its occurrence appears to be rare, and mechanistically is related to IgE reactivity to the mannan or enolase content of the cell rather than beta-glucan (Kortekangas-Savolainen *et al.*, 1993; Nittner-Marszalska *et al* , 2001)

In general, systemic toxicity is limited to parenteral administration as, with the exception of small amounts of gut-associated-lymphoid-tissue-mediated uptake, beta-glucans are not absorbed from the gastrointestinal tract and thus, systemic exposure to yeast beta-glucan following oral administration will not occur to any significant degree Therefore, the observations associated with the administration of various beta-glucans *via* parenteral routes would not be expected under the proposed conditions of use of yeast beta-glucan in foods

The safety of the yeast beta-glucan is supported by its essentially equivalent composition to bakers yeast glycan, which is the comminuted, washed, pasteurized, and dried cell walls of *S cerevisiae* and is approved for use by the FDA for direct addition to food as a multi-purpose food additive Both ingredients are derived from the cell walls of *S. cerevisiae*, contain at least 80% carbohydrate (dry weight basis), and are produced consistent with cGMP, and with similar product specifications, both ingredients meet food-grade specifications This essential equivalency between yeast beta–glucan and bakers yeast glycan supports the GRAS status of the proposed uses of Biothera's yeast beta-glucan

CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the data and information summarized above and conclude that the proposed uses of Biothera's yeast beta-glucan derived from bakers yeast, *Saccharomyces cerevisiae*, meeting appropriate food-grade specifications and manufactured in accordance with cGMP, are safe and suitable. We further conclude that these proposed uses of Biothera's yeast beta-glucan are Generally Recognized as Safe (GRAS) based on scientific procedures It is our opinion that other qualified experts would concur with these conclusions

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Attachment 1

ATTACHMENT 1

***CURRICULUM VITAE* OF EXPERT PANEL MEMBERS**

Pages 000039-000090 removed under Exemption B(6) of the Freedom of Information Act.

SUBMISSION END