

21 July 2021

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Dear Dr. Gaynor:

Re: Amendment to GRAS Notice for Fiber Extracted from White Button Mushrooms (GRN 000997)

Chinova Bioworks Inc. (Chinova hereafter), as the notifier, has previously concluded that the company's fiber extracted from white button mushrooms has Generally Recognized as Safe (GRAS) status for use as a food ingredient for addition to a number of food and beverage products across multiple categories. Chinova's GRAS conclusion was notified to the offices of the United States Food and Drug Administration (U.S. FDA) on March 4th, 2021 and was filed by the agency under GRN No. 997 on July 13th, 2021. In accordance with 21 CFR §170.280, Chinova hereby submits the following amendment to GRN 997, which includes addition of a new subcategory of egg substitutes, which falls under the existing category of 'Plant Protein Products'. Further description of this amendment and the results of the intake analysis conducted with the new subcategory and in accordance with the most recent cycle of NHANES (2017-2018) are discussed in the attached amendment.

Should you have any questions or concerns regarding this amendment, 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,



David Brown
Chief Operating Officer
Chinova Bioworks Inc.

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AMENDMENT TO GRN 997

GRAS STATUS OF FIBER EXTRACTED FROM WHITE BUTTON MUSHROOMS (*AGARICUS BISPORUS*)

Chinova Bioworks Inc.
50 Crowther Lane, Suite 100
Fredericton, New Brunswick, Canada
E3C 0J1

DATE:
21 July 2021

GRAS Status of Fiber Extracted from White Button Mushrooms (*Agaricus Bisporus*)

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GRAS Status of Fiber Extracted from White Button Mushrooms (*Agaricus Bisporus*)

1.0 INTRODUCTION

Chinova Bioworks Inc. (Chinova hereafter), as the notifier, has previously concluded that the company's fiber extracted from white button mushrooms has Generally Recognized as Safe (GRAS) status for use as a food ingredient for addition to a number of food and beverage products across multiple categories. Chinova's GRAS conclusion was notified to the offices of the United States Food and Drug Administration (U.S. FDA) on March 4th, 2021, and was filed by the agency under GRN No. 997 on July 13th, 2021. In accordance with 21 CFR §170.260 – Steps you may take before FDA responds to your GRAS notice, Chinova hereby submits the following amendment to GRN 997, which includes addition of a new subcategory of egg substitutes, which falls under the existing category of 'Plant Protein Products'. Further description of this amendment and the results of the intake analysis conducted with the new subcategory and in accordance with the most recent cycle of NHANES (2017-2018) are discussed in the attached amendment.

The original assessment of the anticipated intake of Chinova's fiber extracted from white button mushrooms (*A. bisporus*) as an ingredient under proposed antimicrobial food uses was conducted using consumption data available in the 2015-2016 cycle of the U.S. National Center for Health Statistics' National Health and Nutrition Examination Survey (NHANES) (CDC, 2020a,b; USDA, 2019). In this amendment, Chinova's fiber extracted from white button mushrooms is additionally proposed for use in egg substitutes. In addition, revised estimates for dietary exposure were based on the updated proposed food uses and use levels for Chinova's fiber extracted from white button mushrooms (see Table 2-1) in conjunction with more recent food consumption data included in the NHANES 2017-2018 cycle (CDC, 2021a,b; USDA, 2021). A summary along with the pertinent results is presented herein.

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2017-2018. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (CDC, 2021a,b; USDA, 2021). The NHANES data were employed to assess the mean and 90th percentile intake of Chinova's fiber extracted from white button mushrooms for each of the following population groups:

- Infants and young children, aged <2 years;
- Children, ages 2 to 5;
- Children, ages 6 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (ages 2 years and older, and both gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of Chinova's fiber extracted from

white button mushrooms by the U.S. population¹. Estimates for the daily intake of Chinova's fiber extracted from white button mushrooms represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2017-2018; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of Chinova's fiber extracted from white button mushrooms averaged over all individuals surveyed, regardless of whether they consumed food products in which Chinova's fiber extracted from white button mushrooms is proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing Chinova's fiber extracted from white button mushrooms during the 2 survey days). "Consumer-only" intake refers to the estimated intake of Chinova's fiber extracted from white button mushrooms by those individuals who reported consuming food products in which the use of Chinova's fiber extracted from white button mushrooms is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which Chinova's fiber extracted from white button mushrooms is proposed for use on either Day 1 or Day 2 of the survey.

As with the previous assessment, the estimates for the intake of Chinova's fiber extracted from white button mushrooms were generated using the maximum FEMA GRAS approved use level of 2,000 ppm (0.20 g/100g) for each intended food use, as presented in Table 2-1, together with food consumption data available from the 2017-2018 NHANES datasets. The results for this assessment are presented in Section 3.1 and a comparison to the previous assessment is detailed in Section 3.2.

2.0 INTENDED CONDITION OF USE OF CHINOVA'S FIBER EXTRACTED FROM WHITE BUTTON MUSHROOMS (*A. BISPORUS*)

The proposed conditions of use for antimicrobial purposes for Chinova's fiber extracted from white button mushrooms are summarized in Table 2-1. The use levels of Chinova's fiber extracted from white button mushrooms (*A. bisporus*) for its intended food uses as an antimicrobial ingredient will range from 0.01 to 0.150 g/100 g (equivalent to 100 to 1,500 ppm), which are much lower than the FEMA GRAS-approved use levels, which range from 1,500 to 2,000 ppm. Therefore, as the antimicrobial use levels are lower than the FEMA-GRAS conditions of use for chitosan for flavoring purposes (up to 2,000 ppm), the use level of 0.2% was applied in the current intake analysis to all proposed food use categories for antimicrobial purposes (See Table 2-1). Food codes representative of each proposed food use were chosen from the NHANES 2017-2018 (CDC, 2021b). Food codes were grouped in food use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (U.S. FDA, 2020). If necessary, product-specific adjustment factors were developed for composite foods/mixtures based on data provided in the Food and Nutrient Database for Dietary Studies (USDA ARS, 2021) or the Food Commodity Intake Database (U.S. EPA & USDA, 2020).

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

Table 2-1 Summary of the Individual Proposed Antimicrobial Food Uses and Use Levels for Chinova’s Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*) and the Maximum FEMA GRAS-Approved Use Level for Proposed Food Uses in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020)	Food Uses^a	Proposed Antimicrobial Use Levels (g/100 g)	Maximum FEMA GRAS- Approved Use Level (g/100g)
Baked Goods and Baking Mixes	Bagels and English Muffins	0.100	0.200
	Bread (excluding sweet type breads and rolls)	0.100	0.200
	Cakes	0.100	0.200
	Light weight cakes	0.100	0.200
	Medium weight cakes	0.100	0.200
	Heavy weight cakes	0.100	0.200
	Cornbread, corn muffins, or tortillas	0.100	0.200
	Croissants	0.100	0.200
	Doughnuts (Donuts)	0.100	0.200
	French toast, pancakes, and waffles	0.100	0.200
	Muffins	0.100	0.200
	Pastries	0.100	0.200
	Pies	0.100	0.200
Beverages, Alcoholic	Cocktail drinks	0.040	0.200
Beverages and Beverage Bases	Energy drinks	0.040	0.200
	Enhanced or fortified waters	0.040	0.200
	Flavored or carbonated waters	0.040	0.200
	Soft drinks (regular and diet)	0.040	0.200
	Sport or electrolyte drinks, fluid replacement drinks	0.040	0.200
Cheeses	Cheese-based sauces	0.100	0.200
	Cottage cheese	0.100	0.200
	Cream cheese and cheese-based spreads	0.100	0.200
	Natural cheese	0.150	0.200
	Processed cheese or cheese mixtures	0.150	0.200
Coffee and Tea	Ready-to-drink coffees	0.015	0.200
	Ready-to-drink tea beverages	0.040	0.200
Condiments and Relishes	Ketchup	0.040	0.200
	Mustard	0.040	0.200
	Relish	0.080	0.200
Confections and frostings	Coatings	0.100	0.200
	Frostings and icings	0.040	0.200
Dairy Product Analogs	Imitation cheese	0.150	0.200
Fats and oils	Fat-based sauces	0.100	0.200
	Margarine and margarine-like spreads	0.100	0.200

Table 2-1 Summary of the Individual Proposed Antimicrobial Food Uses and Use Levels for Chinova’s Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*) and the Maximum FEMA GRAS-Approved Use Level for Proposed Food Uses in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020)	Food Uses ^a	Proposed Antimicrobial Use Levels (g/100 g)	Maximum FEMA GRAS- Approved Use Level (g/100g)
	Mayonnaise and mayonnaise-type dressings	0.100	0.200
	Salad dressings	0.100	0.200
Gelatins, Puddings, and Fillings	Flans, custards, and other egg-based desserts	0.080	0.200
Grain Products and Pastas	Cereal and granola bars	0.020	0.200
	Energy bars or protein bars or meal replacement bars	0.020	0.200
	Macaroni and noodle products	0.020	0.200
Gravies and Sauces	Gravies	0.020	0.200
	Tomato-based sauces	0.020	0.200
	White sauces	0.100	0.200
Jams and Jellies	Jams, jellies, preserves, and marmalades	0.100	0.200
Milk Products	Plain or flavored yogurt	0.100	0.200
Processed Fruits and Fruit Juices	Fruit drinks and ades and smoothies	0.060	0.200
	Fruit juices	0.060	0.200
	Fruit nectars	0.060	0.200
	Fruit-based desserts	0.080	0.200
Plant Protein Products	Meat analogs	0.150	0.200
	Egg substitutes	0.080	0.200
Processed Vegetables and Vegetable Juices	Vegetable juices	0.040	0.200
	Vegetable purees ^b	0.040	0.200
Soups and Soup Mixes	Prepared and canned soups	0.040	0.200
Sugar Substitutes	Sugar substitutes	0.100	0.200
Sweet sauces, toppings, and syrups	Sweet sauces, syrups, and toppings (including fruit-based)	0.100	0.200
	Cocoa syrups	0.100	0.200

CFR = *Code of Federal Regulations*; U.S. = United States.

^a Chinova’s mushroom-derived fiber is intended for use in unstandardized products when standards of identity, as established under 21 CFR §130 to 169, do not permit its addition.

^b New proposed food uses are **bolded**.

^c Food codes for vegetable mixtures and vegetable combinations (which are likely to be used to make purees) were included as a surrogate for ‘vegetable purees’.

3.0 ESTIMATED DIETARY CONSUMPTION OF CHINOVA'S FIBER EXTRACTED FROM WHITE BUTTON MUSHROOMS (*A. BISPORUS*)

3.1 Intake Estimates for Chinova's Fiber Extracted from White Button Mushrooms (*A. bisporus*)

Table 3.1-1 summarizes the estimated total intake of Chinova's fiber extracted from white button mushrooms (g/person/day) from all proposed food uses in the U.S. population groups. Table 3.1-2 presents this data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of consumers was high among all age groups evaluated in the current intake assessment; more than 62.0% of the population groups consisted of consumers of food products in which Chinova's fiber extracted from white button mushrooms is currently proposed for use (Table 3.1-1). Children and female teenagers had the greatest proportion of consumers at 100%. The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (ages 2 years and older), the mean and 90th percentile consumer-only intakes of Chinova's fiber extracted from white button mushrooms were determined to be 1.2 and 2.6 g/person/day, respectively. Of the individual population groups, children aged 6 to 11 were determined to have the greatest decrease in mean and 90th percentile in consumer-only intakes of Chinova's fiber extracted from white button mushrooms, at 5.5 and 9.3%, respectively. Male teenager had the greatest increase at mean, of 1.7%, while female adults had the greatest increase in 90th percentile consumer-only intakes of 2.9% (Table 3.1-1).

Table 3.1-1 Summary of the Estimated Daily Intake of Chinova's Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*) from Proposed Antimicrobial Food Uses at the Maximum FEMA GRAS-Approved Use Level in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group (Years)	Per Capita Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90 th Percentile	%	n	Mean	90 th Percentile
Infants and Toddlers	0 to <2	0.3	0.8	62.0	252	0.5	1.0
Children	2 to 5	0.8	1.4	100	470	0.8	1.4
Children	6 to 11	1.0	1.7	100	681	1.0	1.7
Female Teenagers	12 to 19	1.0	2.0	100	450	1.0	2.0
Male Teenagers	12 to 19	1.3	2.6	99.7	440	1.3	2.6
Female Adults	20 and older	1.1	2.3	99.4	2,150	1.1	2.3
Male Adults	20 and older	1.5	3.2	99.4	1,962	1.5	3.2
Total Population	2 and older	1.2	2.6	99.5	6,153	1.2	2.6

n = sample size; FEMA; Flavor and Extract Manufacturers Association of the United States; GRAS = Generally recognized as Safe; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (ages 2 years and older) mean and 90th percentile consumer-only intakes of Chinova's fiber extracted from white button mushrooms were determined to be 19.1 and 39.0 mg/kg body weight/day, respectively. Among the individual population groups, children aged 2 to 5 years were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, at 47.4 and 82.6 mg/kg body weight/day, respectively. Female adults had the lowest

mean and 90th percentile consumer-only intakes of 14.7 and 28.7 mg/kg body weight/day, respectively (Table 3.1-2).

Table 3.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Chinova’s Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*) from Proposed Antimicrobial Food Uses at the Maximum FEMA GRAS-Approved Use Level in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group (Years)	Per Capita Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90 th Percentile	%	n	Mean	90 th Percentile
Infants and Toddlers	0 to <2	25.9	73.7	61.8	250	41.9	81.8
Children	2 to 5	47.4	82.6	100	462	47.4	82.6
Children	6 to 11	30.9	55.9	100	679	30.9	55.9
Female Teenagers	12 to 19	17.2	32.0	100	443	17.2	32.0
Male Teenagers	12 to 19	21.2	41.3	99.7	437	21.2	41.3
Female Adults	20 and older	14.6	28.7	99.4	2,132	14.7	28.7
Male Adults	20 and older	17.1	34.8	99.4	1,946	17.2	34.8
Total Population	2 and older	19.0	38.9	99.5	6,099	19.1	39.0

bw = body weight; FEMA; Flavor and Extract Manufacturers Association of the United States; GRAS = Generally recognized as Safe; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

3.2 Comparison of Amended Intakes Assessment *versus* Previous Dietary Exposure Assessment

The revised estimated daily intake of Chinova’s Fiber Extracted from White Button Mushrooms from the updated proposed conditions of use (Table 2-1) conducted using the most recent cycle of NHANES (2017-2018) is compared to that of the original assessment, which did not include egg substitutes and was conducted using the 2015-2016 cycle of NHANES. Table 3.2-1 summarizes the comparison on an absolute basis (g/person/day).

Among the total population (ages 2 years and older), the change in consumer-only intakes of Chinova’s fiber extracted from white button mushrooms range from a 2.2% decrease to a 1.8% increase, observed at the mean and 90th percentile, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of Chinova’s fiber extracted from white button mushrooms on an absolute basis, at 1.5 and 3.2 g/person/day, respectively, while infants and toddlers had the lowest mean and 90th percentile consumer-only intakes of 0.5 and 1.0 g/person/day, respectively (Table 3.1-1).

Table 3.2-1 Comparison of Consumer-Only Estimated Daily Intake of Chinova’s Fiber Extracted from White Button Mushrooms from Previous Assessment (2015-2016 NHANES Data) with Estimated Daily Intake from Updated Assessment (2017-2018 NHANES Data) in the U.S. by Population Group

Population Group	Age Group (Years)	Original Assessment Intake (g/day)		Revised Assessment Intake (g/day)		Change from Previous Assessment (%)	
		Mean	90 th Percentile	Mean	90 th Percentile	Mean	90 th Percentile
Infants and Toddlers	0 to <2	0.5	1.0	0.5	1.0	-0.9	-2.9
Children	2 to 5	0.8	1.5	0.8	1.4	-1.7	-7.8

Table 3.2-1 Comparison of Consumer-Only Estimated Daily Intake of Chinova’s Fiber Extracted from White Button Mushrooms from Previous Assessment (2015-2016 NHANES Data) with Estimated Daily Intake from Updated Assessment (2017-2018 NHANES Data) in the U.S. by Population Group

Population Group	Age Group (Years)	Original Assessment Intake (g/day)		Revised Assessment Intake (g/day)		Change from Previous Assessment (%)	
		Mean	90 th Percentile	Mean	90 th Percentile	Mean	90 th Percentile
Children	6 to 11	1.1	1.9	1.0	1.7	-5.5	-9.3
Female Teenagers	12 to 19	1.0	1.9	1.0	2.0	+1.2	+2.3
Male Teenagers	12 to 19	1.3	2.6	1.3	2.6	+1.7	+2.0
Female Adults	20 and older	1.1	2.2	1.1	2.3	-2.8	+2.9
Male Adults	20 and older	1.6	3.2	1.5	3.2	-1.9	-0.2
Total Population	2 and older	1.3	2.6	1.2	2.6	-2.2	+1.8

n = sample size; NC = no change; FEMA; Flavor and Extract Manufacturers Association of the United States; GRAS = Generally recognized as Safe; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

In summary, the change in intakes from the previous assessment among individual population groups ranged from a 9.3% decrease, observed at the 90th percentile in children aged 6 to 11, to a 2.9% increase in intakes, observed at the 90th percentile in female adults. In conclusion, the addition of the proposed use in egg substitutes and the update to the most recent cycle of NHANES (2017-2018) did not result in a significant change to the anticipated intake estimates in the U.S. population. As such, the addition of the new subcategory of egg substitutes would not impact the safety of the ingredient or change the GRAS status of Chinova’s Fiber Extracted from White Button Mushrooms.

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Santos, Marissa

From: David Brown <dave@chinovabioworks.com>
Sent: Thursday, September 30, 2021 4:20 PM
To: Santos, Marissa
Subject: [EXTERNAL] Re: GRN 000997 - Questions for the Notifier
Attachments: Response to GRN997 FDA Questions - September 30'2021.pdf

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

Good afternoon Marissa,

Please find attached here the responses to the questions you sent in regards to our GRAS submission GRN#997.

If you have any questions or would like any clarification please let me know!

Warm regards,

Dave

On Tue, Sep 21, 2021 at 12:46 PM Santos, Marissa <Marissa.Santos@fda.hhs.gov> wrote:

Dear Mr. Brown,

During our review of GRAS Notice No. 000997, we noted several 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 responses.

If you have any questions or need further clarification, please feel free to reach out to me.

Regards,

Marissa

Marissa Santos, M.S.

Regulatory Review Scientist

Division of Food Ingredients

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Center for Food Safety and Applied Nutrition

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30 September 2021

Marissa Santos, MS
Regulatory Review Scientist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Dear Ms. Santos

Re: FDA Questions on GRN 997

Chemistry

Question 1. On page 8, Chinova Bioworks states that the proposed ingredient is composed of approximately 95% chitosan and up to 5% beta-1,3-D-glucans. FDA requests that the notifier establish a specification for the minimum content of chitosan and for the minimum content of beta-1,3-D-glucans, provide an updated Table 2.3-1 including these specifications, and provide an updated Table 2.4-1 including the results of the analyses for chitosan and beta-1,3-D-glucans from a minimum three (preferably five) non-consecutive batches.

Response 1. The specifications have been established for chitosan ($\geq 95\%$) and beta-1,3-D-glucans ($\leq 5\%$). The revised specifications table is provided in Table 1. The results of analysis for chitosan and beta-1,3-D-glucan content are provided in Table 2.

Table 1 Product Specifications for Chinova's Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*)

Specification Parameter	Specification Limit	Method of Analysis
Identification	Positive	FTIR, H-NMR
Color of powder	White to beige	Validated Internal (visual)
Appearance of 1% solution of 1% HAC	Clear	Validated Internal (visual)
Chitosan (% w/w)	$\geq 95\%$	H-NMR
Beta-1,3-D-glucan (% w/w)	$\leq 5\%$	H-NMR
Degree of deacetylation (mol%)	≥ 80	Validated Internal
Molecular weight average (kDa)	10 to 400	HPLC
Moisture (% w/w)	≤ 10	Validated Internal
Total ash (% w/w)	≤ 3	Validated Internal
Solubility (% w/w)	≥ 99.5	Validated Internal
Heavy Metals		
Arsenic (mg/kg)	≤ 0.2	AOAC 2015.01
Lead (mg/kg)	≤ 1.0	AOAC 2015.01
Cadmium (mg/kg)	≤ 0.2	AOAC 2015.01
Mercury (mg/kg)	≤ 0.2	AOAC 2015.01
Microbiological Parameters		
Aerobic microbial count (CFU/g)	≤ 100	ISO 4833 Part 2 2013

Table 1 **Product Specifications for Chinova’s Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*)**

Specification Parameter	Specification Limit	Method of Analysis
Yeast and mold count (CFU/g)	≤100	ISO 21527-2
<i>Escherichia coli</i> (CFU/10 g)	Absent	ISO 7251
<i>Salmonella</i> (CFU/25 g)	Absent	AOAC 2013.01

AOAC = Association of Official Agricultural Chemists; CFU = colony-forming units; FTIR = Fourier-transform infrared spectroscopy; H-NMR = proton nuclear magnetic resonance; HAc = acetic acid; HPLC = high-performance liquid chromatography; ISO = International Organization for Standardization; kDa = kilodaltons; mg/kg = milligram per kilogram.

Table 2 **Summary of the Product Analysis for 5 Lots of Chinova’s Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*)**

Specification Parameter	Specification Limit	Manufacturing Lot No.				
		CH20210103	CH20210108	CH20210123	CH20210218	CH20210320
Chitosan content (% w/w)	≥95 %	96	96	95	96	95
Beta-1,3-D-glucan content (% w/w)	≤5 %	4	4	5	4	5

Question 2. The ingredient is composed primarily of chitosan. Please provide the CAS registry number for chitosan.

Response 2. The CAS number of chitosan is 9012-76-4.

Question 3. On page 10 (Table 2.3-1) Chinova Bioworks states that the elemental analysis was performed using ISO 11885, a method that uses ICP-OES for the determination of elements in water, which is not suitable for elemental quantification in biological matrices. Please provide the results of the elemental analyses obtained using a suitable method such as FDA EAM 4.7 or AOAC 2015.01.

Response 3. The results of analysis of 5 lots of the fiber extracted from white button mushrooms using AOAC 2015.01 are provided in Table 3.

Table 3 **Summary of the Product Analysis for 5 Lots of Chinova’s Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*)**

Specification Parameter	Specification Limit	Manufacturing Lot No.				
		CH20210103	CH20210108	CH20210123	CH20210218	CH20210320
Identification	Positive	Positive	Positive	Positive	Positive	Positive
Color of powder	White to beige	White	White	White	White	White
Appearance of 1% solution of 1% HAc	Clear	Clear	Clear	Clear	Clear	Clear
Chitosan content (% w/w)	≥95 %	96	96	95	96	95
Beta-1,3-D-glucan content (% w/w)	≤5 %	4	4	5	4	5
Degree of deacetylation (mol%)	≥80	96	95	96	96	95
Molecular weight average (kDa)	10 to 400	60±5	60±5	60±5	60±5	60±5
Loss on drying (% w/w)	≤10	5.1	4.9	4.8	5.3	5.5
Total ash (% w/w)	≤3	1.3	1.2	1.4	1.3	1.4
Solubility (% w/w)	≥99.5	100	100	100	100	100
Heavy Metals						
Arsenic (mg/kg)	≤0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Lead (mg/kg)	≤1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Cadmium (mg/kg)	≤0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Mercury (mg/kg)	≤0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Microbiological Parameters						
Aerobic microbial count (CFU/g)	≤100	<5	<5	<5	<5	<5
Yeast and mold count (CFU/g)	≤100	<5	<5	<5	<5	<5
<i>Escherichia coli</i> (CFU/10 g)	Absent	Absent	Absent	Absent	Absent	Absent
<i>Salmonella</i> (CFU/25 g)	Absent	Absent	Absent	Absent	Absent	Absent

CFU = colony-forming units; HAc = acetic acid; kDa = kilodaltons; ppm = parts per million.

Question 4. In Appendix A, Chinova Bioworks provides a letter issued by the Flavor Extract Manufacturers Association (FEMA) stating that the ingredient is GRAS for use as a flavor in selected food categories at the maximum average use level of 2000 mg/kg (FEMA No. 4946). FDA notes that the notifier provides estimates of dietary exposure to the ingredient from the intended use as an antimicrobial agent, but at FEMA’s use level of 2000 mg/kg specified for flavor use. At the current time, FEMA’s GRAS determination for the ingredient has not yet been published. Therefore, FDA requests that the notifier provide a dietary exposure estimate based on the food categories and use levels listed in Table 1.3-1 specified for the intended use of the ingredient as an antimicrobial agent. As the submission contains a discussion of the FEMA proposed use levels, the notifier should also provide an estimate of the cumulative dietary exposure from the intended uses of the ingredient as an antimicrobial agent and as a flavor. Please note that the FEMA uses include additional food categories (e.g., meat products, soft candies) that should be included in the cumulative dietary exposure.

Response 4. As discussed in Section 3.3.1 of GRN 997, in evaluating the dietary exposure to Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*), the maximum FEMA GRAS-approved use level of 2,000 ppm (0.20 g/100g) was applied to *all* proposed antimicrobial food uses of the ingredient. This conservative approach took into account that the ingredient is already permitted for use as a flavouring ingredient in all food categories where Fiber Extracted from White Button Mushrooms is proposed to be added as an antimicrobial ingredient. The proposed use levels for antimicrobial uses range from 0.01 to 0.150 g/100 g (equivalent to 100 to 1,500 ppm), which are much lower than the FEMA GRAS-approved use levels of 1,500 to 2,000 ppm. Considering this, exposure to the ingredient from flavouring and antimicrobial uses would not be considered cumulative, as the higher flavouring use levels would already be achieving the antimicrobial function. It is the notifier's understanding that addition of the 90th percentile exposure from the proposed uses of Fiber Extracted from White Button Mushrooms to the 90th percentile exposure from the proposed uses as a flavouring is not appropriate, as the population that is a 90th percentile consumer of the proposed uses is not the same population that is a 90th percentile consumer for the use of the ingredient as a flavouring substance. In addition, the FEMA GRAS food categories are very broad and include additional categories, which are beyond those that are proposed for use of the ingredient as an antimicrobial. A separate intake assessment using NHANES database based on the proposed uses as a flavouring ingredient would result in a gross overestimation of exposure. Considering the foregoing and given the fact that the ingredient's use as an antimicrobial will not be in an additive/cumulative manner to its approved uses as a flavoring, it is the notifier's opinion that taking a cumulative approach for exposure assessment would not be suitable under the intended uses of the ingredient.

Toxicology

Question 1. In section 6.3.5 Short-Term Tests for Genotoxicity, the notifier states the following:

"The genotoxic potential of chitosan (derived from *Aspergillus bisporus*) and chitosan oligosaccharides was investigated in in vitro and in vivo studies and reviewed in GRN 000397."


Please address how the genotoxicity potential (or lack thereof) of chitosan and beta-glucan derived from *Aspergillus bisporus* shows the genotoxicity potential (or lack thereof) of chitosan and beta-glucan derived from *Agaricus bisporus*.

Response 1. We note that the source of chitosan subject to the bacterial reverse mutation (Ames) test discussed in GRN 397 was likely to be erroneously reported as *Aspergillus bisporus*. As discussed in Section II.A of GRN 397, the subject of the notice, chitosan, was derived from the cell walls of non-genetically modified *Aspergillus niger*. A search of the Applicant's website¹ indicates the main sources of fungal chitosan in KitoZyme's chitosan portfolio are *Aspergillus niger* or *Agaricus bisporus*. In addition, an application for the authorization of the same chitosan for use as a processing aid was submitted to Food Standards Australia and New Zealand (FSANZ) for review (Application A1077). The publicly available information on this application indicates that the KitoZyme chitosan (KiOmedine-CsU) subject to the bacterial reverse mutation assay as discussed in GRN 397 was obtained from *Agaricus bisporus*, rather than *Aspergillus bisporus*. It is noted that the results of this unpublished bacterial reverse mutation test have since been published by Douette *et al.* (2020), in addition to an *in vitro* mouse lymphoma assay conducted at concentrations up to 10% for 4 hours in the presence and absence of metabolic activation (Douette *et al.*, 2020). No genotoxic or mutagenic potential were reported by the study authors.

¹ <https://www.kitozyme.com/en/>

It is noted that the publication by Douette *et al.* (2020) was not identified in the literature search due to the fact that this study was published in the Journal of Biomaterials, which is not indexed by any of the databases that were searched. As discussed in Section 6.1 of the GRAS Notice, a comprehensive search of the published scientific literature was conducted to identify publications that were published since GRN 397. The time period for this search spanned from 2011 through March 2021. The search was conducted using the electronic search tool, ProQuest Dialog™, with several databases, including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. Nevertheless, the findings of Douette *et al.* (2020) are consistent with the GRAS conclusions that chitosan does not have genotoxic or mutagenic potential.

Yours sincerely



David Brown
Chief Operating Officer
Chinova Bioworks Inc.

8 November 2021

Marissa Santos, MS
Regulatory Review Scientist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Dear Ms. Santos

Re: Toxicology Comments on GRN 997

In the safety section, there are two types of studies provided: the human studies and the animal studies.

The human studies. Focusing on studies conducted for 90 days or longer, two aspects of the studies are apparent:

- (1) The studies are all efficacy studies, not safety (toxicology) studies.
- (2) In all studies, except for the 24-week study, the number of subjects was low.
- (3) The endpoints studied focused on features like body weight reduction, obesity, and the effects on lipid profile (TC, HDL-C, LDL-C, TG).
- (4) Two studies that measured the serum 25(OH)D showed a reduction.

The bottom line of all the human studies in the notice is that none of them qualifies as a safety study because of the lack of measurement of appropriate apical endpoints. The 24-week study even uses the words 'dietary supplement' in the study title.

Conclusion from the human studies: The human studies are clearly meant to support the dietary supplement use (infrequent, does not assume lifelong use, does not include the whole population), which is quite different from the use as a food ingredient (assumes consumption by the general population throughout life). That is why the GRAS program focuses on the safety data (toxicology aspect), not the efficacy data. Under this situation, even if the notifier wants to use the chitosan dose in the longest duration-highest dose human study (Mhurchu et al., 2004) as the starting point to determine the ADI and EDI, the intraspecies safety factor must be applied. Ideally, if the ingredient were a chemical of unknown nature, another safety factor would have been needed to account for the lack of safety endpoints.

In this case, the notifier may justify avoiding the use of additional safety factor by providing a good, and logical discussion on the totality of evidence (animal and human studies, and any other evidence), as well as the nature of the ingredient. The **intraspecies safety factor** still needs to be applied.

Conclusion from the animal studies: The NTP study is the most thoroughly conducted and most well characterized of all animal studies. The study identified a LOEL of 450 mg/kg. Thus, using the totality of

evidence and the nature of the ingredient, the notifier may justify using the LOEL and apply the proper (inter- and intraspecies) safety factors to arrive at the ADI. Ideally, the EDI should be lower than the ADI.

Response:

In the NTP study, dietary exposure to chitosan for 6 months resulted in decreased fat digestion and depletion of some fat-soluble vitamins in male and female rats. There were no histological findings associated with the observed decreases in vitamin levels. Based on the effects of chitosan on serum vitamin E levels, the authors concluded the “*lowest-observed-effect level for chitosan exposure was 1% (approximately equivalent to 450 mg/kg) in male and 9% (approximately equivalent to 6,000 mg/kg) in female rats*”. These effects are considered to be indirect consequences of the recognized fat binding properties of chitosan¹ resulting in excretion of dietary fat and reduced absorption of fat-soluble vitamins. Furthermore, as also discussed in Section 6.3.3 of GRN 997, generalized effects of resistant dietary fibers, such as chitosan, on nutrient absorption have been long known, are well characterized, and are not considered nutritionally relevant at levels that are commonly consumed in the diet. Considering the foregoing, these effects are not considered to be a direct toxic effect of chitosan on organ systems or a finding of toxicological or nutritional significance. The reported fatty change is considered to be a biological adaptive response to depletion of fat-soluble vitamins and minerals and contingent upon consumption of supraphysiological intakes that would affect lipid absorption.

In order to address the concerns raised by the FDA’s review team with respect to the inadequate margin of safety between the EDI and the LOEL from the NTP study, Chinova have made revisions to the proposed use levels of the notifiable substance, fiber extracted from white button mushrooms, in order to reduce the EDI. The revised proposed use levels are listed in Table 1 below.

INTENDED CONDITION OF USE OF CHINOVA’S FIBER EXTRACTED FROM WHITE BUTTON MUSHROOMS (*A. BISPORUS*)

The proposed conditions of use for Chinova’s fiber extracted from white button mushrooms are summarized in Table 1 below. Chinova’s fiber extracted from white button mushrooms (*A. bisporus*) is intended for use in the same food categories as those that previously received GRAS status by the Flavor and Extract Manufacturers Association (FEMA) for use as an ingredient with flavor modifying properties (FEMA No. 4946). While the FEMA approval for Chinova’s fiber extracted from white button mushrooms is up to 2,000 ppm in the food and beverage categories listed in Table 1, post market monitoring data related to Chinova’s fiber extracted from white button mushrooms demonstrated that the flavor modifying effects of the ingredient can be achieved at much lower levels, which are also aligned with its antimicrobial activity. Therefore, Chinova’s ingredient functions as both an antimicrobial and a flavor modifier at use levels ranging from 10 to 150 ppm (*i.e.*, 0.01 to 0.1%). Therefore, the dual function of Chinova’s fiber extracted from white button mushrooms is not considered additive. For the purposes of the intake assessment, the maximum use level between the two applications was applied to all proposed food use categories, as this would be the maximum amount required in the final food product to meet the dual function of the ingredient as an antimicrobial and flavor modifier ingredient (See Table 1).

¹ Chitosan is marketed as a dietary supplement for weight loss, and the USP monograph for chitosan includes fat binding capacity as a qualitative specification parameter for the ingredient.

Food codes representative of each proposed food use were chosen from the NHANES 2017-2018 (CDC, 2021b). Food codes were grouped in food use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (U.S. FDA, 2020). If necessary, product-specific adjustment factors were developed for composite foods/mixtures based on data provided in the Food and Nutrient Database for Dietary Studies (USDA ARS, 2021) or the Food Commodity Intake Database (U.S. EPA & USDA, 2020).

Table 1 Summary of the Individual Proposed Food Uses and Use Levels for Chinova’s Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*) in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020)	Food Uses ^a	Proposed Antimicrobial Use Levels (g/100 g)	Proposed Flavor Modification Use Levels (g/100g)	Maximum Proposed Use Levels (g/100g)
Baked Goods and Baking Mixes	Bagels and English Muffins	0.060	0.060	0.060
	Bread (excluding sweet type breads and rolls)	0.060	0.060	0.060
	Cakes			
	Light weight cakes	0.060	0.060	0.060
	Medium weight cakes	0.060	0.060	0.060
	Heavy weight cakes	0.060	0.060	0.060
	Cornbread, corn muffins, or tortillas	0.060	0.060	0.060
	Muffins	0.040	0.040	0.040
Beverages, Alcoholic	Pastries	0.050	0.050	0.050
	Cocktail drinks	0.020	0.020	0.020
	Energy drinks	0.010	0.010	0.010
	Flavored or carbonated waters	0.010	0.010	0.010
	Soft drinks (regular and diet)	0.010	0.010	0.010
	Sport or electrolyte drinks, fluid replacement drinks	0.010	0.010	0.010
	Cheese-based sauces	0.100	0.100	0.100
	Cottage cheese	0.050	0.050	0.050
Cheeses	Cream cheese and cheese-based spreads	0.100	0.100	0.100
	Processed cheese or cheese mixtures	0.060	0.060	0.060
Coffee and Tea	Ready-to-drink tea beverages	0.010	0.010	0.010

Table 1 Summary of the Individual Proposed Food Uses and Use Levels for Chinova’s Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*) in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020)	Food Uses^a	Proposed Antimicrobial Use Levels (g/100 g)	Proposed Flavor Modification Use Levels (g/100g)	Maximum Proposed Use Levels (g/100g)
Condiments and Relishes	Relish	0.080	0.050	0.080
Confections and frostings	Coatings	0.100	0.100	0.100
	Frostings and icings	0.040	0.100	0.100
Dairy Product Analogs	Imitation cheese	0.150	0.150	0.150
Fats and oils	Fat-based sauces	0.050	0.050	0.050
	Margarine and margarine-like spreads	0.050	0.050	0.050
	Mayonnaise and mayonnaise-type dressings	0.060	0.060	0.060
	Salad dressings	0.080	0.080	0.080
Gelatins, Puddings, and Fillings	Flans, custards, and other egg-based desserts	0.080	0.100	0.100
Grain Products and Pastas	Energy bars or protein bars or meal replacement bars	0.020	0.020	0.020
Gravies and Sauces	Gravies	0.020	0.020	0.020
	White sauces	0.100	0.100	0.100
Jams and Jellies	Jams, jellies, preserves, and marmalades	0.100	0.100	0.100
Milk Products	Plain or flavored yogurt	0.060	0.060	0.060
Processed Fruits and Fruit Juices	Fruit drinks and ades and smoothies	0.020	0.020	0.020
	Fruit-based desserts	0.080	0.100	0.100
Plant Protein Products	Meat analogs	0.150	0.150	0.150
	Egg substitutes	0.080	0.080	0.080
Processed Vegetables and Vegetable Juices	Vegetable purees ^c	0.040	0.040	0.040
Sugar Substitutes	Sugar substitutes	0.100	0.100	0.100
Sweet sauces, toppings, and	Sweet sauces, syrups, and toppings (including fruit-based)	0.060	0.060	0.060

Table 1 Summary of the Individual Proposed Food Uses and Use Levels for Chinova’s Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*) in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020)	Food Uses ^a	Proposed Antimicrobial Use Levels (g/100 g)	Proposed Flavor Modification Use Levels (g/100g)	Maximum Proposed Use Levels (g/100g)
syrops	Cocoa syrops	0.100	0.100	0.100

CFR = *Code of Federal Regulations*; U.S. = United States.

^a Chinova’s mushroom-derived fiber is intended for use in unstandardized products when standards of identity, as established under 21 CFR §130 to 169, do not permit its addition.

^b Food codes for vegetable mixtures and vegetable combinations (which are likely to be used to make purees) were included as a surrogate for ‘vegetable purees’.

ESTIMATED DIETARY CONSUMPTION OF CHINOVA’S FIBER EXTRACTED FROM WHITE BUTTON MUSHROOMS (*A. BISPORUS*)

Among the total population (ages 2 years and older), the mean and 90th percentile consumer-only intakes of Chinova’s fiber extracted from white button mushrooms were determined to be 0.11 and 0.21 g/person/day, respectively (Table 2). Of the individual population groups, male adults were determined to have the highest mean and 90th percentile consumer-only intakes of Chinova’s fiber extracted from white button mushrooms, at 0.13 and 0.25 g/person/day respectively.

Table 2 Summary of the Estimated Daily Intake of Chinova’s Fiber Extracted from White Button Mushrooms (*Agaricus bisporus*) from Proposed Food Uses at the Maximum Use Level in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group (Years)	Per Capita Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90 th Percentile	%	n	Mean	90 th Percentile
Children	2 to 5	0.07	0.13	96.7	446	0.07	0.13
Children	6 to 11	0.09	0.16	99.3	675	0.09	0.16
Female Teenagers	12 to 19	0.09	0.16	98.4	439	0.09	0.16
Male Teenagers	12 to 19	0.10	0.21	96.8	426	0.11	0.21
Female Adults	20 and older	0.10	0.19	97.6	2,107	0.10	0.19
Male Adults	20 and older	0.12	0.25	97.9	1,928	0.13	0.25
Total Population	2 and older	0.10	0.20	97.8	6,021	0.11	0.21

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

Taking into consideration a 100-fold safety factor to account for inter- and intraspecies variations, the LOEL of 450 mg/kg bw/day from the NTP study corresponds to an ADI of 0.31 g/day for a 70 kg individual. The highest 90th percentile consumer-only intakes of Chinova's fiber extracted from white button mushrooms was estimated to be 0.25 g/person/day, which is lower than the ADI of 0.31 g/day, indicating that an adequate margin of safety (126-fold) can be established.

The safety of Chinova's fiber extracted from white button mushrooms is further corroborated by a number of human studies wherein chitosan was consumed at doses of 0.54 to 6.75 g/day for a duration of 2 to 24 weeks and was reported to be well tolerated without significant treatment-related adverse effects. Although these studies were mainly efficacy-related studies evaluating the use of chitosan in food supplement products due to its fat sequestering properties, the results of these studies demonstrated that concerns regarding chitosan reducing the absorption of lipid and other nutrients were mainly reported in studies with rats, and no significant decreases in fat-soluble vitamins were reported in human studies. As such, these studies can provide corroborative evidence to the safety of Chinova's fiber extracted from white button mushrooms.

We hope that the above has adequately addressed the concerns raised by the FDA's review team. If you have any further questions, please do not hesitate to contact me.

Yours sincerely



David Brown
Chief Operating Officer
Chinova Bioworks Inc.

1 December 2021

Marissa Santos, MS
Regulatory Review Scientist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Dear Ms. Santos

Re: Chemistry Comments on GRN 997

Please find our response to the reviewer's questions below in bolded lettering:

1. Based on the response provided in the amendment dated November 8, 2021, please indicate if the intended uses in GRN 000997 would be additive to the FEMA GRAS uses of chitosan.
Chinova's Response - The proposed conditions of use for Chinova's fiber extracted from white button mushrooms as indicated in GRN 000997 are 'substitutional' for the FEMA GRAS uses; therefore, they are not considered additive.
2. Please provide a statement that the additional food categories listed in the FEMA GRAS (fish products, meat products, milk products, other grains, seasonings and flavorings, soft candy, and soups) list are no longer intended uses of the chitosan ingredient.
Chinova's Response – The additional food categories listed in the FEMA GRAS, including fish products, meat products, milk products, other grains, seasonings and flavorings, soft candy, and soups are no longer intended uses of the Chinova's fiber extracted from white button mushrooms, as there are no market opportunities for such products.
3. Please indicate if the intended uses in GRN 000997 would be substitutional for the FEMA GRAS uses and therefore, there would be no increase in the cumulative exposure to chitosan.
Chinova's Response – The intended uses of Chinova's fiber extracted from white button mushrooms, as listed in GRN 000997, would be substitutional for the FEMA GRAS uses and as such no increase in the cumulative exposure to chitosan would be expected.

We hope that the above has adequately addressed the concerns raised by the FDA's review team. If you have any further questions, please do not hesitate to contact me.

Yours sincerely



David Brown
Chief Operating Officer
Chinova Bioworks Inc.