



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

Re: GRAS Notice No. GRN 000997

Dear Mr. Brown:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000997. We received Chinova Bioworks Inc.'s (Chinova) notice on March 4, 2021, and filed it on July 13, 2021. Chinova submitted an amendment to the notice on July 21, 2021, adding the subcategory of egg substitutes. Additionally, Chinova submitted amendments dated September 9, 2021, November 8, 2021, and December 1, 2021, providing additional information regarding the intended uses, specifications, analytical methods, dietary exposure, and safety of the notified substance.

The subject of the notice is chitosan from white button mushrooms (*Agaricus bisporus*) for use as an antimicrobial at levels ranging from 0.015 g to 0.15 g per 100 g of food. The intended uses and use levels are shown in Table 1.¹ The notice informs us of Chinova's view that the use of chitosan is GRAS through scientific procedures.

Table 1. Proposed uses and use levels for chitosan

Food Category	Food Uses	Maximum Use Level (g/100 g)
Baked goods	Bagels and English muffins	0.06
	Bread (excluding sweet type)	0.06
	Cakes	0.06
	Cornbread, corn muffins, tortillas	0.06
	Muffins	0.04
	Pastries	0.05
Alcoholic beverages	Cocktail drinks	0.02
Beverages and beverage bases	Energy Drinks	0.01
	Flavored and carbonated waters	0.01
	Soft drinks	0.01
	Sport, electrolyte and fluid replacement	0.01
Cheese	Cheese-based sauces	0.1
	Cottage cheese	0.05

¹ Chinova states that chitosan is not intended for use in infant formula, infant foods, and in products under the jurisdiction of the United States Department of Agriculture.

	Cream cheese and cheese spreads	0.1
	Processed cheese and cheese mixtures	0.06
Coffee and tea	Ready-to-drink tea	0.01
Condiments and relishes	Relish	0.08
Confections and frostings	Coatings	0.1
	Frostings and icings	0.1
Dairy product analogs	Imitation cheese	0.15
Fats and oils	Fat-based sauces	0.05
	Margarine and margarine-like spreads	0.05
	Mayonnaise and mayonnaise-type dressings	0.06
	Salad dressings	0.08
Gelatins, puddings, and fillings	Flans, custards and egg-based desserts	0.08
Grain products	Energy, protein or meal-replacement bars	0.02
Gravies and sauces	Gravies	0.02
	White sauces	0.1
Jams and jellies	Jams, jellies, preserves, and marmalades	0.1
Milk Products	Yogurt	0.06
Processed fruit	Fruit drinks, ades and smoothies	0.02
	Fruit-based desserts	0.1
Plant protein products	Meat analogs	0.15
	Egg substitutes	0.08
Processed vegetables	Vegetable purees	0.04
Sugar substitutes	Sugar substitutes	0.1
Sweet sauces, toppings and syrups	Sweet sauces, syrups and toppings	0.06
	Cocoa syrups	0.1

Our use of the term “chitosan” in this letter is not our recommendation of that term as an appropriate common or usual name for declaring the substance in accordance with FDA’s labeling requirements. Under 21 CFR 101.4, each ingredient must be declared by its common or usual name. In addition, 21 CFR 102.5 outlines general principles to use when establishing common or usual names for nonstandardized foods. Issues associated with labeling and the common or usual name of a food ingredient are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition. The Office of Food Additive Safety (OFAS) did not consult with ONFL regarding the appropriate common or usual names for “chitosan.”

Chinova provides information on the identity and composition of chitosan [(1,4)-2-amino-2-desoxy-beta-D-glucan] extracted from white button mushrooms (*Agaricus bisporus*). They describe it as a white to beige powder containing 95% chitosan and up to 5% beta-1,3-D-glucans. The identity of the two components is confirmed using ¹H-NRM and infrared spectroscopy. Chinova states that chitosan is a linear polycationic polysaccharide composed of glucosamine and N-acetyl glucosamine monomers linked

with a 1,4-beta-linkage and having a molecular weight ranging from 10 to 400 kDa and a CAS registry number of 9012-76-4.

Chinova describes the manufacturing process for chitosan as a multistep extraction and purification from white button mushrooms. Chinova states that chitosan is manufactured in accordance with current good manufacturing practices and that all raw materials, processing aids and food contact articles used in the manufacturing are food grade and approved for their respective uses in accordance with an appropriate regulation, are the subject of an effective food contact notification, or are GRAS for that use. Chitosan is produced from white button mushrooms via thermal deacetylation with sodium hydroxide followed by collection of the biomass and repeated rinsing with water. The chitosan is separated from the biomass by extraction with vinegar. The remaining solids are removed by centrifugation and the chitosan is precipitated by adjusting the pH with sodium hydroxide. The precipitated substance is collected via centrifugation and repeatedly washed with water. The chitosan is then dried using a drum dryer and milled into a fine powder.

Chinova provides specifications for chitosan that include total chitosan (≥ 95 % w/w), beta-1,3-glucans (≤ 5 % w/w), degree of deacetylation (≥ 80 mol %), moisture (≤ 10 %), total ash (≤ 3 %), solubility (≥ 99.5 %), total arsenic (≤ 0.2 mg/kg), cadmium (≤ 0.2 mg/kg), mercury (≤ 0.2 mg/kg), lead (≤ 1 mg/kg) and limits for microorganisms including aerobic microbial count (≤ 100 colony forming units (CFU)/g), yeast and mold (≤ 100 CFU/g), *Escherichia coli* (absent in 10 g), and *Salmonella* (absent in 25 g). Chinova provides the results of five non-consecutive batch analyses to demonstrate that chitosan can be manufactured to meet the specifications.

Chinova provides the results of stability studies for three batches of chitosan stored at either 25 °C and 60% relative humidity (recommended storage conditions) or 40 °C and 70% relative humidity (accelerated storage conditions). Based on the results, Chinova notes that chitosan is stable for at least 9 months when stored under both the recommended and accelerated storage conditions and up to 24 months based on the results from the accelerated storage conditions.

Chinova states that the technical effect for chitosan is as an antimicrobial. Chinova provides results demonstrating this technical effect in beverages, baked goods, and dairy products.

Chinova discusses the estimated dietary exposure to chitosan for the intended uses and use levels described in Table 1. Chinova notes that chitosan's intended use as an antimicrobial is not additive to its use as flavoring agent. Based on food consumption data from the 2017-2018 National Health and Examination Survey (NHANES), Chinova estimates the mean and 90th percentile eaters-only dietary exposure to chitosan to be 0.11 g and 0.21 g/person (p)/day, respectively, for the U.S. population aged 2 years and older. Chinova states that the intended uses in GRN 000997 would be substitutional for the use as a flavoring agent and therefore, there would be no increase in the cumulative dietary exposure to chitosan.

Chinova states that chitosan is not digested by human digestive enzymes; absorption and systemic exposure to intact chitosan molecules consumed in the diet will not occur. However, chitosan is subjected to microbial fermentation in the gastrointestinal tract, as is seen for dietary fibers. Chinova discusses a published six-month study in rats where rats of both sexes were fed either control diets or diets containing seafood shell-derived chitosan at concentrations of 1%, 3%, or 9%. Chinova agrees with the study authors' conclusion that no effects were observed when chitosan was fed at less than 1% (approximately equivalent to 450 mg/kg bw) in male rats and 9% (approximately equivalent to 6,000 mg/kg bw) in female rats. Chinova states that Chitosan does not have any reproductive or developmental toxicity and is not mutagenic or genotoxic. Chinova discusses human studies in which subjects consumed shrimp or shellfish derived chitosan ranging from 0.5 to 6.75 g/day for 4 to 24 weeks. There was no decrease in serum fat soluble vitamins (A, E, D), α -carotene, and β -carotene, or changes in clinically relevant serum parameters.

Chinova includes the statement of a panel of individuals (Chinova's GRAS panel). Based on its review, Chinova's GRAS panel concluded that chitosan is safe under the conditions of its intended use.

Based on the totality of the data and information described above, Chinova concludes that chitosan is GRAS for its intended use.

Standards of Identity

In the notice, Chinova states its intention to use chitosan in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of Chinova's notice concluding that chitosan is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing chitosan. Accordingly, our response should not be construed to be a statement that foods containing chitosan, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Chinova provided, as well as other information available to FDA, we have no questions at this time regarding Chinova's conclusion that chitosan

is GRAS under its intended conditions of use. This letter is not an affirmation that chitosan is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 000997 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

 Digitally signed by Susan J.
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Susan Carlson, Ph.D.
Director
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