Ankit Rathi
Specialty Enzymes & Probiotics (a subsidiary of Advanced Enzymes USA)
13591 Yorba Avenue
Chino, CA 91710

Re: GRAS Notice No. GRN 000991

Dear Mr. Rathi:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000991. We received Advanced Enzyme Technologies Ltd. (Advanced Enzymes)'s GRAS notice on December 15, 2020, and filed it on June 9, 2021. Advanced Enzymes submitted amendments to the notice on October 13, 2021, January 19, 2022, and June 3, 2022 to update contact information, provide additional information on the production strain, analytical methods, technical effect and enzyme activity, and clarify the status of information designated as confidential.[1]

The subject of the notice is chitosanase enzyme preparation produced by *Bacillus subtilis* (chitosanase enzyme preparation) for use as an enzyme at up to 2.55 g Total Organic Solids (TOS)/kg chitosan in the manufacture of water-soluble chitosan (WSC) used as a preservative for shrimp. The notice informs us of Advanced Enzymes' view that this use of chitosanase enzyme preparation is GRAS through scientific procedures.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component that catalyzes the chemical reaction, as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. Advanced Enzymes' notice provides information about the components in the chitosanase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, chitosanase is identified by the Enzyme Commission Number 3.2.1.132.[2] Chitosanase is identified by the Chemical Abstracts Service Number 51570-20-8. Advanced Enzymes provides the amino acid sequence of chitosanase and determined the molecular mass of the notified enzyme to be 29 kDa.

[1] The June 3, 2022 amendment states that information previously designated as confidential in GRN 000991 is not considered confidential by Advanced Enzymes.
[2] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/132.html

Advanced Enzymes states that the *B. subtilis* production organism is non-pathogenic non-toxigenic, and a well-characterized production organism with a history of safe use in the food industry.

Advanced Enzymes states that the chitosanase enzyme preparation is manufactured by submerged fermentation of a pure culture of the *B. subtilis* CSSC production strain under controlled conditions. The chitosanase enzyme is secreted into the medium and then recovered by filtration and concentrated by ultrafiltration and diafiltration steps. The enzyme concentrate is filtered to remove any insoluble materials and residual production strain. The enzyme concentrate is stabilized with glycerol and then spray-dried and formulated with food grade ingredients (e.g., maltodextrin) to produce a liquid chitosanase enzyme preparation. Advanced Enzymes states that the entire process is performed using food grade raw materials in accordance with current good manufacturing practices. Advanced Enzymes states that soy flour, an ingredient in the fermentation medium, is absent in the final enzyme preparation.

Advanced Enzymes has established food grade specifications and states that the chitosanase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 12th edition, 2021), and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). Advanced Enzymes provides data from analyses of three batches of chitosanase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met. Advanced Enzymes states that the steps during manufacturing ensure the absence of the production organism and antibiotic activity in the final product and include these as specifications.

Advanced Enzymes intends to use chitosanase enzyme preparation for use as an enzyme at up to 2.55 g TOS/kg chitosan in the manufacture of WSC. Advanced Enzymes states that chitosanase catalyzes the hydrolysis of β-1,4 linkages between D-glucosamine residues in a partially acetylated chitosan. WSC is intended to be used as a preservative for shrimp, at levels of 5 g WSC/kg shrimp. Although not removed from the WSC, the chitosanase enzyme preparation is not expected to have a technical effect in the final food. If it is assumed that the chitosanase enzyme preparation is completely retained in the WSC, the resulting level of chitosanase enzyme preparation is 12.74 mg TOS/kg shrimp. Advanced Enzymes estimates a dietary exposure to chitosanase enzyme preparation to be 0.245 mg TOS/person/day (mg TOS/p/d), corresponding to 0.0035 mg TOS/kg body weight per day (mg TOS/kg bw/d). In their dietary exposure estimate Advanced Enzymes assumes that all seafood consumed in the US, based on USDA ERS 2016 food availability data, is in the form of shrimp. Advanced Enzymes states that the enzymatic activity is not expected to produce any reaction products that are not already part of the human diet, and that the chitosanase itself is expected to be digested and metabolized in the human gastrointestinal system.

Advanced Enzymes relies on published information to demonstrate the safety of the *B. subtilis* production organism and the safety, in general, of microbial enzyme preparations used in food processing. Advanced Enzymes summarizes corroborative

evidence of safety of chitosanase. Tests conducted with bacterial cells showed that the chitosanase enzyme concentrate is not mutagenic at the highest dose tested both in the presence and absence of metabolic activation. Advanced Enzymes also demonstrates that the chitosanase enzyme concentrate is not clastogenic based on results from *in vitro* mammalian cell micronucleus test. A 90-day oral toxicity study in rats using the chitosanase enzyme concentrate at the highest dose tested (1000 mg TOS/kg bw/d) showed no treatment-related effects. Based on the highest dose tested in the unpublished 90-day study (1000 mg TOS/kg bw/d) and the estimated dietary exposure from the intended uses of the chitosanase enzyme preparation (0.0035 mg TOS/kg bw/d), Advanced Enzymes estimates a margin of exposure of 285,000.[3]

Advanced Enzymes discusses publicly available literature, as well as the conclusions of several organizations and working groups, concerning the low risk of allergenicity posed by oral consumption of enzymes to address the potential for allergenicity to chitosanase. Based on bioinformatic analyses, Advanced Enzymes reports no matches between the amino acid sequences of the chitosanase and the primary sequences of known allergens based on the guidelines developed by Codex Alimentarius Commission (FAO/WHO, 2001).[4] Advanced Enzymes also discusses unpublished results of an *in vitro* gastric digestion assay to support their conclusion as to the lack of potential for oral allergenicity. Based on the totality of the information available, Advanced Enzymes concludes that it is unlikely that oral consumption of chitosanase enzyme from the intended use will result in allergenic responses.

Based on the data and information summarized above, Advanced Enzymes concludes that chitosanase enzyme preparation is GRAS for its intended use.

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 Advanced Enzymes' notice concluding that chitosanase enzyme preparation 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 chitosanase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing chitosanase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

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[3] FDA notes the margin of exposure is based on unpublished safety studies and is corroborative of the published information regarding safety of enzyme preparations used in food processing.

[4] Advanced Enzymes used sequence homology with an 80 amino acid sliding window with more than 35% identity match and 8 continuous amino acids based on the FAO/WHO report on allergenicity of genetically modified foods (FAO/WHO, 2001) and received no hits with known allergens.

Conclusions

Based on the information that Advanced Enzymes provided, as well as other information available to FDA, we have no questions at this time regarding Advanced Enzymes' conclusion that chitosanase enzyme preparation produced by *B. subtilis* is GRAS under its intended conditions of use. This letter is not an affirmation that chitosanase enzyme preparation produced by *B. subtilis* 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 000991 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2022.08.31 16:22:52 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Center for Food Safety
and Applied Nutrition