Mel Drozen
Keller and Heckman LLP
1001 G Street, NW
Suite 500 West
Washington, DC 20001

Re: GRAS Notice No. GRN 000743

Dear Mr. Drozen:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000743. We received the GRAS notice that you submitted on behalf of Amano Enzyme Inc. (Amano) on November 8, 2017 and filed it on December 21, 2017. Amano submitted an amendment containing additional safety information and clarification on information initially designated confidential[1] on May 14, 2018.

The subject of the notice is beta-galactosidase enzyme preparation produced by *Papiliotrema terrestris* (beta-galactosidase enzyme preparation) for use as an enzyme at up to 827 mg/kg of lactose starting material in the production of galactooligosaccharides (GOS) from lactose. The notice informs us of Amano's view that this use of beta-galactosidase 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. Amano's notice provides information about the components in the beta-galactosidase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, beta-galactosidase is identified by the Enzyme Commission Number 3.2.1.23. The common name for the enzyme is beta-galactosidase and the systematic name is beta-D-galactoside galactohydrolase. Beta-galactosidase is also known as lactase; beta-lactosidase; maxilact; hydrolact; beta-D-lactosidase; S 2107; lactozym; trilactase; beta-D-galactanase; oryzatym; sumiklat. The CAS Registry Number for beta-galactosidase is 9031-11-2. Beta-galactosidase catalyzes the hydrolysis of terminal non-reducing beta-D-galactose residues in beta-D-galactosides. In the presence of high lactose concentrations and specific processing conditions, the beta-galactosidase enzyme can utilize lactose as a substrate to form GOS. Amano states that it has determined the beta-galactosidase preparation contains

[1] GRN 000743 included information in Appendices 5 and 6 that Amano initially designated confidential in the notice. In the May 14, 2018, amendment, Amano confirms that the information was incorrectly marked confidential and that this information is not confidential.

three beta-galactosidase enzymes: the full-size protein consisting of 566 amino acids, and two proteins with N-terminal truncations of 6 and 11 amino acids each. Aside from the N-terminal truncations, the three enzymes are identical in sequence. Based on the amino acid sequences, Amano calculates the molecular weights of these enzymes to be 63.9 kDa, 63.3 kDa, and 62.8 kDa respectively.[2]

Amano describes *P. terrestris* as a non-pathogenic and non-toxigenic fungus based on published data. Amano states that *P. terrestris* does not appear on any public registries of hazardous microorganisms,[3] and that it is classified as BSL-1 by ATCC. Amano states that the *P. terrestris* production strain was obtained via mutagenesis of the parent strain[4] with N-methyl-N'-nitro-N-nitrosoguanidine.

Amano states that the beta-galactosidase enzyme preparation is manufactured from a pure culture of the production strain by fermentation under controlled conditions. The culture is monitored to ensure production strain identity and purity. The beta-galactosidase is secreted into the fermentation media. Amano states that after fermentation the production organism is removed from the supernatant by filtration. The enzyme is then recovered by several filtration and concentration steps. The supernatant containing the enzyme is concentrated by ultrafiltration followed by additional filtration steps. The concentrated enzyme solution is then filtered again to ensure removal of the production organism. Amano provides analytical data from three representative batches of beta-galactosidase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production strain. The liquid enzyme concentrate is used for toxicological studies. The enzyme is spray dried and formulated with milk-derived lactose. Amano also states that the final enzyme preparation contains no major food allergens from the fermentation media. Amano states that the entire process is performed in accordance with current good manufacturing practices using raw materials of food grade quality. Amano notes that the beta-galactosidase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 10th edition, 2016), 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).

Amano intends to use beta-galactosidase enzyme preparation at levels up to 827 mg/kg in the production of GOS from lactose. GOS may be used in a variety of food applications including products consumed by infants. Amano states that there is no residual beta-galactosidase activity at the limit of detection (LOD)[5] in the final GOS. However, based on the maximum intended use levels and the assumption that all the beta-galactosidase enzyme preparation will remain in the final food, Amano estimates the dietary exposure to beta-galactosidase enzyme preparation to be 0.55 mg Total Organic Solids (TOS)/kg bodyweight (bw)/day (d) in infants zero to six months old, 0.86 mg/kg bw/d in infants and children six months to 2 years old, and 2.7 mg TOS/kg bw/d in individuals 2+ years old.

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[2] Amano calculated the molecular weights using Expasy Bioinformatics (http://web.expasy.org/compute_pi/).

[3] The public registries include the list of pathogens in Annex III of Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agent at work, the list of microbiological hazards of the French Agency for Food, Environmental and Occupational Health & Safety, and the list of pathogens on the Belgian Biosafety Server.

[4] The parent strain was isolated from soil, and originally identified to correspond to *Cryptococcus terrestris* (also called *P. terrestris*) based on phenotypic properties.

[5] Amano states that the LOD of the validated method to measure the activity of residual beta-galactosidase in GOS is 5 mLU/g.

Amano summarizes published toxicological studies using the beta-galactosidase enzyme concentrate. Amano states that the beta-galactosidase enzyme is not mutagenic based on results from a bacterial reverse mutation assay. A 13-week sub-chronic oral toxicity study of the beta-galactosidase enzyme concentrate did not cause any treatment-related adverse effects in rats at up to the highest dose tested (equivalent to 1800 mg TOS/kg bw/d). Based on the highest dose tested in the 13-week study and the estimated dietary exposure from the intended use of the beta-galactosidase enzyme preparation, Amano calculates a margin of exposure to be 2093, 3273, and 652, in infants 0-6 months old, in infants and children 6 months to 2 years old, and in individuals 2+ years of age, respectively. Additionally, Amano relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism. Amano also states that the safety of the beta-glucosidase produced by *P. terrestris* is corroborated by published toxicological studies of a related beta-galactosidase produced by a different microorganism.

Amano discusses the potential food allergenicity of beta-galactosidase enzyme by comparing the amino acid sequence of beta-galactosidase to primary sequences of known allergens within the Structural Database of Allergenic Proteins. In their searches, Amano did not find any matches to known allergens in the database with greater than 35% sequence homology over an 80-amino acid window or any sequence identity to potential allergens over contiguous stretches of eight amino acids. Additionally, Amano performed an *in silico* digestion of the beta-galactosidase using Expasy Peptide Cutter. Amano states that no matches were found when the resulting peptides were compared to known toxins and toxic peptides in the Toxic Exposome Database using standard parameters. Amano also states that within the amino acid sequence of the beta-galactosidase enzyme, no sequence homologies to toxic peptides were found using ToxinPred. Based on all these analyses, Amano concludes that the beta-galactosidase and its metabolized products are not expected to be toxigenic. Amano further discussed the conclusions of several organizations and working groups about the low-risk of allergenicity posed by enzymes due to their low use levels and the extensive processing of enzyme-containing foods during manufacturing. Based on the totality of the information available, Amano concludes that it is unlikely that oral consumption of beta-galactosidase enzyme will result in allergenic or toxic responses.

Based on the data and information summarized above, Amano concludes that beta-galactosidase enzyme preparation is GRAS for its intended uses.

Allergen Labeling

The Federal Food, Drug, and Cosmetic Act (FD&C Act) requires that the label of a food that is or contains "major food allergen" declare the allergen's presence (section 403(w)). The FD&C Act defines a "major food allergen" as one of eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. Beta-galactosidase enzyme preparation may require labeling under the FD&C Act, because it contains milk-derived lactose. Questions about petitions or notifications for exemptions from food allergen labeling requirements should be directed to the Division of Biotechnology and GRAS Notice Review in the Office of Food Additive Safety. Questions related to food labeling in general should be directed to the Office of Nutrition and Food Labeling.

Section 301(ll) of the 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 Amano's notice concluding that beta-galactosidase 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 beta-galactosidase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing beta-galactosidase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Amano provided, as well as other information available to FDA, we have no questions at this time regarding Amano's conclusion that beta-galactosidase enzyme preparation produced by *P. terrestris* is GRAS under its intended conditions of use. This letter is not an affirmation that beta-galactosidase enzyme preparation produced by *P. terrestris* 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 000743 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
Date: 2018.06.01 14:00:35 -04'00'

Dennis M. Keefe, Ph.D.
Director
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