Claire L. Kruger, PhD, DABT, CFS
Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Re: GRAS Notice No. GRN 001039

Dear Dr. Kruger:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001039. We received Godo Shusei Co., Ltd. (Godo Shusei)'s notice on September 22, 2021, and filed it on March 11, 2022. Godo Shusei submitted an amendment to the notice December 6, 2022, providing additional information on the enzyme identity, production organism, and bioinformatic analyses.

The subject of the notice is lactase[1] enzyme preparation produced by *Aspergillus oryzae* (lactase enzyme preparation) for use as an enzyme at up to 130 mg Total Organic Solids/kg raw material (TOS/kg) in the processing of milk or whey used in milk powders, fermented milk products and yogurt, fresh cheeses, milk-based desserts, whey products, baked goods, confectionary, cereal bars, and soft drinks, and at 36 mg TOS/kg raw material in the processing of milk used for non-exempt infant formulas for term infants and milk-based formulas and drinks for young children ages 12 to 36 months. The notice informs us of Godo Shusei's view that these uses of lactase enzyme preparation are 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. Godo Shusei's notice provides information about the components in the lactase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, lactase is identified the Enzyme Commission Number 3.2.1.23;[2] the CAS Number for lactase is 9031-11-2. Godo Shusei states that the primary amino acid sequence of the lactase consists of 1005 amino acids and has a calculated molecular weight of 110 kDa.

[1] "Lactase" is also referred to as β-galactosidase.
[2] https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/23.html

Godo Shusei states that the *A. oryzae* production organism is a non-pathogenic and non-toxigenic fungus with a history of safe use in food production. The GD-FAL production strain was not subjected to genetic engineering.[3]

Godo Shusei states that the lactase enzyme preparation is manufactured by controlled fermentation of a pure culture of the *A. oryzae* production strain. The enzyme is secreted into the fermentation medium. After fermentation, the medium containing the enzyme is separated from the biomass, recovered, and concentrated by precipitation and ultrafiltration steps, followed by polish and germ filtration. The resulting lactase enzyme concentrate is formulated with glycerin to a lactase enzyme preparation. Godo Shusei states that the entire process is performed in accordance with current Good Manufacturing Practices and with raw materials that are food-grade. Godo Shusei notes that the manufacturing process is designed to remove the production organism from the enzyme preparation. Godo Shusei also states that final lactase enzyme preparation does not contain any major food allergens.

Godo Shusei has established food-grade specifications and states that the lactase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 11th edition, 2018),[4] 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). Godo Shusei provides data from analyses of three non-consecutive batches of lactase enzyme preparation to demonstrate that the manufacturing acceptance criteria can be met. Godo Shusei also provides data to confirm absence of potential mycotoxins.

Godo Shusei intends to use lactase enzyme preparation at a maximum level of 130 mg TOS/kg raw material to catalyze the hydrolysis of terminal non-reducing β-D-galactose residues in lactose in milk and whey products. By this activity, lactose is reduced to monosaccharides glucose and galactose. Godo Shusei notes that the lactase enzyme will be heat denatured or inactivated during food processing (e.g., pasteurization). Godo Shusei intends to use lactase enzyme preparation as a substitute for other lactase enzymes that are GRAS for their intended uses (e.g., the subject of GRN 000825[5]) and incorporates the dietary exposure estimate from GRN 000825. Godo Shusei estimates a maximum dietary exposure to lactase enzyme preparation to be 9.6 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the use in the processing of milk used in non-

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[3] Good Shusei states that *Aspergillus oryzae* GD-FAL was deposited to the Biological Resource Center (NBRC) on April 19, 2021 and given the designation National Institute of Technology and Evaluation (NITE) No. NITE SD 00458.

[4] Specifications for enzymes remain the same in the most recent edition of the Food Chemicals Codex (FCC 13th edition, 2022).

[5] GRN 000825 describes uses of beta-galactosidase (lactase) enzyme preparation produced by a genetically engineered strain of *Kluyveromyces lactis* to express a modified synthetic gene encoding beta-galactosidase from *K. lactis*, for use as an enzyme at up to 130 mg TOS/kg in the processing of milk and whey for use in conventional foods and at 36 mg TOS/kg raw material in the processing of milk for non-exempt infant formulas for ages 0 to 12 months and milk-based products for ages 12 to 36 months. We evaluated this notice and responded in a letter dated November 7, 2019, stating that we had no questions at that time regarding the notifier's GRAS conclusion. Uses in GRN 001039 are the same as those in GRN 000825.

exempt infant formulas for term infants and milk-based formulas for children 12 to 36 months of age, and 3.7 mg TOS/kg bw/day from the use in dairy products, baked goods, confectionaries and beverages, with the assumption that the added lactase enzyme preparation will remain in the final food.

Godo Shusei relies on published information that discusses the safety of the *A. oryzae* production organism and the safety of microbial enzyme preparations used in food processing. Godo Shusei states that active β-galactosidases of microbial and human origin are naturally present in the gastrointestinal tract. Godo Shusei states that the amino acid sequence for the lactase described in this notice is identical to the lactase that was the subject of GRN000510.[6] Godo Shusei discusses results from published toxicity studies with the lactase enzyme concentrate. These include tests conducted with bacterial cells to show that the lactase is not mutagenic at the highest dose tested, both in the presence and absence of metabolic activation. Godo Shusei also demonstrates that the lactase enzyme concentrate is not clastogenic based on results from *in vitro* mammalian cell micronucleus test. Finally, Godo Shusei discusses their published 90-day oral repeat dose toxicity study in rats, in which the highest dose tested of 2000 mg TOS/kg bw/d was determined to be the No Observed Adverse Effect Level. Godo Shusei also discusses published toxicology studies performed using other sources of lactase as the test article to support their GRAS conclusion.

Godo Shusei discusses publicly available literature, as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes from their intended use, to address potential allergenicity due to lactase. Based on bioinformatic analyses using criteria recommended by FAO/WHO in 2001 (Food and Agriculture Organization of the United Nations, January 2001) and the Codex Alimentarius Commission in 2009 (Codex, 2009), Godo Shusei reports no results with >35 % sequence homology using a window of 80 amino acids or exact matches using a window of 8 amino acids. Based on the totality of the information available, Godo Shusei concludes that it is unlikely that oral consumption of lactase will result in allergenic responses from its intended uses.

Based on the data and information summarized above, Godo Shusei concludes that lactase enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, Godo Shusei states its intention to use lactase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. 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.

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[6] GRN 000510 describes uses of acid lactase from *Aspergillus oryzae* expressed in *Aspergillus niger*. We evaluated this notice and responded in a letter dated September 29, 2014, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

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

Conclusions

Based on the information that Godo Shusei provided, as well as other information available to FDA, we have no questions at this time regarding Godo Shusei's conclusion that lactase enzyme preparation produced by *A. oryzae* is GRAS under its intended conditions of use. This letter is not an affirmation that lactase enzyme preparation produced by *A. oryzae* 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 001039 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.04.06 16:54:44 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
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