Janet Oesterling
Novozymes North America Inc.
PO BOX 576
77 Perry Chapel Church Road
Franklinton, NC 27525

Re: GRAS Notice No. GRN 000774

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000774. We received Novozymes North America Inc.'s (Novozymes) notice on April 10, 2018, and filed it on May 3, 2018.

The subject of the notice is L-glutaminase enzyme preparation produced by *Bacillus licheniformis* expressing the L-glutaminase gene from *B. licheniformis* (L-glutaminase enzyme preparation) for use as an enzyme in the manufacture of wheat proteins, casein, whey protein, soy, breads, noodles, tofu, fish, cheese, and seasonings, at up to 0.17 mg TOS per g of dry protein solids. The notice informs us of Novozymes' view that these uses of L-glutaminase 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. Novozymes' notice provides information about the components in the L-glutaminase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, L-glutaminase is identified by the Enzyme Commission Number 3.5.1.2. The accepted name for this enzyme is L-glutaminase and the systematic name is L-glutamine amidohydrolase. The enzyme is also known as glutaminase, glutaminase I, and glutamine aminohydrolase. L-glutaminase catalyzes the hydrolysis of glutamine to yield L-glutamate and ammonia. The CAS No. for L-glutaminase is 9001-47-2. Novozymes states that the primary amino acid sequence of L-glutaminase enzyme has been determined.

Novozymes describes *B. licheniformis* as a non-pathogenic, non-toxigenic, well-characterized production organism with a history of safe use in the food industry. Novozymes also states that the *B. licheniformis* production strain SJ13263 was derived from the *B. licheniformis* parental strain DSM 9552 *via* the recipient strain PP1897-3. Novozymes states that the recipient strain was modified at several chromosomal loci to improve product purity and stability. These modifications result in inactivating genes encoding proteases, eliminating the ability to sporulate, and deleting additional genes encoding unwanted proteins in the culture supernatant.

Novozymes describes the construction of SJ13263 by targeted homologous recombination using a plasmid containing an expression cassette carrying the *ggt* gene encoding the L-glutaminase gene from *B. licheniformis*, under the control of the *B. licheniformis* promoter and a transcriptional terminator. Novozymes states that only the expression cassette with elements between the promoter and the terminator are present in the final production strain as confirmed by Southern hybridization, PCR, and DNA sequencing. Novozymes also confirmed the absence of functional antibiotic resistance genes in the final production strain by Southern hybridization.

Novozymes states that L-glutaminase enzyme is produced by submerged fed-batch fermentation of a pure culture of the production strain. Novozymes states that fermentation is carried out under controlled conditions and that the enzyme is secreted into the fermentation medium After fermentation, the microbial biomass is removed and the supernatant containing the enzyme is clarified by adjusting pH and adding appropriate flocculants. The liquid enzyme is concentrated and filtered further to remove residual production strain. This is further used for the safety studies. The liquid enzyme concentrate is then stabilized by the addition of glycerol and preserved with potassium sorbate and sodium benzoate to obtain the L-glutaminase enzyme preparation.

Novozymes states that the entire process is performed in accordance with current good manufacturing practices. Novozymes also states that the L-glutaminase 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). Novozymes provides analytical data from three representative batches of L-glutaminase enzyme preparation to demonstrate consistency with the specifications. Novozymes states that the final L-glutaminase enzyme preparation does not contain any major food allergens from the fermentation media.

Novozymes intends to use L-glutaminase enzyme preparation during the manufacture of wheat proteins, casein, whey protein, soy, breads, noodles, tofu, fish, cheese, and seasonings, at up to 0.17 mg TOS per g of dry protein solids. Novozymes states that L-glutaminase will be used in manufacturing these foods. Novozymes states that several factors during manufacturing would render the glutaminase enzyme inactive in the final food. However, Novozymes assumes that all the L-glutaminase enzyme preparation will remain in the final food and, estimates the dietary exposure to L-glutaminase enzyme preparation to be 0.21 mg TOS/kg body weight per day based on the maximum intended use level.[1]

Novozymes relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism. Glutaminase enzymes sourced from *Bacillus amyloliquefaciens* have a long history of use in Japan as they were first reported in the publicly available literature in 1988. Specifically, glutaminase has been used in the production of soy sauces since 1991, the production of miso since 1992 and the production of hydrolyzed vegetable protein since 2003.

Novozymes discusses potential food allergenicity of L-glutaminase enzyme. Novozymes states that naturally occurring food enzymes, if present in the final food, are unlikely to have allergenic

[1] Novozymes uses the Budget Method to calculate estimated dietary exposure to L-glutaminase enzyme preparation. Novozymes assumes consumption of a maximum of 25 g of solid foods per person per day and that 50% of these solid foods will be processed. Novozymes further assumes these foods to contain 10% of protein hydrolsates that will be treated by the L-glutaminase enzyme preparation at the maximum intended level.

potential because they are present in low concentrations, have history of safe use, or are denatured during food processing, and are susceptible to digestion in the gastrointestinal system. Novozymes further cites the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes used as processing aids. Additionally, Novozymes states that no homology to food allergens were found when they conducted sequence homology searches using the peptide sequence of the L-glutaminase against known allergens stored in the FARRP allergen protein database as well as the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee. Novozymes also states that homology searches of the L-glutaminase sequence using UNIPROT database did not identify any significant homologies to known toxins. Based on the totality of the information available, Novozymes concludes that it is unlikely that oral consumption of L-glutaminase will result in any allergenic or toxigenic responses. In addition, Novozymes discussed the results of unpublished toxicity studies of the L-glutaminase enzyme concentrate to corroborate safety. Based on No-Observed-Adverse-Effect-Level of 702 mg TOS/kg bw/day from a 13-week oral toxicity study in rats and an estimated theoretical maximum daily intake of 0.21 mg TOS/kg bw/day, Novozymes calculated a margin of exposure of 3303. FDA notes that the margin of exposure is based on unpublished information and serves only to corroborate the published information regarding safety of enzyme preparations used in food.

Based on the data and information summarized above, Novozymes concludes that L-glutaminase 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 Novozymes's notice concluding that L-glutaminase 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 L-glutaminase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing [notified substance], if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Novozymes provided, as well as other information available to FDA, we have no questions at this time regarding Novozymes' conclusion that L-glutaminase enzyme preparation produced by *B. licheniformis* expressing the L-glutaminase gene from *B. licheniformis* is GRAS under its intended conditions of use. This letter is not an affirmation that L-glutaminase enzyme preparation produced by *B. licheniformis* expressing the L-glutaminase gene from *B. licheniformis* 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 000774 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Dennis M. Keefe -S

Digitally signed by Dennis M. Keefe -S
Date: 2018.10.26 12:11:56 -04'00'

Dennis M. Keefe, Ph.D.
Director
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