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

Re: GRAS Notice No. GRN 000675

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000675. We received the notice, dated October 10, 2016, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal) on July 17, 2016, and filed it on October 14, 2016. We received amendments containing clarifying information on February, 22, 2017 and March 09, 2017.

FDA published the GRAS final rule on August 17, 2016 (81 FR 54960), with an effective date of October 17, 2016. As GRN 000675 was pending on the effective date of the GRAS final rule, we requested some additional information consistent with the format and requirements of the final rule. We received an amendment responding to this request on October 24, 2016.

The subject of the notice is xylanase enzyme preparation produced by *Trichoderma reesei* carrying a xylanase gene from *Talaromyces leycettanus* (xylanase enzyme preparation). The notice informs us of the view of Novozymes North America, Inc. (Novozymes) that xylanase enzyme preparation is GRAS, through scientific procedures, for use as an enzyme at levels up to 48.33 mg Total Organic Solids (TOS) per kg raw material in brewing, cereal beverage processing, baking, and processing of cereal grains such as corn, wheat, barley, and oats.

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 components derived from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. Novozymes' notice provides information about each of these components in the xylanase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, endo-1,4-β-xylanase is identified by the Enzyme Commission Number 3.2.1.8. The accepted name for the enzyme is endo-1,4-β-xylanase, and the systematic name is 4-β-δ-xylan xylanohydrolase. Endo-1,4-β-xylanase is also known as endo-(1→4)-β-xylan 4-xylanohydrolase; endo-1,4-xylanase; xylanase; β-1,4-xylanase; endo-1,4-xylanase; endo-β-1,4-xylanase; endo-1,4-β-d-xylanase; 1,4-β-xylan xylanohydrolase; β-xylanase; β-1,4-xylan xylanohydrolase; endo-1,4-β-xylanase; β-d-xylanase. The CAS Registry Number for endo-1,4-β-xylanase is 9025-57-4. Endo-1,4-β-xylanase catalyzes the endohydrolysis of (1→4)-β-d-xylosidic linkages in arabinoxylans resulting in depolymerization of the arabinoxylan into smaller oligosaccharides.

Novozymes states that the *T. reesei* production strain is constructed using the recipient strain

T. reesei BTR213. *T. reesei* BTR213 was developed from its parent strain *via* classical mutagenesis techniques for optimal xylanase production. [1]Novozymes describes *T. reesei* as a non-pathogenic, non-toxigenic, and well-characterized production organism with a history of safe use in the food industry. Novozymes also states that *T. reesei* is classified as a Biosafety level 1 microorganism by the ATCC.

Novozymes describes the construction of the production strain from BTR213 by the targeted integration of an expression cassette carrying the xylanase gene from *T. leycettanus,* a fragment of the *T. reesei* cellobiohydrolase 1 (*cbh1)* promoter, the transcriptional terminator of cbh1 and an acetamidase selectable marker. Novozymes confirmed the sequence of the inserted expression cassette and the flanking regions at the integration locus. Novozymes also confirmed that the introduced DNA is stable during production via Southern blot hybridization, and free of any functional antibiotic resistance genes via genome sequence analysis.

Novozymes states that the xylanase enzyme preparation is produced by submerged fermentation of a pure culture of the production strain, controlled to ensure production strain identity, purity, and enzyme-generating ability. After fermentation, the enzyme is secreted to the fermentation broth, and recovered by pH adjustment and flocculation as needed, followed by a primary separation step of filtration or centrifugation. The filtrate containing the enzyme is then concentrated via ultrafiltration or evaporation. The resulting liquid concentrate is further filtered to remove any residual production organisms followed by a final concentration step. The liquid enzyme concentrate is then standardized with sucrose and preserved with the addition of potassium sorbate and sodium benzoate. Novozymes states that the entire process is performed in accordance with current good manufacturing practices using raw materials of food grade quality. Novozymes also states that the final enzyme preparation contains no major food allergens from the fermentation medium.

Novozymes has established food grade specifications and notes that the xylanase 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 Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006). Novozymes provides analytical data from three batches of xylanase enzyme preparation to demonstrate consistency with the specifications. Novozymes states that absence of the production microorganism is an established specification for the commercial product.

Novozymes intends to use the xylanase enzyme preparation to aid in the separation of grains into the germ, starch, gluten, and fibers for use in brewing, processing of cereal beverages, baking, and grain processing, at levels up to 48.33 mg TOS/kg raw material. Novozymes states that the xylanase enzyme will be inactivated during processing, and is not expected to be functional in the final food. However, Novozymes estimates dietary exposure to xylanase enzyme preparation based on the maximum intended use levels and the assumption that all of the enzyme preparation will remain in the final food, to be 0.715 mg TOS/kg bw/d.

Novozymes relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism. Additionally, Novozymes summarizes unpublished toxicological studies using the xylanase

[1] *T. reesei* BTR213 is a natural isolate of its parent strain, RUTC30. RUTC30, deposited as (ATCC) 56765, was developed from the well characterized wild type strain QM6a. QM6a has been used in the construction of several enzyme production strains used for industrial scale food processing applications.

enzyme liquid concentrate to corroborate the safety of the enzyme preparation for its intended uses. Novozymes states that the xylanase enzyme is not mutagenic based on results from a bacterial reverse mutation assay and on results from an *in vitro* mouse micronucleus assay in cultured human lymphocytes. A 90-day oral toxicity study in rats using the xylanase enzyme concentrate did not cause any treatment-related adverse effects up to the highest dose tested (equivalent to 1051 mg TOS/kg bw/d). Based on the highest dose tested in the 90-day study and the estimated dietary exposure from the intended use of the xylanase enzyme preparation, Novozymes calculates a margin of safety to be 1469.

Novozymes discusses potential food allergenicity of xylanase enzyme. Novozymes states that naturally occurring food enzymes, if present in the final food, are unlikely to have allergenic potential because they are present in low concentrations and are susceptible to digestion in the gastro-intestinal system. Additionally, Novozymes conducted an 80-amino acid sequence homology search for xylanase enzyme against known allergens stored in the FARRP allergen protein database, and found no sequence homology over 35% to known allergens using a window of 80 amino acids, at the recommended default settings. Novozymes also did not find any significant matches of contiguous stretches of eight or more amino acids in the xylanase sequence that would be cross reactive with an allergenic protein. Novozymes cites 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, Novozymes concludes that it is unlikely that oral consumption of xylanase enzyme will result in any allergenic responses.

Based on the data and information summarized above, Novozymes concludes that xylanase enzyme preparation is GRAS for its intended use in food.

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 its review of Novozymes' notice that xylanase enzyme preparation is GRAS for the intended uses, FDA did not consider whether section 301(ll) or any of its exemptions apply to foods containing xylanase enzyme preparation. Accordingly, this response should not be construed to be a statement that foods that contain xylanase enzyme preparation, 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 xylanase enzyme preparation produced by *T. reesei* carrying a xylanase gene from *T. leycettanus* is GRAS under its intended conditions of use. This letter is not an affirmation that xylanase enzyme preparation 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 000675 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300042713, cn=Michael A. Adams -S
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