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

Re: GRAS Notice No. GRN 000891

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000891. We received Novozymes North America Inc. (Novozymes)'s GRAS notice on November 5, 2019 and filed it on January 22, 2020. Novozymes submitted amendments to the notice on July 10, 2020 and August 21, 2020 that clarified the intended use, enzyme identities, dietary exposure, and test article used for toxicological studies.

The subject of the notice is cellulase enzyme preparation produced by *Trichoderma reesei* genetically engineered to express genes encoding cellobiohydrolase and beta-glucosidase from *Aspergillus fumigatus*, and endo-glucanase from *T. reesei* (cellulase enzyme preparation), for use as an enzyme preparation at up to 166 mg Total Organic Solids (TOS)/kg starch raw material in the processing of fruits and vegetables, starch and grain, wine, brewing and other cereal based beverages, and potable alcohol. The notice informs us of Novozymes' view that these uses of cellulase 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. Novozymes' notice provides information about the components in the cellulase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, cellobiohydrolase, beta-glucosidase, and endo-glucanase are identified by the Enzyme Commission Numbers 3.2.1.176[1], 3.2.1.21[2], and 3.2.1.4[3] and CAS numbers 37329-65-0, 9001-22-3, and 9012-54-8, respectively.

[1] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/176.html
[2] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/21.html
[3] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/4.html

Novozymes states that the molecular weights of these enzymes are 54, 91, and 48 kDa, respectively.[4]

Novozymes states that the *T. reesei* production strain AyGm61-2C-2 is non-pathogenic and non-toxigenic. AyGm61-2C-2 was derived from the *T. reesei* recipient strain BTR213.[5]

Novozymes describes the construction of the *T. reesei* production strain as a targeted integration of an expression cassette consisting of the genes encoding cellobiohydrolase and modified beta-glucosidase[6] from *A. fumigatus*, and endo-glucanase from *T. reesei,* as well as promoters, and terminators from *T. reesei* and a gene coding for acetamidase from *A. nidulans* used for selection of transformants. Novozymes states that the integrations were confirmed by PCR and DNA sequencing. Novozymes also states that the stability of the introduced DNA has been confirmed by gel electrophoresis. Novozymes states that the final production strain does not contain any functional antibiotic resistance genes.

Novozymes states that cellulase enzyme preparation is manufactured 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. The enzyme is then recovered from the fermentation medium by filtration or centrifugation and concentrated by ultrafiltration or evaporation. The resulting liquid enzyme concentrate is used as the test article for toxicity studies. The liquid enzyme concentrate is further stabilized with sucrose and standardized with water to a liquid enzyme preparation and preserved with potassium sorbate and sodium benzoate. Novozymes states that the entire process is performed in accordance with current good manufacturing practices. Novozymes also states that no major food allergens from the fermentation medium are expected to be present in the final cellulase enzyme preparation.

Novozymes states that the established food grade specifications for cellulase enzyme preparation conform to that established for enzyme preparations in the Food Chemical Codex (FCC, 11th edition, 2018), 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 batches of cellulase enzyme preparation to demonstrate that it meets the manufacturing acceptance criteria. Novozymes also states that the production organism is not present in the final enzyme preparation.

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[4] In an amendment dated July 10, 2020, Novozymes states that the activity measured for cellulase preparation represents activities of all three cellulases produced.

[5] Novozymes states that the BTR213 recipient strain was derived from the RUT-C30 parental strain by classical mutagenesis and genetic modifications, including deletion of a parcelsin gene. RUT-C30 (ATCC 56765) was derived from the well-known wild-type strain, QM6a.

[6] Novozymes states that beta-glucanase gene encodes beta-glucanase enzyme that is modified at four amino acids.

Novozymes intends to use cellulase enzyme preparation at levels up to 166 mg TOS/kg starch raw material in the manufacture of foods listed on Page 1. The enzyme preparation is intended for use in the hydrolysis of the 1,4-beta-D-glycosidic linkages in cellulose, hemicellulose, lichenin and cereal beta-D-glucans. Novozymes notes that the cellulase enzyme preparation will be deactivated and/or removed during production or refining. In estimating dietary exposure, Novozymes assumes that all the cellulase enzyme preparation will remain in the final food. Novozymes estimates dietary exposure to cellulase enzyme preparation to be 3.8 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses based on daily consumption of beer (11.4 g/kg bw/d), wine (9.9 g/kg bw/d), potable alcohol (0.28 g/kg bw/d), cereal-based beverages (1.2 g/kg bw/d), fruit juices (33 g/kg bw/d), and grains (5.62 g starch derived dry matter/kg bw/d).[7]

Novozymes relies on published information that discusses the safety of the *T. reesei* production organism, safety of the donor organisms, and the safety of microbial enzyme preparations used in food processing, in general. Further, Novozymes discusses safety of the three enzymes present in the cellulase enzyme preparation. Novozymes discusses results from unpublished toxicological studies[8] that did not show any treatment-related adverse effects up to the highest level of liquid enzyme concentrate tested. This includes a 14-day oral toxicity study in rats; the highest dose, equivalent to 1314 mg TOS/kg bw, was considered as the no observed adverse effect level (NOAEL). Novozymes calculates a margin of exposure to be 343 using the NOAEL and the estimated dietary exposure for the intended uses of the cellulase enzyme preparation. FDA notes the margin of exposure is based on unpublished safety studies and is corroborative.

Novozymes cites publicly available literature, as well as the conclusions of several organizations and working groups to support a lack of allergenic potential for the cellulases from *T. reesei*. Furthermore, Novozymes reports no matches between the amino acid sequences of the cellulases and the primary sequences of known allergens based on the guidelines developed by FAO/WHO (FAO, 2001) and modified by Codex Alimentarius Commission (FAO, 2009). Based on the information available, Novozymes concludes that it is unlikely that oral consumption of cellulases will result in an allergenic response.

Based on the totality of the information available, Novozymes concludes that the cellulase enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, Novozymes states its intention to use cellulase 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

[7] Novozymes uses the Budget method to estimate dietary exposure to cellulase enzyme preparation based on a maximum use level of cellulase enzyme preparation of 166 mg TOS/ kg raw material and consumption of a maximum of 12.5 g of solid foods and 25 g of beverages per kg body weight per day.
[8] In an amendment dated July 10, 2020, Novozymes states that the test article used for this study was a mixture of the three cellulases produced.

added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Section 301(ll) of the Food Drug & Cosmetic 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' notice concluding that cellulase 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 cellulase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing cellulase 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's conclusion that cellulase enzyme preparation produced by *Trichoderma reesei* genetically engineered to express genes encoding the cellulases, cellobiohydrolase and beta-glucosidase from *Aspergillus fumigatus*, and endo-glucanase from *T. reesei* is GRAS under its intended conditions of use. This letter is not an affirmation that cellulase enzyme preparation produced by *Trichoderma reesei* genetically engineered to express genes encoding the cellulases, cellobiohydrolase and beta-glucosidase from *Aspergillus fumigatus*, and endo-glucanase from *T. reesei* 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 000891 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Mical E. Honigfort -S

Digitally signed by Mical E. Honigfort -S
Date: 2020.10.20 08:43:04 -04'00'

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