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

Re: GRAS Notice No. GRN 000699

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000699. We received Novozymes North America, Inc.'s (Novozymes) notice on April 3, 2017, and filed it on May 30, 2017. We received amendments addressing questions regarding the use and exposure levels of the subject of the notice on June 28, 2017, and November 2, 2017.

The subject of the notice is trehalase enzyme preparation produced by *Aspergillus niger* expressing a trehalase gene from *Myceliophthora sepedonium* (trehelase enzyme preparation) for use as an enzyme to increase available glucose for ethanol production in grain slurries at 125 mg Total Organic Solids (TOS)/kg dry solids. The notice informs us of Novozymes' view that this use of trehelase 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 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 trehalase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, trehalase is identified by the Enzyme Commission Number 3.2.1.28. The accepted name for the enzyme is α,α-trehalase and the systematic name is α,α-trehalose glucohydrolase. The CAS No. for trehalase is 9025-52-9. Trehalase is an anomer-inverting glucosidase that catalyzes the hydrolysis of disaccharide α,α-trehalose, releasing glucose. Novozymes states that the molecular weight of the trehalase enzyme is approximately 100kDa by SDS PAGE analysis.

Novozymes' states that the *A. niger* production strain[1] is constructed from the recipient strain *A. niger* C3085, modified at several loci to inactivate genes that encode amylases

[1] The production strain was derived from a natural isolate of *A. niger* strain B0-1.

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Center for Food Safety & Applied Nutrition
5001 Campus Drive
College Park, MD 20740

and proteases. Additionally, the fumonisin gene cluster and the oxaloacetate hydrolase gene were deleted. The expression plasmid carries the treMS gene encoding the trehalase sequence from *M. sepedonium*, a fragment of the *A. niger* promoter, a transcriptional terminator and a selective marker. The plasmid was integrated at two sites by targeted homologous recombination. Novozymes states that it confirmed the sequence of the inserted expression cassettes and the flanking regions at each of the integration loci in the production strain by Southern blot and PCR analyses, followed by DNA sequencing. Novozymes states that the transformed DNA is stably integrated into the *A. niger* chromosome and is mitotically stable and therefore unlikely to be mobilized for genetic transfer to other organisms. Novozymes states that there were no functional antibiotic resistance genes left in the strain after genetic modifications; Novozymes confirmed the absence of any antibiotic resistance genes by genomic sequence analysis. Novozymes also states that the modified production organism complies with the criteria set by the Organization for Economic Co-operation and Development for Good Industrial Large Scale Practice microorganisms (1993).

Novozymes states that the trehalase enzyme preparation is manufactured by submerged fermentation of a pure culture of the production strain, controlled to ensure production strain identity, purity, and enzyme-generating ability. The enzyme is recovered by separating the cell mass from the supernatant after pH adjustment, followed by filtration or centrifugation and concentration steps. The enzyme is stabilized with glycerol, and the final liquid product is formulated with water, potassium sorbate, sodium bisulfite, and sodium benzoate. Novozymes states that the entire process is performed in accordance with current good manufacturing practices using food grade raw materials. 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 trehalase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 9th edition, 2014), 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 trehalase enzyme concentrate to demonstrate consistency with the specifications. Novozymes confirms the absence of the production microorganism with an established specification for the commercial product at a detection limit of <1 CFU/g.

Novozymes intends to use the trehalase enzyme preparation in grain slurries, either during starch hydrolysis or added directly to the fermenter, to facilitate the degradation of starch and non-starch polysaccharides into fermentable sugars, improve ethanol yield and to enhance ethanol production efficiency. Novozymes states that due to the nature of the ethanol distillation process the trehalase enzyme is inactivated and no trehalase enzyme TOS is expected to be transferred with the distillate. However, to calculate the dietary exposure from trehalase enzyme preparation, Novozymes assumes that the trehalase enzyme preparation will be used at the maximum intended levels, and that all the enzyme preparation will remain in the final food. Based on these assumptions,

Novozymes estimates a maximum dietary exposure of trehalase enzyme preparation from all intended uses to be 0.0313 mg TOS/kg body weight per day (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 discusses unpublished toxicological studies using the trehelase enzyme. Tests conducted using the mouse micronucleus assay showed that trehelase enzyme is not mutagenic. Novozymes also demonstrates that the trehelase enzyme is not clastogenic based on *in vitro* chromosomal aberration testing. A 14-day sub-chronic oral toxicity study conducted in rats showed that consumption of trehalase enzyme did not cause any treatment-related adverse effects up to the highest dose tested**,** equivalent to 1298 mg TOS /kg bw/d. Based on the highest dose tested in the 14-day study and the estimated dietary exposure from the intended uses of the trehalase enzyme preparation, the margin of safety (MOS) is 41500.[2]

Novozymes discusses potential food allergenicity of trehalase enzyme. Novozymes conducted an 80-amino acid sequence homology search for trehalase enzyme against known allergens stored in the FARRP allergen protein database, and found no sequence identity matches over 35% to known allergens. Additionally, Novozymes did not find any matches of contiguous stretches of eight or more amino acids in the trehalase sequence that would be cross reactive with an allergenic protein. Novozymes further 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 trehalase enzyme will result in allergenic responses.

Based on the data and information summarized above, Novozymes concludes that trehalase 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' notice concluding that trehelase 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 trehelase enzyme preparation. Accordingly, our response should not be construed to be

[2] The MOS was calculated by FDA based on the data provided by the notifier. FDA also notes that the MOS is based on unpublished safety studies, and is corroborative with published information regarding enzyme preparations used in food processing.

a statement that foods containing trehelase 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 trehelase enzyme preparation produced by *A. niger* expressing a trehalase gene from *M. sepedonium* is GRAS under its intended conditions of use. This letter is not an affirmation that trehelase enzyme preparation produced by *A. niger* expressing a trehalase gene from *M. sepedonium* 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 000699 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
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Dennis M. Keefe, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition