Vincent Sewalt, Ph.D.
Senior Director, Product Stewardship & Regulatory
Danisco US Inc. (Operating as DuPont Industrial Biosciences)
925 Page Mill Road
Palo Alto, CA 94304

Re: GRAS Notice No. GRN 000727

Dear Dr. Sewalt:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000727. We received Danisco US Inc., operating as DuPont Industrial Biosciences' (DuPont), notice on August 18, 2017, and filed it on October 19, 2017. We received an amendment that addressed our questions regarding the molecular weight of trehalase on December 12, 2017.

The subject of the notice is trehalase enzyme preparation produced by *Trichoderma reesei* expressing a trehalase gene from *T. reesei* (trehalase enzyme preparation) for use as an enzyme in the manufacture of organic acids *(e.g.,* lactic, citric, and succinic acid), monosodium glutamate, and potable alcohol, at levels up to 36.7 mg Total Organic Solids (TOS) trehalase enzyme preparation/kg starch. The notice informs us of DuPont's view that these uses of trehalase 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 components derived from the manufacturing process, *e.g*., constituents of the fermentation media or the residues of processing aids. DuPont's 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 Registry Number for trehalase is 9025-52-9. Trehalase is an anomer-inverting glucosidase that catalyzes the hydrolysis of the disaccharide α,α-trehalose, with the release of glucose. DuPont provides the amino acid sequence of trehalase. DuPont states that the molecular weight of the trehalase enzyme is 114 kDa as predicted by its amino acid sequence, and confirmed by SDS-PAGE.

DuPont states that the host *T. reesei*[1] RL-P37 is well characterized; it is a cellulase over-producing strain obtained from the wild type *T. reesei* strain QM6a[2] via several classical mutagenesis steps. The *T. reesei* production strain is constructed by incorporating an expression cassette containing the trehalase gene under the regulation of the native *T. reesei*

[1] *T. reesei* is classified as a Biosafety Level 1 (BSL1) microorganism by the American Type Culture Collection (ATCC), and as Good Industrial Large-Scale Practice; *T. reesei* is an anamorph of *Hypocrea jecorina.*

[2] *T. reesei* strain QM6a has been deposited in the ATCC as ATCC 13631.

cellobiohydrolase *(cbhI)* promoter, the native *T. reesei* cellobiohydrolase (*cbhI)* transcription terminator, and the native *T. reesei* orotate phosphoribosyl transferase *(pyr2)* gene as a selectable marker into the chromosome. DuPont states that it verified the final construct by Southern blot analysis. DuPont also states that the transformed DNA is stably integrated into the *T. reesei* chromosome for a minimum of 60 generations, and no antibiotic resistance genes were used in the construction of the production strain.

DuPont states that the trehalase enzyme preparation is manufactured by submerged fermentation of a pure culture of the *T. reesei* production strain, controlled to ensure strain identity and purity. The trehalase enzyme is recovered from the fermentation broth by separating the cell mass from the supernatant by filtration or centrifugation, followed by concentration. The enzyme is stabilized with dextrose, sodium chloride, sodium benzoate, and potassium sorbate at pH 4.5-5.0. DuPont states that the entire process is performed in accordance with current good manufacturing practices using food grade raw materials. DuPont also states that the final enzyme preparation contains no major food allergens.

DuPont 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, 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). DuPont provides analytical data from three batches of trehalase enzyme concentrate to demonstrate consistency with the specifications. DuPont confirms the absence of the production microorganism with an established specification for the commercial product.

DuPont intends to use trehalase enzyme preparation in the manufacture of organic acids, monosodium glutamate, and potable alcohol, at levels up to 36.7 mg TOS trehalase enzyme preparation/kg starch. The trehalase enzyme converts trehalose to glucose during the fermentation process; glucose serves as a yeast-fermentable substrate. To estimate dietary exposure to the trehalase enzyme preparation, DuPont assumes that the enzyme preparation will always be used at the maximum intended levels, and that the trehalase enzyme preparation will remain in the final food. DuPont estimated dietary exposure from all uses of trehalase enzyme preparation to be 0.02 mg TOS/kg body weight per day (mg TOS/kg bw/d).

DuPont relies on published information to discuss the safety of microbial enzyme preparations used in food processing, including the safety of *T. reesei*. DuPont states that the trehalase enzyme will be deactivated or removed during processing and that even if the enzyme preparation were ingested, the enzyme would be digested and metabolized to normal metabolic constituents and thus would not be expected to pose any human health risk. DuPont provided a summary of unpublished toxicological studies for 22 enzyme preparations derived from *T. reesei* to corroborate safety of the trehalase enzyme preparation. Based on these studies DuPont states that enzyme preparations from all *T. reesei* production strains were non-toxic, non-mutagenic, and not clastogenic.

DuPont discusses potential food allergenicity of trehalase enzyme. DuPont 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 any known allergens. Additionally, DuPont did not find any matches of contiguous stretches of eight or more amino acids in the trehalase sequence that would be expected to cross-react with an allergenic protein. DuPont 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, DuPont concludes that it is unlikely that oral consumption of trehalase enzyme will result in allergenic responses.

Based on the data and information summarized above, DuPont 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 DuPont's 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 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 DuPont provided, as well as other information available to FDA, we have no questions at this time regarding DuPont's conclusion that trehelase enzyme preparation produced by *T. reesei* expressing a trehalase gene from *T. reesei* is GRAS under its intended conditions of use. This letter is not an affirmation that trehelase enzyme preparation produced by *T. reesei* expressing a trehalase gene 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 000727 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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