



Vincent Sewalt, Ph.D.
Danisco US Inc. (operating as DuPont Industrial Biosciences)
925 Page Mill Road
Palo Alto, CA 94304

Re: GRAS Notice No. GRN 000703

Dear Dr. Sewalt:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000703. We received Danisco US Inc. (operating as DuPont Industrial Biosciences) (DuPont)'s notice on May 3, 2017, and filed it on June 26, 2017.

The subject of the notice is alpha-glucosidase enzyme preparation produced by *Trichoderma reesei* expressing the alpha-glucosidase gene from *Aspergillus niger* (alpha-glucosidase enzyme preparation) for use as an enzyme in the production of potable alcohol, organic acids, and monosodium glutamate at a maximum level of 235 mg Total Organic Solids (TOS)/kg starch raw material. The notice informs us of DuPont's view that these uses of alpha-glucosidase 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. DuPont's notice provides information about the components in the alpha-glucosidase enzyme preparation.

Per the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, alpha-glucosidase is identified by the Enzyme Commission Number 3.2.1.20.¹ Alpha-glucosidase catalyzes the hydrolysis of terminal, non-reducing (1→4)-linked α -D-glucose residues with release of α -D-glucose. The accepted name is alpha-glucosidase. The systematic name for this enzyme is α -D-glucoside glucohydrolase. The CAS No. for alpha-glucosidase is 9001-42-7. DuPont states that the primary amino acid sequence of the expressed alpha-glucosidase enzyme has been determined to be 985 amino acids. DuPont states that the *T. reesei* production

¹ FDA notes that alpha-glucosidase has an additional transglucosidase activity, which is identified by the Enzyme Commission Number, EC 2.4.1.24. Transglucosidase enzyme preparation is the subject of GRN 000315.

strain was derived from *T. reesei* strain RL-P37.² RL-P37 has been used by DuPont for production of cellulases for over fifteen years, and is non-toxigenic, and non-pathogenic. *T. reesei* is classified as a Biosafety Level 1 (BSL1) microorganism by ATCC.³

DuPont describes the construction of the production strain from *T. reesei* strain RL-P37 by the targeted integration of an expression cassette containing the gene encoding the mature alpha-glucosidase from *A. niger* fused to a *T. reesei* CBHI signal peptide, *T. reesei cbh1* promoter and transcriptional terminator, and a selectable marker gene from *A. nidulans*. DuPont confirmed the insertion of the expression cassette by Southern blot analysis. DuPont states that the production strain is stable for at least 60 generations of fermentation, based on alpha-glucosidase production. DuPont states that the final production strain does not contain any antibiotic resistance genes.

DuPont states that alpha-glucosidase enzyme is produced by submerged fermentation of a pure culture of the production strain. DuPont states that fermentation is carried out under controlled conditions and that the enzyme is secreted into the culture medium. The enzyme is recovered from the culture medium by centrifugation or filtration of the supernatant carrying the enzyme, and concentration by ultrafiltration. The enzyme concentrate is stabilized and formulated with water, dextrose, and sodium chloride, and preserved with sodium benzoate. 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 does not contain any major food allergens from the culture medium.

DuPont states that the alpha-glucosidase 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 alpha-glucosidase enzyme concentrate to demonstrate consistency with the manufacturing specifications. DuPont also confirms that a test for absence of any production organism in the final product is an established specification.

DuPont proposes to use alpha-glucosidase enzyme preparation to hydrolyze terminal, non-reducing (1->4)-linked alpha D-glucose residues during the manufacture of potable alcohol, organic acids, and monosodium glutamate. To estimate dietary exposure, DuPont relied on information such as the amount of alpha-glucosidase enzyme preparation in each of the uses based on the maximum concentration of the alpha-glucosidase enzyme preparation and the manufacturing processes. DuPont assumes that the alpha-glucosidase 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, DuPont estimates a maximum dietary exposure of alpha-glucosidase enzyme preparation from all intended uses to be 0.44 mg TOS/kg body weight per day

² *T. reesei* strain RL-P37 is a commercial production strain that was derived from the well-known wild-type *T. reesei* strain QM6a using several classical mutagenesis steps.

³ BSL1 microorganisms are not known to cause disease in healthy humans.

(mg TOS/kg bw/d) based on the theoretical maximum daily intake of solid and liquid foods that could contain alpha-glucosidase enzyme preparation.⁴

DuPont relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism. Additionally, DuPont summarizes unpublished toxicological studies using the alpha-glucosidase enzyme liquid concentrate to corroborate safety. DuPont states that the alpha-glucosidase enzyme is not mutagenic based on results from a bacterial reverse mutation assay, and on results from an *in vitro* chromosomal aberration assay in cultured human lymphocytes. In a two-phase 18-week oral toxicity study in rats, DuPont observed that the alpha-glucosidase enzyme concentrate did not cause any treatment-related adverse effects up to the highest dose tested (equivalent to 74.8 mg TOS/kg bw/d). Based on the highest dose tested in the 18-week study and the estimated dietary exposure from the intended uses of the alpha-glucosidase enzyme preparation, DuPont calculates a margin of safety to be 169. FDA notes the margin of safety is based on unpublished safety studies, and is corroborative with published information regarding enzyme preparations used in food processing.

DuPont discusses potential food allergenicity of alpha-glucosidase enzyme. DuPont 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 gastrointestinal system. DuPont 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. Additionally, DuPont conducted a sequence homology search with a window of 80 amino acids from the peptide sequence of the alpha-glucosidase against known allergens stored in the FARRP allergen protein database and found no significant homology over 35% to known allergens. DuPont did not find any significant homology between sequences of eight contiguous amino acids of alpha-glucosidase and known allergenic proteins. Based on the totality of the information available, DuPont concludes that it is unlikely that oral consumption of alpha-glucosidase enzyme will result in any allergenic responses.

Based on the data and information summarized above, DuPont concludes that the alpha-glucosidase enzyme preparation is GRAS for its intended use.

Section 301(II) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Section 301(II) 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(II)(1)-(4) applies. In our evaluation of DuPont's notice concluding that

⁴ DuPont states that the dietary exposure estimation considers exposure to alpha-glucosidase enzyme preparation, as well as to the transglucosidase enzyme preparation from intended uses described in GRN 000315 due to the dual enzyme activities in the product.

alpha-glucosidase 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 alpha-glucosidase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing alpha-glucosidase 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 alpha-glucosidase enzyme preparation produced by *T. reesei* expressing the alpha-glucosidase gene from *A. niger* is GRAS under its intended conditions of use. This letter is not an affirmation that alpha-glucosidase enzyme preparation produced by *T. reesei* expressing the alpha-glucosidase gene from *A. niger* 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 000703 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,
Dennis M.
Keefe -S

Dennis M. Keefe, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

Digitally signed by Dennis M. Keefe -S
DN: c=US, o=U.S. Government, ou=HHS,
ou=FDA, ou=People,
0.9.2342.19200300.100.1.1=1300072773,
cn=Dennis M. Keefe -S
Date: 2017.11.09 16:42:52 -05'00'