Vincent Sewalt, Ph.D.
DuPont Industrial Biosciences
925 Page Mill Road
Palo Alto, CA 94304

Re: GRAS Notice No. GRN 000664

Dear Dr. Sewalt:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000664. We received the notice, dated July 15, 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 August 25, 2016.

FDA published the GRAS final rule on August 17, 2016 (81 FR 54960), with an effective date of October 17, 2016. As GRN 000664 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 α-amylase enzyme preparation produced by *Bacillus licheniformis* carrying a modified α-amylase gene from *Cytophaga* sp. (α-amylase enzyme preparation). The notice informs FDA of the view of DuPont Industrial Biosciences (DuPont) that α-amylase enzyme preparation is GRAS, through scientific procedures, for use as an enzyme in carbohydrate processing to produce sugar syrups, during fermentation to produce citric acid and lactic acid, and in the manufacture of potable alcohol, up to a maximum level of 32 mg Total Organic Solids per kg (mg TOS/kg) raw material.

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 α-amylase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, α-amylase is identified by the Enzyme Commission Number 3.2.1.1. The accepted name for the enzyme is α-amylase, and the systematic name is 4-α-D-glucan glucanohydrolase. Alpha amylase is also known as glycogenase, endoamylase, Taka-amylase A, and 1,4-α-D-glucan glucanohydrolase. The CAS Registry Number for α-amylase is 9000-90-2. Alpha amylase hydrolyzes the (1→4)-α-D-glucosidic linkage in polysaccharides containing three or more (1→4)-α-linked D-glucose units.

DuPont states that the *B. licheniformis* production strain is constructed using the recipient strain *B. licheniformis* Bra7.[1] Bra7 was developed from its wild-type parent *via* classical strain improvement techniques for optimal α-amylase production and lowered protease production; strain development included the inactivation of the genes encoding for α-amylase, chloramphenicol resistance, a sporulation gene, the subtilisin gene and the glutamic acid specific protease gene. DuPont describes *B. licheniformis* as a non-pathogenic, non-toxigenic, and well-characterized production organism with a history of safe use in the food industry. DuPont also states that *B. licheniformis* is considered suitable for Good Industrial Large Scale Practice worldwide.

DuPont describes the construction of the production strain from Bra7. A plasmid carrying a modified α-amylase gene from *Cytophaga* sp. was generated in the first step.[2] DuPont integrated the resulting plasmid into Bra7 at the *cat* locus. After integration, DuPont states that all vector sequences of the plasmid were deleted and the expression cassette was amplified through multiple generations using increasing concentrations of chloramphenicol until the final production strain was obtained. DuPont measured incorporation of the desired functional genetic information and the intended chromosomal modifications by PCR analyses. DuPont states that the production strain is stable after industrial scale fermentation as measured by the α-amylase production.

DuPont states that the α-amylase enzyme preparation is manufactured by submerged fermentation of a pure culture of the production strain, controlled to ensure production strain identity and purity, as well as enzyme-generating ability. After fermentation, the cell mass is removed from the culture broth by a primary separation step, the filtrate is then concentrated *via* ultrafiltration concentration. The ultrafiltered liquid concentrate is stabilized by the addition of sodium benzoate, potassium sorbate, sodium chloride and up to 33% glycerol at pH 6–6.5 and subsequently filtered. DuPont states that the entire process is performed in accordance with current good manufacturing practices using raw materials of food grade quality. DuPont also states that the final enzyme preparation contains no major food allergens from the fermentation medium.

DuPont has established food grade specifications and notes that the α -amylase 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 α-amylase enzyme preparation to demonstrate consistency with the specifications. DuPont confirms the absence of the production microorganism with an established specification for the commercial product at a detection limit of <1 CFU/g.

DuPont intends to use the α-amylase enzyme preparation in combination with other enzymes in carbohydrate processing to obtain glucose-rich syrups from granular starch derived from corn, wheat, milo, tapioca, barley, rice, potato, and cassava. These syrups will be used in the manufacture of dextrose and high fructose corn syrup. DuPont states that the α-amylase enzyme preparation will also be used to treat liquefied starch in the manufacture of specialty starch syrups and in combination with other enzymes (glucoamylase, proteases, etc.) to

[1] Bra7 and strains developed from it have been used for industrial scale food processing applications.
[2] The α-amylase gene from *Cytophaga* sp. was optimized and synthesized *in vitro* including seven amino acid modifications.

produce potable alcohol and organic acids such as citric and lactic acid. DuPont states that the α-amylase is expected to be inactivated and/or removed during manufacturing in all the intended uses. DuPont also states that α-amylase enzyme, if present in the final food, will be broken down like all other proteins in the human digestive system. However, DuPont estimates dietary exposure to α-amylase 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 0.248 mg TOS/kg bw/d.

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 α-amylase enzyme liquid concentrate to corroborate safety in the intended uses. Tests conducted using the α-amylase enzyme with bacterial cells, showed that the α-amylase is not mutagenic at the highest dose tested both in the presence and absence of metabolic activation. DuPont also demonstrates the α-amylase enzyme is not clastogenic based on results from *in vitro* chromosomal aberration tests. A 90-day oral toxicity study in rats using the α-amylase enzyme concentrate did not cause any treatment-related adverse effects up to the highest dose tested (500 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 α-amylase enzyme preparation, DuPont calculates a margin of safety to be approximately 2016.

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

Based on the data and information summarized above, DuPont concludes that α-amylase 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 its review of DuPont's notice that α-amylase 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 α-amylase enzyme preparation. Accordingly, this response should not be construed to be a statement that foods that contain α-amylase 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 α-amylase enzyme preparation produced by *Bacillus licheniformis* carrying a modified α-amylase gene from *Cytophaga* sp. is GRAS under its intended conditions of use. This letter is not an affirmation that α-amylase 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 000664 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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