Annie Han
Danisco US Inc.
925 Page Mill Road
Palo Alto, CA 94304

Re: GRAS Notice No. GRN 001054

Dear Ms. Han:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001054. We received Danisco US Inc.'s[1] (Danisco) notice on January 7, 2022 and filed it on June 30, 2022. Danisco submitted amendments to the notice on June 10, 2022, March 8, 2023, and April 11, 2023, that provided clarifications on the intended uses, identity, manufacturing, specifications, and safety narrative.

The subject of the notice is glucose oxidase enzyme preparation produced by *Aspergillus niger* expressing the gene encoding glucose oxidase from *A. niger* (glucose oxidase enzyme preparation) for use as an enzyme at up to 7.72 mg total organic solids (TOS)/kg raw material, in baking, cheese processing, and egg processing, including de-sugared eggs, mayonnaise, and salad dressing. The notice informs us of Danisco's view that this use of glucose oxidase 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 from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. Danisco's notice provides information about the components in the glucose oxidase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, glucose oxidase is identified by the Chemical Abstracts Service number 9001-37-0 and the Enzyme Commission Number 1.1.3.4.[2] Danisco states that the primary sequence of glucose oxidase consists of 553 amino acids with a calculated molecular weight of 64 kDa.

Danisco states that the *A. niger* production organism is non-pathogenic and non-toxigenic and is a well-characterized production organism with history of safe use in the food industry. Danisco states that the production strain, GICC03206, was constructed through chromosomal integration of multiple copies of the glucose oxidase gene from *A.*

[1] Danisco US Inc. is a wholly owned subsidiary of International Flavors & Fragrances Inc.
[2] https://iubmb.qmul.ac.uk/enzyme/EC1/1/3/4.html

niger strain NRRL3 and an acetamidase gene from *A. nidulans* as a selection marker.[3,4] Danisco states that they confirmed sequence integration by Southern blot analysis. Danisco evaluated the genetic stability of the production strain by measuring enzyme production. Danisco verified the absence of functional or transferable antibiotic resistance genes in the final production strain genome.

Danisco states that the glucose oxidase enzyme preparation is produced by submerged fermentation of a pure culture of the *A. niger* production strain under controlled conditions. The glucose oxidase enzyme is secreted into the fermentation medium and then recovered by centrifugation or filtration before being concentrated via ultrafiltration. The resulting enzyme concentrate is formulated to a liquid enzyme preparation with microcrystalline cellulose, sodium benzoate, wheat flour, L-ascorbic acid, and potassium sorbate, or is spray dried and formulated with wheat flour or potato starch to a powdered enzyme preparation. Danisco states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices.

Danisco has established food grade specifications and states that the glucose oxidase enzyme preparation conforms to the specifications set in the Food Chemicals Codex (FCC, 12th ed., 2020)[5] 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). Danisco provides results from analyses of three non-consecutive batches of glucose oxidase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism and antibiotic activity.

Danisco intends to use glucose oxidase enzyme preparation at a maximum use level of 7.72 mg TOS/kg raw material, as the powdered form in baking, and as the liquid form for processing of cheese, de-sugared eggs, mayonnaise, and salad dressing. Danisco states that glucose oxidase catalyzes the oxidation of D-glucose to D-glucono-1,5-lactone and hydrogen peroxide while consuming molecular oxygen. Danisco notes that the final enzyme is inactivated or removed during baking and egg processing, while the enzyme will remain active in cheese processing applications. Danisco states that any ingested inactive or active glucose oxidase in the final food is expected to be metabolized into small peptides and amino acids and will not pose any human health risk. Danisco estimates a maximum dietary exposure to glucose oxidase enzyme preparation to be 0.03 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses, and assumes that all of the glucose oxidase enzyme preparation will be active and remain in

[3] Danisco states that the parental strain, *A. niger* AGME9, was obtained from Solvay (Elkhart, IN) and was produced by classical UV mutagenesis of *A. niger* strain ATCC 14916 and selection for improved glucoamylase production.
[4] Danicso states that the production strain possesses a mutant GOX gene that presumably results in production of a truncated protein.
[5] Specifications for enzymes remain the same in the most recent edition of the Food Chemicals Codex (FCC, 13th edition, 2022).

the final food.[6]

In support of the safety of the glucose oxidase enzyme preparation, Danisco highlights published information that supports the history of safe use of glucose oxidase in food. Danisco states that any active or inactive glucose oxidase remaining in the final food will be digested in the gastrointestinal system of the consumer into small peptides and amino acids and thus will not pose any safety risk. Additionally, Danisco states that a literature search did not identify any information that would contradict a general recognition of safety of the glucose oxidase enzyme preparation. Danisco discusses unpublished studies using glucose oxidase enzyme preparation and concludes that the subject of this notice is not genotoxic and did not show any toxicologically significant adverse effects at any dose tested in a 90-day repeat dose oral toxicity study in rats. Based on the highest dose tested from this rat study and the estimated dietary exposure, Danisco states that they estimated the margin of safety to be 651.[7] Danisco relies on published information that discusses the safety of the *A. niger* production organism, including safe strain lineage and use of the parent strain for production of food ingredients.

Danisco discusses publicly available literature to address potential allergenicity due to glucose oxidase. Based on bioinformatic analysis, Dansico reports no significant matches between the amino acid sequences of the glucose oxidase and the primary sequences of known food allergens based on the guidelines developed by the Codex Alimentarius Commission (Codex, 2009). Based on the totality of information available, Danisco concludes that it is unlikely that oral consumption of glucose oxidase preparation from the intended uses will result in allergic responses.

Based on the data and information summarized above, Danisco concludes that glucose oxidase enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, Danisco states its intention to use glucose oxidase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Allergen Labeling

The FD&C Act requires that the label of a food that is or contains an ingredient that contains a "major food allergen" declare the allergen's presence (section 403(w)). The

[6] Danisco uses the Budget method to estimate the dietary exposure to glucose oxidase enzyme preparation based on the consumption of 25 g of solid foods per kg bw/d. Danisco assumes that 50% of the solid foods (12.5 g/kg bw/d) will be baked goods (worst case scenario) and contain the glucose oxidase enzyme preparation at the recommended use level.

[7] FDA notes that the margin of safety stated by the notifier is based on unpublished safety studies and is corroborative of the published information regarding enzyme preparations used in food processing.

FD&C Act defines a "major food allergen" as one of nine foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame) or a food ingredient that contains protein derived from one of those foods. The glucose oxidase enzyme preparation requires labeling under the FD&C Act because it contains wheat flour.

Use in Products under USDA Jurisdiction

As provided under 21 CFR 170.270, during our evaluation of GRN 001054, we coordinated with the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture. Under the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act, FSIS determines the efficacy and suitability of ingredients used in meat, poultry, and egg products, and prescribes safe conditions of use. Suitability relates to the ingredient's effectiveness in performing its intended technical effect and the assurance that the ingredient's use will not result in products that are adulterated or misleading for consumers.

FSIS has completed its review and concurs with the submitter's position that the glucose oxidase enzyme preparation produced by *A. niger* desugars egg products. Egg products are required to include a statement ("Glucose removed for stability" or "Stabilized, glucose removed") immediately after the product name that indicates the glucose content was reduced.

FSIS requested that we advise you to seek regulatory guidance from its Risk Management and Innovations Staff (RMIS) about the use of glucose oxidase enzyme preparation in meat, poultry, and egg products. You should direct such an inquiry to Dr. Stephanie Hretz, Director, RMIS, Office of Policy and Program Development, FSIS by email at Stephanie.Hretz@usda.gov.

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 Danisco's notice concluding that glucose oxidase 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 glucose oxidase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing glucose oxidase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Danisco provided, as well as other information available to FDA, we have no questions at this time regarding Danisco's conclusion that glucose oxidase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that glucose oxidase 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 001054 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
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Susan Carlson, Ph.D.
Director
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cc: Stephanie Hretz, Ph.D.
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