



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

Re: GRAS Notice No. GRN 001210

Dear Ms. Han:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 1210. We received Danisco US Inc.'s¹ (Danisco) notice on August 8, 2024, and filed it on November 19, 2024. Danisco submitted amendments to the notice on April 2, 2025, May 29, 2025, June 12, 2025, July 7, 2025, and August 12, 2025, providing additional information on identity, manufacturing, specifications, dietary exposure, and the safety narrative.

The subject of the notice is glucosyltransferase enzyme preparation produced by *Bacillus subtilis* expressing the gene encoding glucosyltransferase from *Streptococcus salivarius* (glucosyltransferase enzyme preparation) for use as an enzyme at up to 7 mg total organic solids (TOS)/g sucrose in conventional food processing. The notice informs us of Danisco's view that this use of glucosyltransferase 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 glucosyltransferase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, glucosyltransferase is identified by the Chemical Abstracts Service number 9032-14-8 and the Enzyme Commission Number 2.4.1.5.² Danisco states that the primary sequence of glucosyltransferase consists of 1,341 amino acids with a calculated molecular weight of 149 kDa.

¹ Danisco US Inc. is a wholly owned subsidiary of International Flavors & Fragrances Inc.

² <https://iubmb.qmul.ac.uk/enzyme/EC2/4/1/5.html>

Danisco states that the *B. subtilis* production organism is non-pathogenic and non-toxicogenic and is a well-characterized production organism with history of safe use in the food industry. Danisco states that the production strain, “GICCO3620,” was constructed through transformation with an expression cassette carrying a modified, synthetic glucosyltransferase gene from *S. salivarius*.³ Danisco states that they confirmed sequence integration by whole genome sequencing and polymerase chain reaction. Danisco evaluated the genetic stability of the production strain by whole genome sequencing. Danisco confirmed the absence of functional or transferable antibiotic resistance genes in the final production strain genome.

Danisco states that the glucosyltransferase enzyme preparation is produced by submerged fermentation of a pure culture of the *B. subtilis* production strain under controlled conditions. The *B. subtilis* cells are lysed and the glucosyltransferase enzyme is released into the fermentation medium and then recovered by centrifugation or filtration, concentration via ultrafiltration, and a final polish filtration. The enzyme preparation is then formulated with glycerol, calcium chloride, and water, resulting in a brown hazy liquid. 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 including a limit for lead (0.5 mg/kg) and states that the glucosyltransferase enzyme preparation conforms to the specifications set in the Food Chemicals Codex (FCC, 13th edition, 2022) 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 glucosyltransferase 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 glucosyltransferase enzyme preparation at a maximum use level of 7 mg total organic solids (TOS)/g sucrose in the processing of conventional food. Danisco states that glucosyltransferase catalyzes the conversion of sucrose into polysaccharides and oligosaccharides, and, to a lesser extent, maltose isomers (e.g., nigerose) and sucrose isomers (e.g., leucrose) during the processing of food. Danisco notes that the final enzyme is expected to be inactivated or removed during production of the final food. Danisco conservatively estimates a dietary exposure to glucosyltransferase enzyme preparation at the 90th percentile for the U.S. population aged 1 years and older to be 6.3 mg TOS/kg body weight per day (mg TOS/kg bw/d)

³ Danisco states that there were nine amino acid modifications to the *S. salivarius* glucosyltransferase gene was to enhance selectivity for glucan production and activity at lower pH and higher temperature compared to the native gene; Danisco notes that the expression cassette also included an *alrA* gene for selection of transformants.

from the intended uses of glucosyltransferase enzyme preparation.⁴

In support of the safety of the glucosyltransferase enzyme preparation, Danisco highlights published information that supports the general safe use of enzymes in food for human consumption. Danisco states that enzymes are generally added at the lowest level to catalyze the desired reaction, and that exposure is generally low. Danisco states that the glucosyltransferase derived from *S. salivarius* contains nine amino acid modifications but notes that these are not expected to alter the safety profile of the enzyme. Danisco summarizes data from a literature search that did not identify any information that would contradict a general recognition of safety of the glucosyltransferase enzyme preparation. Danisco summarizes corroborative, unpublished toxicological studies on the notified glucosyltransferase enzyme preparation. This includes a bacterial reverse mutation assay, an *in vitro* micronucleus assay, and a 90-day repeated dose oral toxicity study in rats. Danisco states that the glucosyltransferase enzyme preparation was not mutagenic, and there were no treatment-related adverse effects up to the highest dose tested (1000 mg TOS/kg bw/d). Danisco calculates a margin of exposure to be 158 using the no observed adverse effect level and the estimated dietary exposure for the intended uses of the glucosyltransferase enzyme preparation.⁵ Danisco further relies on published information that discusses the safety of the *B. subtilis* 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 glucosyltransferase. Based on bioinformatic analysis, Danisco reports no significant matches between the amino acid sequences of the glucosyltransferase 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 glucosyltransferase preparation from the intended uses will result in allergic responses.

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

Standards of Identity

In the notice, Danisco states its intention to use glucosyltransferase enzyme preparation in several food categories, including foods for which standards of identity exist, located

⁴ Danisco uses the 90th percentile sucrose consumption data from "Estimation of Sugars Intake of U.S. Population Groups" (The Journal of Nutrition, Volume 116, Supplement 11, 1986, Pages S150-S216) to estimate the dietary exposure to glucosyltransferase enzyme preparation, based on the assumption that 50% of total sucrose comes from processed foods in a worst-case scenario. For comparison, FDA prepared its own estimate of glucosyltransferase enzyme preparation to be 6.2 mg TOS/kg bw/d at the 90th percentile for the U.S. population aged 2 years and older using 2021-2023 NHANES total sugar consumption data for all conventional foods, with the assumption that refined sugar accounts for approximately 55% of total caloric sweeteners used in the United States (ERS USDA Sugar and Sweeteners Yearbook Tables, 2021-2023).

⁵ 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.

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.

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 glucosyltransferase 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 glucosyltransferase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing glucosyltransferase 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 glucosyltransferase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that glucosyltransferase 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 1210 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

 Digitally signed by Susan J.
Carlson -S
Date: 2025.08.15 16:52:20
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Susan Carlson, Ph.D.
Director
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