Vincent Sewalt
Danisco US Inc. (a wholly owned-subsidiary of International Flavors & Fragrances Inc.)
925 Page Mill Road
Palo Alto, CA 94304

Re: GRAS Notice No. GRN 000964

Dear Dr. Sewalt:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000964. We received Danisco US Inc., a wholly owned-subsidiary of International Flavors & Fragrances Inc. (Danisco)'s GRAS notice on August 6, 2020 and filed it on November 25, 2020. We received amendments to the notice on May 26, 2021 and June 10, 2021 that provided the notifier's name change and clarifying information.

The subject of the notice is lysophospholipase enzyme preparation produced by *Trichoderma reesei* expressing a gene encoding lysophospholipase from *Aspergillus niger* (lysophospholipase enzyme preparation) for use as an enzyme at a maximum level of 24.16 mg Total Organic Solids (TOS)/kg raw material in carbohydrate processing (e.g., production of high fructose corn syrup). The notice informs us of Danisco's view that this use of lysophospholipase 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 lysophospholipase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, lysophospholipase is identified by the Chemical Abstracts Service number 9001-85-8 and the Enzyme Commission Number 3.1.1.5.[1] Danisco provides the amino acid sequence of lysophospholipase and states that the molecular weight of the notified enzyme is 67.14 kDa.

[1] https://enzyme.expasy.org/EC/3.1.1.5

Danisco states that the *T. reesei* production organism is non-pathogenic and non-toxigenic. Danisco also states that the production strain was derived from the host strain, RLP-37,[2] by targeted integration of an expression cassette.[3] Danisco states it confirmed the insertion of the expression cassettes by Southern blot, PCR, and whole genome sequencing. Danisco evaluated the stability of the integration as determined by the lysophospholipase production. Danisco states that the final production strain does not contain any antibiotic resistance genes.

Danisco states that the lysophospholipase enzyme preparation is manufactured by submerged fermentation of a pure culture of the *T. reesei* production strain, under controlled conditions, and that the enzyme is recovered from the fermentation broth by filtration or centrifugation, and then concentrated via ultracentrifugation. The resulting liquid enzyme concentrate is preserved and formulated to an enzyme preparation with glycerol, sodium chloride, sodium benzoate, potassium sorbate, and water. After formulation, the enzyme preparation is subjected to polish filtration. 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 lysophospholipase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 12th edition, 2020), 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 data from analyses of three batches of lysophospholipase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism.

Danisco intends to use lysophospholipase enzyme preparation at a maximum level of 24.16 mg TOS/kg raw material for carbohydrate processing. Danisco notes that the lysophospholipase will be denatured or removed during processing. Danisco estimates a maximum dietary exposure to lysophospholipase enzyme preparation to be 0.087 mg TOS/kg body weight per day (mg TOS/kg bw/d) from all the intended uses, on the assumptions processed liquid and solid foods contain a maximum of 2.9 mg TOS/L and 1.21 mg TOS/kg, respectively, and the enzyme will remain in the final food.[4]

Danisco relies on published information that discusses the safety of the *T. reesei* production organism and the safety of microbial enzyme preparations from *T. reesei* used in food processing. In discussing safety of lysophospholipase, Danisco also states

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[2] The *T. reesei* host strain RL-P37 is a cellulase over-producing strain obtained from the wild-type *T. reesei* strain QM6a by classical mutagenesis. QM6a is deposited in the American Type Culture Collection as ATCC 13631.

[3] The expression cassette contained the lysophospholipase gene from *A. niger* under control of the endogenous *T. reesei cbh1* promoter and transcriptional terminator, and the *T. reesei* gene encoding orotate phosphoribosyl transferase selectable marker.

[4] Danisco uses the Budget method to estimate dietary exposure to lysophospholipase enzyme preparation based on consumption of a maximum of 12.5 g of solid foods and 25 mL of beverages per kg bw/d, reflecting the assumption that 50% of all solid foods and 25% of all beverages are processed foods treated with lysophospholipase.

that almost all amyloglucosidase enzyme preparations, which have a history of safe use in food, contain a significant amount of lysophospholipase as a secondary enzyme activity. Danisco discusses unpublished toxicological studies using a trehalase enzyme produced by a related *T. reesei* production strain to support safety of their lysophospholipase enzyme preparation.[5] Danisco further states that lysophospholipase enzyme preparation produced by *T. reesei* was a subject of GRN000653.[6]

Danisco discusses publicly available literature, as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by oral consumption of enzymes, to address potential allergenicity due to lysophospholipase. Based on bioinformatic analyses, Danisco reports no matches between the amino acid sequences of the lysophospholipase and the primary sequences of known allergens using the guidelines developed by Codex Alimentarius Commission (FAO, 2009). Based on the totality of the information available, Danisco concludes that it is unlikely that oral consumption of lysophospholipase enzyme from the intended use will result in allergenic responses.

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

Allergen Labeling

The Federal Food, Drug, and Cosmetic Act (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 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 (effective January 1, 2023)) or a food ingredient that contains protein derived from one of those foods. Lysophospholipase enzyme preparation may require labeling under the FD&C Act because it may contain protein derived from wheat in the fermentation medium. Questions about petitions or notifications for exemptions from the food allergen labeling requirements should be directed to the Division of Food Ingredients in the Office of Food Additive Safety. Questions related to food labeling in general should be directed to the Office of Nutrition and Food Labeling in the Center for Food Safety and Applied Nutrition.

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

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[5] FDA notes that these studies only serve to corroborate the safety of the *T. reesei* production strain and not the lysophospholipase produced by it.

[6] GRN 000653 describes uses of lysophospholipase enzyme preparation from *A. nishimurae* expressed in *T. reesei* for use as an enzyme in the production of syrups from wheat and corn starches, at up to 1 mg TOS/kg of starch raw material. We evaluated GRN 000653 and responded in a letter dated October 13, 2016, stating that we had no questions at the time regarding the notifier's GRAS conclusion.

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 lysophospholipase 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 lysophospholipase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing lysophospholipase 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 lysophospholipase enzyme preparation produced by *T. reesei* expressing a gene encoding lysophospholipase from *A. niger* is GRAS under its intended conditions of use. This letter is not an affirmation that lysophospholipase enzyme preparation produced by *T. reesei* expressing a gene encoding lysophospholipase 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 000964 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2021.09.09 17:10:32 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
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and Applied Nutrition