



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

Re: GRAS Notice No. GRN 000808

Dear Dr. Sewalt:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000808. We received Danisco US Inc. (Operating by DuPont Industrial Biosciences) (DuPont)'s GRAS notice on September 17, 2018, and filed it on October 25, 2018. We received an amendment containing additional safety information on April 4, 2019.

The subject of the notice is triacylglycerol lipase enzyme preparation produced by *Trichoderma reesei* expressing a gene encoding triacylglycerol lipase from *Aspergillus tubigenis* (triacylglycerol lipase enzyme preparation) for use as an enzyme at up to 52 mg Total Organic Solids (TOS)/kg of raw material during baking, brewing, and in the manufacture of cereal beverages, pasta, and potable alcohol. The notice informs us of DuPont's view that these uses of triacylglycerol lipase 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 triacylglycerol lipase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, triacylglycerol lipase is identified by the Enzyme Commission Number 3.1.1.3¹ and CAS number 9001-62-1. DuPont states that the molecular weight of the triacylglycerol lipase is 28.9 kDa and provides the amino acid sequence in the notice.

DuPont states that the *T. reesei* production strain, Morph Lip3, is non-pathogenic and non-toxicogenic. Morph Lip3 is derived from the recipient strain, *T. reesei* RL-P37.²

¹ <https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/1/1/3.html>

² DuPont states that *T. reesei* RL-P37 is commercially produced from the wild-type strain QM6a via several classical mutagenesis steps. QM6a has been deposited in ATCC as 13631.

DuPont describes the construction of the production strain as targeted integration of an expression cassette carrying a native Lipase 3 gene encoding a native (unmodified) amino acid sequence from *A. tubingensis*, the promoter and the terminator elements from *T. reesei* (cbhI), and an *A. nidulans* acetamidase gene (amdS) as a selectable marker. DuPont states that the genetic modification is confirmed by Southern blot analysis. DuPont also states that the stability of the introduced DNA is confirmed by the consistency of the enzyme production on an industrial scale. DuPont states that the final production strain does not contain any functional or transferable antibiotic resistance genes.

DuPont states that triacylglycerol lipase enzyme preparation is manufactured by submerged fermentation of a pure culture of the *T. reesei* production strain. DuPont states that fermentation is carried out under controlled conditions and that the enzyme is secreted into the fermentation media. Flocculants are added to enable enzyme separation from the media components and the enzyme is recovered by filtration or centrifugation, followed by ultrafiltration to concentrate. The enzyme concentrate was used for the toxicological testing. To make the enzyme preparation, potassium sorbate and sodium benzoate are added to stabilize the enzyme concentrate at pH 4.3-5.0 in a liquid preparation. Alternatively, the enzyme concentrate is spray-dried to a powder with potato or wheat starch. DuPont states that the entire process is performed using food-grade raw materials and in accordance with current good manufacturing practices. DuPont also states that the final triacylglycerol lipase enzyme preparation does not contain any major food allergens from the fermentation media.

DuPont has established food grade specifications and states that the triacylglycerol lipase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 11th edition, 2018), 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 analyses of three batches of triacylglycerol lipase enzyme to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production strain.

DuPont intends to use triacylglycerol lipase enzyme preparation at levels that result in a maximum of 24 mg TOS/kg in the final food. The enzyme preparation is intended for use in baking and brewing processes, and in the manufacture of cereal beverages, pasta, and potable alcohol. DuPont notes that the triacylglycerol lipase enzyme preparation will be deactivated or removed during production or refining. However, in estimating dietary exposure, DuPont assumes that all the triacylglycerol lipase enzyme preparation will remain in the final food. DuPont estimates dietary exposure to triacylglycerol lipase enzyme preparation to be 0.4 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses.³

³ DuPont uses the Budget method to calculate estimated dietary exposure to triacylglycerol lipase enzyme preparation based on consumption of a maximum of 25 g of solid foods and 25 mL of liquid foods per kg bw per day. In their estimate, DuPont assumes that 50% of all solid foods and 25% of all liquid foods will be processed and will contain the triacylglycerol lipase enzyme preparation.

DuPont relies on published information that discusses the safety of the *T. reesei* production organism and the safety of microbial enzyme preparations used in food processing. Additionally, DuPont summarizes unpublished toxicological studies using the triacylglycerol lipase enzyme concentrate to corroborate safety of the intended uses of the triacylglycerol enzyme preparation. These studies include a bacterial reverse mutation assay and *in vitro* micronucleus assay in cultured human lymphocytes. DuPont also discusses results from an unpublished 90-day oral toxicity study in rats. DuPont did not observe any treatment-related adverse effects at up to the highest dose of triacylglycerol lipase enzyme concentrate tested; this is equivalent to 123.15 mg TOS/kg bw/d and considered as the No Observed Adverse Effect Level (NOAEL). DuPont calculates a margin of exposure using the NOAEL and the estimated dietary exposure values for the intended uses of the triacylglycerol enzyme preparation to be 300. FDA notes that the margin of exposure is based on unpublished safety studies and that it is corroborative of the published information regarding enzyme preparations used in food processing.

DuPont discusses publicly available literature, as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes, to address potential allergenicity due to triacylglycerol lipase. Further, based on bioinformatic analyses, DuPont reports that the triacylglycerol lipase does not share any biologically meaningful sequence homology or sequence identity to potential oral allergens. Based on the totality of the information available, DuPont concludes that it is unlikely that oral consumption of triacylglycerol lipase enzyme will result in allergenic responses.

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

Standards of Identity

In the notice, DuPont states its intention to use triacylglycerol lipase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. 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 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 eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. The triacylglycerol lipase enzyme spray-dried preparation with wheat starch requires labeling under the FD&C Act because it contains protein derived from wheat.

Section 301(ll) of the 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 DuPont's notice concluding that triacylglycerol lipase 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 triacylglycerol lipase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing triacylglycerol lipase 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 triacylglycerol lipase enzyme preparation produced by *T. reesei* expressing a gene encoding triacylglycerol lipase from *A. tubingensis* is GRAS under its intended conditions of use. This letter is not an affirmation that triacylglycerol lipase enzyme preparation produced by *T. reesei* expressing a gene encoding triacylglycerol lipase from *A. tubingensis* 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 000808 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

Susan Carlson, Ph.D.
Director

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

Digitally signed by Susan J.
Carlson -S
Date: 2019.05.28 15:50:20
-04'00'