Kazunori Harada
Meito Sangyo Co., Ltd.
2973-2 Ishikawa-machi
Hachioji 192-8509
Tokyo, JAPAN

Re: GRAS Notice No. GRN 001161

Dear Mr. Harada:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001161. We received Meito Sangyo Co., Ltd. (Meito)'s GRAS notice on September 7, 2023, and filed it on January 5, 2024. Meito submitted amendments to the notice on June 11, 2024, July 25, 2024, and September 11, 2024 containing additional information on enzyme identity, manufacturing, specifications, analytical methods, and safety narrative.

The subject of the notice is triacylglycerol lipase enzyme preparation produced by *Limtongozyma cylindracea*[1] (lipase enzyme preparation) for use as an enzyme at up to 1500 mg TOS/kg raw material in the manufacture of cheeses and edible oils, as well as in various brewing, flavoring, and baking applications. The notice informs us of Meito's view that this use of lipase 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. Meito's notice provides information about the components in the lipase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, lipase is identified by the Enzyme Commission Number 3.1.1.3,[2] and the Chemical Abstracts Service Number 9001-62-1. Meito states that the primary amino acid sequence of each of the five lipase enzymes consists of 548 or 549 amino acids with a calculated molecular weight of 60 kDa.

Meito states that the *L. cylindracea* production organism is a non-pathogenic and non-

[1] *Limtongozyma cylindracea* was reclassified in 2019 and previously identified as *Candida cylindracea*.
[2] https://iubmb.qmul.ac.uk/enzyme/EC3/1/1/3.html

toxigenic yeast. Meito states that the *L. cylindracea* production strain "MS-5-OF" was produced by conventional mutagenesis. Meito also states that based on the results of genome sequencing the production strain does not contain any functional antibiotic resistance genes.

Meito states that lipase enzyme preparation is manufactured by controlled fed-batch fermentation of a pure culture of the *L. cylindracea* production strain "MS-5-OF." The enzyme is secreted into the fermentation medium. After fermentation, the medium containing the enzyme is separated from the biomass by centrifugation, dehydrated with acetone, centrifuged, vacuum dried, and sifted and is a white to off-white/tan powder. Meito states that the entire process is performed in accordance with current Good Manufacturing Practices and with food-grade raw materials.

Meito has established food-grade specifications including a limit for lead (< 0.1 mg/kg) and states that the lipase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 13th edition, 2023), 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). Meito provides results from analyses of three non-consecutive batches of lipase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism in the final product.

Meito states lipase enzyme preparation is intended for use as an enzyme at up to 1500 mg TOS/kg raw material in the manufacture of cheeses and edible oils, as well as in various brewing, flavoring, and baking applications. Lipase catalyzes the hydrolysis of ester bonds of fats. Meito notes that the lipase enzyme is removed, denatured, and/or inactivated during food production. Meito estimates a maximum dietary exposure to the lipase enzyme preparation to be 3.2 mg TOS/kg body weight (bw)/day from the use in food and drinks.[3]

Meito relies on published information that discusses the safety of the lipase, the *L. cylindracea* production organism and the safety of microbial enzyme preparations used in food processing. Meito states that a literature search did not identify any information that would contradict a general recognition of safety of the lipase enzyme preparation. Meito discusses the results of published toxicological studies using the lipase enzyme preparation, which included a bacterial reverse mutation assay, an *in vitro* chromosome aberration test, and a 90-day subchronic toxicity study in rats. Meito states that the lipase enzyme concentrate was not mutagenic. Meito notes that in the 90-day toxicity study, male rats in the highest dose group had significantly increased platelet counts and prothrombin time; however, no corresponding test-article related histopathological changes were observed and no dose-response relationship was established. Additionally, no treatment-related adverse events were reported in female rats up to the highest dose tested. Meito states that the authors concluded that the No-Observed-Adverse-Effect-Level (NOAEL) for the lipase enzyme preparation is 1,027 mg TOS/kg bw/d in males

[3] Meito uses the Budget method to estimate the dietary exposure to lipase enzyme preparation based on a maximum use levels of 195 mg TOS/kg in solid foods and 32 mg TOS/kg in liquid foods respectively, and consumption of 12.5 g of solid foods and 25 mL of non-milk beverages/kg bw per day.

and 2,379 mg TOS/kg bw/d in females). Meito calculates a margin of exposure to be at least 317 using the NOAEL and the estimated dietary exposure for the intended uses of the lipase enzyme preparation. As further corroborative evidence of safety, Meito discusses toxicological studies done with another lipase enzyme preparation from the *L. cylindracea* production organism, and notes that this lipase preparation was not mutagenic nor were any test-article related adverse effects noted in the 90-day subchronic toxicity study in rats.

Meito discusses publicly available literature, as well as the conclusions of several organizations and working groups, about the low risk of allergenicity posed by enzymes from their intended use, to address potential allergenicity due to lipase. Based on bioinformatic analyses, using criteria recommended by FAO/WHO (FAO/WHO, 2001; Codex Alimentarius, 2009; JECFA, 2016), Meito reports that no sequence homology of lipase to known allergens that would raise allergenicity concerns were identified. Based on the totality of the information available, Meito concludes that it is unlikely that oral consumption of lipase will result in allergenic responses from its intended uses.

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

Standards of Identity

In the notice, Meito states its intention to use lipase 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 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) or a food ingredient that contains protein derived from one of those foods. Lipase enzyme preparation requires labeling under the FD&C Act because it contains protein derived from soybeans.

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 Meito's notice concluding that 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 lipase enzyme

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

Conclusions

Based on the information that Meito provided, as well as other information available to FDA, we have no questions at this time regarding Meito's conclusion that lipase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that lipase 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 001161 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2024.09.24 17:06:49 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
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