Celia Martin, PhD
Lallemand Inc.
1620 Préfontaine
Montreal, Quebec
CANADA
H1W 2N8

Re: GRAS Notice No. GRN 001154

Dear Dr. Martin,

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 1154. We received Lallemand Inc.'s (Lallemand) notice on September 4, 2023 and filed it on November 16, 2023. Lallemand submitted amendments to the notice on April 17, 2024, and June 19, 2024, that expanded the safety narrative and provided clarifications on identity, manufacturing, specifications, and intended use.

The subject of the notice is triacylglycerol lipase enzyme preparation produced by *Komagataella phaffii*[1] expressing a lipase gene from *Fusarium oxysporum* (lipase enzyme preparation) for use as an enzyme at up to 8.46 mg total organic solids (TOS)/kg flour in baking. The notice informs us of Lallemand'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. Lallemand'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 Chemical Abstracts Service number 9001-62-1 and the Enzyme Commission Number 3.1.1.3.[2] Lallemand states that the primary sequence of lipase consists of 331 amino acids with a calculated molecular weight of 35 kDa.

Lallemand states that the *K. phaffi* production organism is non-pathogenic and non-

[1] Lallemand states that *Pichia pastoris* was reclassified as *K. phaffii* as reported in Kurtzman, C. (2005). Description of *Komagataella phaffii* sp. nov. and the transfer of *Pichia pseudopastoris* to the methylotrophic yeast genus Komagataella. *International Journal of Systematic and Evolutionary Microbiology*, 55, 973-976. doi: 10.1099/ijs.0.63491-0

[2] https://iubmb.qmul.ac.uk/enzyme/EC3/1/1/3.html

toxigenic and is a well-characterized production organism with history of safe use in the food industry. Lallemand states that the recipient strain used in the construction of the production strain, LALL-LI2, is *K. phaffi* strain ATCC 76273. Lallemand states that the production organism was constructed through transformation with an expression cassette carrying a modified *F. oxysporum* lipase gene.[3] Lallemand states that they confirmed sequence integrity by polymerase chain reaction (PCR) and whole genome sequencing. Lallemand evaluated the genetic stability of the production strain by PCR. Lallemand verified the absence of functional or transferable antibiotic resistance genes in the final production strain genome using whole genome sequencing.

Lallemand states that the lipase enzyme preparation is produced by fed batch fermentation of a pure culture of the *K. phaffi* production strain under controlled conditions. The lipase enzyme is secreted into the fermentation medium and then recovered by an initial centrifugation step, clarification via microfiltration, concentration via ultrafiltration, and a polish filtration. The resulting liquid enzyme concentrate is brown in color. The lipase enzyme preparation is then formulated to either a liquid or solid preparation using stabilizing and preserving agents that include sucrose, glycerol, sodium chloride, potassium sorbate, and sodium benzoate. For solid preparations, Lallemand states that the enzyme concentrate is dried to form a powder and then carriers, such as salt, starch or dextrin, may be used. Lallemand states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices.

Lallemand has established food grade specifications including a limit for lead (<1 mg/kg) and states that the lipase 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). Lallemand provides results from analyses of three non-consecutive batches of lipase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism and antibiotic activity.

Lallemand intends to use lipase enzyme preparation at a maximum use level of 8.46 mg total organic solids (TOS)/kg flour as a powder or liquid in baking. Lallemand states that lipase catalyzes the hydrolysis of triglycerides ester bonds into diglycerides and subsequently into monoglycerides and glycerol, as well as free fatty acids. Lallemand notes that the final enzyme is inactivated during baking and any ingested inactive lipase in the final food is expected to be digested and not pose any human health risk. Lallemand estimates a maximum dietary exposure to lipase enzyme preparation of 0.11 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses, assuming

[3] Lallemand states that the modified *F. oxysporum* lipase gene excludes the first 15 amino acids and a sequence encoding a new signal peptide was added to facilitate excretion in *K. phaffi.*

that all of the lipase enzyme preparation will be active and remain in the final food.[4]

In support of the safety of the lipase enzyme preparation, Lallemand highlights published information that supports the history of safe use of lipase in food as well as safety of the donor organism. In addition, Lallemand provides a list of lipases that were previously concluded to be GRAS, including lipases produced in organisms expressing lipase from *F. oxysporum*. Lallemand states that the amino acid sequence of the lipase enzyme preparation that is the subject of this notice is identical to the lipase that was the subject of GRN 001047,[5] and sufficiently similar to lipases that were the subject of GRNs 000075[6] and 000631,[7] such that safety information for those lipases is relevant to safety assessment of this lipase. Lallemand states that enzymes used in food processing are typically inactivated and denatured, making any lipase remaining in the final food susceptible to digestion by consumers and thus it will not pose any safety risk. Additionally, Lallemand states that a literature search did not identify any information that would contradict a general recognition of safety of the lipase enzyme preparation. Lallemand relies on published information that discusses the safety of the *K. phaffi* production organism.

Lallemand discusses publicly available literature to address potential allergenicity due to lipase. Lallemand states that enzymes in general are unlikely to be food allergens. Based on bioinformatic analysis, Lallemand reports no significant matches between the amino acid sequences of the lipase and the primary sequences of known food allergens based on the guidelines developed by the Codex Alimentarius Commission (Codex, 2009). Lallemand also discusses bioinformatics analyses of custom databases containing known toxins and concluded that the lipase sequence showed no significant homology to known toxins. Based on the totality of information available, Lallemand concludes that it is unlikely that oral consumption of lipase preparation from the intended uses will result in allergic responses.

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

---

[4] Lallemand uses the Budget method to estimate the dietary exposure to lipase enzyme preparation based on enzyme preparation based on the consumption of 12.5 g solid foods per kg body weight per day (worst case scenario) containing the lipase enzyme preparation at the recommended use level.

[5] Lipase enzyme preparation produced by *Saccharomyces cerevisiae* expressing lipase from *F. oxysporum* was the subject of GRN 001047. We evaluated this notice and responded in a letter dated May 12, 2023 stating that we had no questions at the time regarding the notifier's GRAS conclusion.

[6] Lipase enzyme preparation produced by *Aspergillus oryzae* expressing lipase from *F. oxysporum* was the subject of GRN 000075. We evaluated this notice and responded in a letter dated August 14, 2001 stating that we had no questions at the time regarding the notifier's GRAS conclusion.

[7] Lipase enzyme preparation produced by *Trichoderma reesei* expressing lipase from *F. oxysporum* was the subject of GRN 000631. We evaluated this notice and responded in a letter dated July 18, 2016 stating that we had no questions at the time regarding the notifier's GRAS conclusion.

Standards of Identity

In the notice, Lallemand states its intention to use lipase enzyme preparation in a food category that includes 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.

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 Lallemand'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 Lallemand provided, as well as other information available to FDA, we have no questions at this time regarding Lallemand'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 1154 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2024.08.15 15:45:01 -04'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition