Andrew Ellis
Biocatalysts Limited
Cefn Coed, Pare Nantgarw
Cardiff, CFlS 7QQ
UNITED KINGDOM

Re: GRAS Notice No. GRN 000940

Dear Dr. Ellis:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000940. We received Biocatalysts Limited (Biocatalysts)'s GRAS notice on May 19, 2020 and filed it on July 30, 2020. Biocatalysts submitted amendments to the notice on September 18, 2020, February 12, 2021, and March 3, 2021, and April 16, 2021 providing additional safety information and clarifying information designated confidential.[1]

The subject of the notice is phospholipase A2 enzyme preparation produced by *Yarrowia lipolytica* genetically engineered to express a synthetic gene for phospholipase A2 from *Sus scrofa* (wild pig; phospholipase A2 enzyme preparation) at levels in accordance with current good manufacturing practices (cGMPs), in the production of bread, baked goods, egg yolk-based sauces and dressings (e.g., mayonnaise) and, in degumming of vegetable oils. The notice informs us of Biocatalysts' view that the uses of phospholipase A2 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. Biocatalysts' notice provides information about the components in the phospholipase A2 enzyme preparation.

[1] The September 18, 2020 amendment stated that information contained in GRN 000940 that was designated confidential is not considered confidential by Biocatalysts. The February 12, 2021 amendment contained information designated confidential, which was revised and submitted as the March 3, 2021 amendment without confidential information.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, phospholipase A2 is identified by the Enzyme Commission Numbers 3.1.1.4[2] and CAS number 9001-84-7. Biocatalysts states that the mature enzyme is 124 amino acids and the molecular weight is 15 kDa.

Biocatalysts notes that *Y. lipolytica* is non-pathogenic and non-toxigenic. Biocatalysts also states that the *Y. lipolytica* production strain JMY1212 was derived from a wild-type recipient strain W29.[3] The notifier describes the construction of the *Y. lipolytica* production strain by targeted integration of an expression cassette.[4] Biocatalysts also states that the stability of the introduced DNA has been confirmed by end of fermentation enzyme activity assay, and that the final production strain does not contain any antibiotic resistance genes.

Biocatalysts states that phospholipase A2 enzyme preparation is manufactured by submerged fed-batch fermentation of a pure culture of the production strain. Biocatalysts states that fermentation is carried out under controlled conditions and that the inactivated enzyme is secreted into the fermentation medium. The enzyme is then recovered and concentrated by centrifugation and filtration, and then activated by treatment with a protease; the added protease is then inactivated by adjusting of the temperature. The enzyme is clarified and concentrated. The liquid enzyme concentrate is standardized to a preparation with water, glycerol, and sodium chloride, and preserved with potassium sorbate. Biocatalysts states that the entire process is performed in accordance with cGMP. Biocatalysts also states that the final phospholipase A2 enzyme preparation does not contain any major food allergens.

Biocatalysts has established food grade specifications and states the phospholipase A2 enzyme preparation conforms to established specifications 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). Biocatalysts provides data from analyses of four batches of phospholipase A2 enzyme concentrate to demonstrate the manufacturing acceptance criteria have been met. Biocatalysts also confirms that the production organism is not present in the final enzyme preparation.

Biocatalysts intends to use phospholipase A2 enzyme preparation at levels up to 33.9 g TOS/1000 kg of egg yolk during the manufacture of sauces and dressings such as mayonnaise, up to 24 g TOS/1000 kg flour in bread processing, and up to 279 ml/1000 kg oil in the degumming of edible crude vegetable oils. Biocatalysts notes that the phospholipase A2 enzyme preparation will be inactivated during production. However, in estimating dietary exposure, Biocatalysts assumes that all of the enzyme preparation will remain in the final food except vegetable oils; this is because the phospholipase A2 will be removed during downstream processing of oils. Biocatalysts estimates dietary exposure to phospholipase A2 enzyme preparation to be

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[2] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/1/1/4.html

[3] The *Y. lipolytica* wild-type strain W29 is deposited at the ATCC with the deposit number ATCC20460.

[4] The expression cassette contains the synthetic phospholipase A2 gene from *S. scrofa*, promoter, transcriptional terminator, and the gene for a selectable marker from *Y. lipolytica*.

1-38 μg TOS/kg body weight per day based on a 60 kg person day (μg TOS/kg bw/d) from the intended uses based on daily consumption of mayonnaise (30 g/person (p)/d) and bread (113 g/p/d).

Biocatalysts relies on published information that discusses the safety of the *Y. lipolytica* production organism and the safety of microbial enzyme preparations in general used in food processing. Further, Biocatalysts discusses safety of the phospholipase enzymes used in food production.

Biocatalysts 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 phospholipase A2. Further, Biocatalysts discusses sequence comparisons with known allergenic proteins using AllergenOnline, Structural Database of Allergenic Proteins (SDAP), and Algpred (prediction of Allergenic Proteins and Mapping of IgE Epitopes) databases. Such bioinformatic analyses showed that while the phospholipase A2 showed some similarities to known allergens, based on the totality of information available, Biocatalysts concludes that it is unlikely that oral exposure of phospholipase A2 will result in an allergenic response.

Based on the totality of the information available, Biocatalysts concludes that the phospholipase A2 enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, Biocatalysts states its intention to use phospholipase A2 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.

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 Biocatalysts' notice concluding that phospholipase A2 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 phospholipase A2 enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing phospholipase A2 enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Biocatalysts provided, as well as other information available to FDA, we have no questions at this time regarding Biocatalysts' conclusion that phospholipase A2 enzyme preparation produced by *Yarrowia lipolytica* genetically engineered to express a synthetic phospholipase A2 gene from *Sus scrofa* is GRAS under its intended conditions of use. This letter is not an affirmation that phospholipase A2 enzyme preparation produced by *Yarrowia lipolytica* genetically engineered to express a synthetic phospholipase A2 gene from *Sus scrofa* 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 000940 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2021.05.12 12:11:35 -04'00'

Susan Carlson, Ph.D.
Director
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