DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
College Park, MD 20740-3835

Ms. Candice Cryne
Feldbergstr. 78
D-64293 Darmstadt
Germany

Re: GRAS Notice No. GRN 000653

Dear Ms. Cryne:

The Food and Drug Administration (FDA) is responding to the notice, dated May 5, 2016, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on May 27, 2016, filed it on June 21, 2016, and designated it as GRAS Notice No. GRN 000653.

The subject of the notice is lysophospholipase enzyme preparation from *Aspergillus nishimurae* expressed in *Trichoderma reesei* (lysophospholipase enzyme preparation). The notice informs FDA of the view of AB Enzymes GmbH (AB Enzymes) that lysophospholipase enzyme preparation is GRAS, through scientific procedures, for use as an enzyme in the production of syrups from wheat and corn starches, at up to 1 milligram Total Organic Solids per kilogram (mg TOS/kg) of starch raw material.

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 components derived from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. AB Enzymes' notice provides information about each of these 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 Enzyme Commission Number 3.1.1.5. The accepted name for the enzyme is lysophospholipase, and the systematic name is 2-lysophosphatidylcholine acylhydrolase. This enzyme is also known as lecithinase B; lysolecithinase; phospholipase B; lysophosphatidase; lecitholipase; phosphatidase B; lysophosphatidylcholine hydrolase; lysophospholipase A1; lysophopholipase L2; lysophospholipase-transacylase; neuropathy target esterase; NTE; NTE-LysoPLA; NTE-lysophospholipase. The CAS Registry Number for lysophospholipase is 9001-85-8. Lysophospholipase catalyzes the hydrolysis of 2-lysophosphatidylcholine to form glycerophosphocholine and carboxylate. The functional lysophospholipase is 588 amino acids in length, has a molecular mass of 63,171.9 Da (non-glycosylated) and an isoelectric point of 5.54. [1]

[1] The encoded protein after expression is 611 amino acids long, and it includes the signal peptide sequence.

AB Enzymes describes the construction of the *T. reesei* production strain, RF7206, [2] from the recipient *T. reesei* strain, RF4847 [3], expressing a recombinant lysophospholipase gene from *A. nishimurae* [4]. RF4847 is a classical mutant strain of *T. reesei* QM6a.[5] The components of the expression cassette used to transform the recipient strain include an *A. nishimurae lpl* gene fused to a native *T. reesei* cellulase promoter and *A. nidulans amdS* marker gene. The *T. reesei* production strain differs from RF4847 in its high lysophospholipase production capability due to overexpression of the *lpl* gene driven by the cbh1 promoter. AB Enzymes confirms that the transformed DNA fragments are well characterized, the fragments are free from any harmful sequences, and that the integration is stable, based on Southern blot analyses of the production strain. AB Enzymes also states that both *T. reesei,* and *A. nishimurae* are nonpathogenic and nontoxigenic microbes, with a history of safe use in the production of enzymes used in food. AB Enzymes also states that the transformed DNA is stably integrated, and does not contain any antibiotic resistance genes.

AB Enzymes states that the lysophospholipase enzyme preparation is manufactured by the submerged fermentation of a pure culture of the *T. reesei* production strain. AB Enzymes states that fermentation is carried out under well-defined process conditions (pH, temperature, mixing, etc.) and that the lysophospholipase is secreted into the culture medium. After fermentation, the enzyme is separated from the cell mass by the addition of a flocculating agent to facilitate the primary solid/liquid separation. This is followed by filtration or centrifugation. The supernatant containing the enzyme is concentrated at controlled pH and temperature, and filtered to remove insoluble material and the production organism. The final enzyme product is standardized as a liquid product with food-grade glycerol and sodium chloride. AB Enzymes states that the entire process is performed in accordance with current good manufacturing practices using raw materials of food-grade quality. AB Enzymes also states that the final enzyme preparation contains no major food allergens from the manufacturing process.

AB Enzymes notes that the lysophospholipase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 10th edition, 2015), 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). AB Enzymes provides analytical data from three batches of lysophospholipase enzyme preparations to demonstrate consistency with the specifications.

AB Enzymes proposes to use the lysophospholipase enzyme preparation as an enzyme in the manufacture of sweeteners such as maltodextrins, glucose, fructose, maltose, and high fructose corn syrups from wheat and corn starches, at up to levels of 1 mg TOS/kg of starch raw material. The enzymatically produced sweeteners are used in various food industries, including beverage, dairy, baking, canning and confectionery. AB Enzymes states that the lysophospholipase enzyme is expected to be inactivated and/or removed during manufacturing in all of the proposed uses. AB Enzymes however, estimates dietary exposure to lysophospholipase enzyme preparation, based on the maximum intended use levels and the assumption that all of the enzyme preparation will remain in the final food, to be 0.006 mg TOS/kg bodyweight per day (mg TOS/kg bw/d). AB Enzymes also states that lysophospholipase enzyme or its reaction products formed during the production or storage of the enzyme treated food would be considered normal constituents of the diet, and no adverse effect on nutrients is expected.

---

[2] The production strain is deposited in the Centraalbureau voor Schimmelcultures (CBS) with the deposit number CBS125079.
[3] RF4847 has been deposited in CBS with the deposit number CBS114041.
[4] The donor strain was first identified as *A. fumigatus* and more recently as *A. nishimurae*.
[5] *T. reesei* QM6a has been identified by CBS based on DNA sequencing.

AB Enzymes relies on published information for the safety of microbial enzyme preparations used in food processing and summarizes corroborative unpublished toxicological studies using the dry ultrafiltered lysophospholipase enzyme concentrate. Tests conducted with bacterial cells showed that the lysophospholipase enzyme is not mutagenic at the highest dose tested both in the presence and absence of metabolic activation. AB Enzymes also demonstrates that the lysophospholipase enzyme concentrate is not clastogenic based on results from *in vitro* chromosomal aberration tests. A ninety-day oral toxicity study (90-day) in rats using the lysophospholipase enzyme concentrate at the highest dose tested (1000 mg/kg bw/d), corresponding to 955 mg TOS/kg bw/d, showed slight effects [6] that were not considered toxicologically relevant by the notifier. Based on the highest dose tested in the 90-day study, and the estimated dietary exposure from the intended uses of the lysophospholipase enzyme preparation, i.e. 955 mg/kg bw/d and 0.006 mg TOS/kg bw/d, respectively, AB Enzymes calculates the margins of safety to be approximately 160,000.

AB Enzymes discusses potential food allergenicity of lysophospholipase enzyme. AB Enzymes conducted an 80-amino acid sequence homology search of the lysophospholipase enzyme against known allergens stored in the publicly available allergen database, Allergen Online. AB Enzymes found no sequence identity matches over 35% to known allergens. Additionally, AB Enzymes found no exact matches in the mature lysophospholipase enzyme sequence to allergenic protein sequences, in analyses performed over contiguous stretches of eight amino acids. AB Enzymes further cites the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes due to their low use levels and the extensive processing of enzyme-containing foods during manufacturing. Based on the totality of the information available, AB Enzymes concludes that oral consumption of lysophospholipase enzyme will not result in allergenic responses.

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

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 its review of AB Enzymes' notice that lysophospholipase enzyme preparation is GRAS for the intended uses, FDA did not consider whether section 301(ll) or any of its exemptions apply to foods containing lysophospholipase enzyme preparation. Accordingly, this response should not be construed to be a statement that foods that contain lysophospholipase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information provided by AB Enzymes GmbH, as well as other information available to FDA, the agency has no questions at this time regarding AB Enzymes GmbH's conclusion that lysophospholipase enzyme preparation from *A. nishimurae* expressed in *T. reesei* is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of lysophospholipase enzyme preparation from *A. nishimurae* expressed in *T. reesei*. As always, it is the continuing responsibility of AB Enzymes GmbH to ensure that food

[6] AB Enzymes noted a slight trend in reduced mean daily and relative food consumptions in animals of both sexes at all dose levels during the treatment period that were not accompanied by concomitant changes in mean body weight.

ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter responding to GRN 000653, as well as a copy of the information in this notice that conforms to the information in the GRAS exemption claim (proposed 21 CFR 170.36(c)(1)), is available for public review and copying at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300042713, cn=Michael A. Adams -S
Date: 2016.10.13 17:14:48 -04'00'

Dennis M. Keefe, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition