Deepti Sood
5th Floor, Sun Magnetica LIC Service Road
Louiswadi, Thane
West Maharashtra
400604 INDIA

Re: GRAS Notice No. GRN 000783

Dear Ms. Sood:

This letter corrects our letter signed December 20, 2018, sent in response to GRN 000783. The purpose of this revised letter is to correct the identity of the promoter used in the construction of the production strain.

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000783. We received the notice that you submitted on behalf of Advanced Enzyme Technologies Limited (AET) on April 30, 2018 and filed it on June 28, 2018. AET submitted an amendment on November 14, 2018, containing additional clarifications regarding the manufacturing process.

The subject of the notice is triacylglycerol lipase enzyme produced by *Aspergillus niger* expressing a triacylglycerol lipase gene from *Rhizopus oryzae* (TAG lipase enzyme preparation) for use as an enzyme at a maximum level of 86 mg TOS/kg of oil, in the production of cocoa butter substitutes, and fat for infant formula. The notice informs us of AET's view that these uses of TAG 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 components derived from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. AET's notice provides information about each of these components in the TAG lipase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, TAG lipase is identified by the Enzyme Commission Number 3.1.1.3. The accepted name for the enzyme is triacylglycerol lipase and the systematic name is triacylglycerol acylhydrolase. The CAS No. for TAG lipase is 9001-62-1. TAG lipase also has numerous synonyms, some containing brand name designations. AET states that the identity of triacylglycerol lipase was confirmed using SDS-PAGE analysis. AET also states that the TAG lipase is 392 amino acids in length with a corresponding molecular weight of 37.8 kDa.

AET states that the *A. niger* production strain, FL100SC, is nonpathogenic and nontoxigenic. FL100SC is constructed from a mutant host strain of *A. niger* [1] and is resistant to 5-fluoro orotic acid. A synthetic codon-optimized lipase gene from a *R. oryzae* donor was transformed into the host strain under the control of a *A. oryzae* amyA promoter and amyA terminator, along with a selection marker. AET confirmed the integration of the TAG lipase gene using Southern blot analysis and stability of the insertion using genetic fingerprinting. AET states that no antibiotic resistance genes were introduced during transformation. AET also states that the production strain lacks potential to produce ochratoxins based on results from PCR-RFLP.

AET states that the TAG lipase enzyme preparation is manufactured by submerged fermentation of a pure culture of the production strain, controlled to ensure identity, purity, and enzyme-generating ability. The enzyme that is secreted into the fermentation medium is recovered by separating the biomass from the supernatant after the addition of filter aids, with pH and temperature adjustments. The enzyme is further concentrated, at controlled pH and temperature. AET states that the concentrated liquid enzyme is filtered to ensure removal of the production organism and stabilized with glycerol. The concentrated enzyme is used for the safety studies discussed in this notice. To make the TAG lipase enzyme preparation, the concentrated enzyme is spray dried and formulated with maltodextrin (sourced from maize). AET states that the entire process is performed using food grade raw materials, and in accordance with current good manufacturing practices. AET also states that the final enzyme preparation does not contain any major food allergens from the fermentation medium.

AET has established food grade specifications and notes that the TAG lipase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 10th edition, 2016), 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). AET provides analytical data from three batches of TAG lipase enzyme preparation to demonstrate manufacturing consistency with the specifications. AET confirms the absence of the production microorganism in the commercial product with an established specification.

AET intends to use the TAG lipase enzyme preparation[2] at a maximum level of 86 mg TOS/kg of oil during the processing of cocoa butter substitutes and fat for infant formula. AET states that the oil treated with the TAG lipase enzyme preparation will be subjected to refining methods and serial filtration that will remove any potential enzyme preparation prior to use in food. However, in the estimation of the dietary exposure AET assumes that all the TAG lipase enzyme preparation will remain in the final food. AET estimates dietary exposure to TAG lipase enzyme preparation from consumption of cocoa butter substitutes to be 0.017 mg TOS/kg bw/d; this is based on the 90th percentile intake of cocoa butter substitutes of 12.7 g as reported in the 2011-12 NHANES food consumption database. AET estimates dietary exposure to TAG lipase enzyme preparation from consumption of fat for infant formula to be 0.96 mg TOS/kg bw/d; this is based on consumption of 34 g of fat per infant (0-6 months) per day.

AET relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the *A. niger* production organism. Additionally, AET discusses corroborative unpublished toxicological studies using the unformulated triacylglycerol lipase enzyme concentrate to corroborate safety. AET states that the

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[1] AET states that the identity of the parental *A. niger* strain, from which the mutant was generated, was determined by DNA sequencing and bioinformatics and that the parental *A. niger* strain was shown not to produce mycotoxins.

[2] AET states that the TAG lipase enzyme preparation is adsorbed to a crosslinked copolymer of methacrylate (resin) prior to use. FDA notes that it is the responsibility of AET, the company immobilizing the TAG enzyme preparation, to ensure that all substances utilized in conjunction with this use are safe and are otherwise in compliance with all applicable legal and regulatory requirements.

triacylglycerol lipase enzyme is neither mutagenic nor clastogenic. AET also discusses results from an unpublished 90-day oral toxicity study conducted in rats that shows consumption of TAG lipase enzyme concentrate did not cause any treatment-related adverse effects up to the highest dose tested**,** equivalent to 847 mg TOS/kg bw/d. AET calculates margins of exposure based on the No Observed Adverse Effect Level of 847 mg TOS/kg bw/d from this study and the estimated maximum dietary exposures from the intended uses of TAG lipase enzyme preparation. FDA notes that the estimated margins of exposure are based on unpublished information, serving only to corroborate published information regarding enzyme preparations used in food processing.

AET 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 TAG lipase. Further, based on bioinformatic analyses, AET reports that the TAG lipase does not share any biologically meaningful sequence homology and that it lacks sequence identity to potential allergens. Additionally, AET provides unpublished results of a simulated gastric fluid digestion to support their conclusion regarding the lack of potential oral allergenicity of TAG lipase enzyme. Based on the totality of the information available, AET concludes that it is unlikely that oral consumption of TAG lipase enzyme will result in allergenic responses.

Based on the data and information summarized above, AET concludes that TAG lipase 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 our evaluation of AET's notice concluding that TAG 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 TAG lipase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing TAG lipase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that AET provided, as well as other information available to FDA, we have no questions at this time regarding AET's conclusion that TAG lipase enzyme produced by *A. niger* expressing a TAG lipase gene from *R. oryzae* is GRAS under its intended conditions of use. This letter is not an affirmation that TAG lipase enzyme produced by *A. niger* expressing a TAG lipase gene from *R. oryzae* 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 00783 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
Date: 2019.05.07 13:46:52 -04'00'

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