Janet Oesterling
Novozymes North America, Inc.
77 Perry Chapel Church Road
Franklinton, NC 27525

Re: GRAS Notice No. GRN 000728

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000728. We received Novozymes North America, Inc.'s (Novozymes') notice on August 25, 2017, and filed it on September 8, 2017. We received an amendment containing additional safety information on October 11, 2017.

The subject of the notice is phosphoinositide phospholipase C enzyme preparation produced by *Bacillus licheniformis* expressing a modified synthetic gene encoding a variant of the wild-type phosphoinositide phospholipase C from *Pseudomonas* sp. 62186 (phosphoinositide phospholipase C enzyme preparation) for use as an enzyme in the degumming of vegetable oils at a maximum level of 1.4 mg Total Organic Solids (TOS)/kg oil. The notice informs us of Novozymes' view that this use of phosphoinositide phospholipase C 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. Novozymes' notice provides information about the components in the phosphoinositide phospholipase C enzyme preparation.

Per the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, phosphoinositide phospholipase C is identified by the Enzyme Commission Number 3.1.4.11. The accepted name is phosphoinositide phospholipase C. The systematic name for this enzyme is 1-phosphatidyl-1D-myo-inositol-4,5-bisphosphate inositoltrisphosphohydrolase. Phosphoinositide phospholipase C is also known as triphosphoinositide phosphodiesterase; phosphoinositidase C; 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase; monophosphatidylinositol phosphodiesterase; phosphatidylinositol phospholipase C; and PI-PLC. Phosphoinositide phospholipase C hydrolyzes phosphatidylcholine present in oil, to its ester, phosphorylinositol. The CAS No. for phosphoinositide phospholipase C is 63551-76-8. Novozymes states that the primary amino acid sequence of the

expressed phosphoinositide phospholipase C has been determined and consists of 327 amino acids. Novozymes states that phosphoinositide phospholipase C has a molecular weight of 36 kDa.

Novozymes states that the *B. licheniformis* production strain MaTa161 was constructed from the recipient strain *B. licheniformis* AEB1953.[1] Novozymes states that AEB1953 was modified at several chromosomal loci to inactivate genes encoding proteases and a gene necessary for sporulation. Novozymes describes *B. licheniformis* as a non-pathogenic, non-toxigenic, well-characterized production organism with a history of safe use in the food industry. Novozymes also states that the production strain is considered suitable for Good Industrial Large Scale Practice worldwide.

Novozymes describes the construction of the production strain MaTa161 from AEB1953 by the targeted integration of an expression cassette carrying a modified synthetic gene encoding a variant of the wild-type phosphoinositide phospholipase C gene[2] from *Pseudomonas* sp. 62186, a hybrid *Bacillus* promoter,[3] and a transcriptional terminator. Novozymes confirmed the sequences of the inserted expression cassettes and the flanking regions at three integration loci. Novozymes also confirmed that the introduced DNA is stable during production using Southern blot hybridization and is free of any functional antibiotic resistance genes by genome sequence analysis.

Novozymes states that phosphoinositide phospholipase C enzyme is produced by submerged fed-batch fermentation of a pure culture of the production strain. Novozymes states that fermentation is carried out under controlled conditions and that phosphoinositide phospholipase C is secreted into the culture medium. After fermentation, the culture medium is subjected to an optional pretreatment step of pH adjustment and flocculation, if required. Phosphoinositide phospholipase C is recovered from the culture medium by vacuum filtration or centrifugation. Following this step, the supernatant containing the enzyme is concentrated by either ultrafiltration or by evaporation. The concentrated enzyme is filtered to ensure the removal of any production organism. The enzyme is further concentrated by ultrafiltration or evaporation, and is then stabilized by the addition of glycerol, and formulated with water, potassium sorbate, and sodium benzoate. Novozymes states that the entire process is performed in accordance with current good manufacturing practices using food grade raw materials. Novozymes also states that the final enzyme preparation does not contain any major food allergens from the fermentation medium.

Novozymes states that the phosphoinositide phospholipase C 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

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[1] Novozymes states strain AEB1953 was derived from *B. licheniformis* DSM 9552.
[2] Novozymes states that the codon optimized phosphoinositide phospholipase C gene is a synthetic gene with a single amino acid residue difference compared to the wild-type phosphoinositide phospholipase C sequence from *Pseudomonas* sp. 62186.
[3] Novozymes states that the hybrid promoter carries promoter elements from *B. licheniformis*, *B. amyloliquefaciens*, and *B. thuringiensis*.

Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). Novozymes provides analytical data from three batches of phosphoinositide phospholipase C enzyme concentrate to demonstrate consistency with the specifications. Novozymes also confirms that a test for absence of any production organism in the final product is an established specification.

Novozymes proposes to use phosphoinositide phospholipase C enzyme preparation to hydrolyze phospholipids in the degumming of crude vegetable oils during the edible oil refining process. The maximum level of phosphoinositide phospholipase C enzyme preparation for the intended use corresponds to 1.4 mg TOS/kg of crude oil. Novozymes notes that the phosphoinositide phospholipase C enzyme preparation will be removed during refining process. If present in the final food, it will be diluted during refining. To estimate dietary exposure to phosphoinositide phospholipase C enzyme preparation, Novozymes assumes that the phosphoinositide phospholipase C enzyme preparation will be used at the maximum intended levels, and that all the enzyme preparation will remain in the final food. Based on these assumptions, Novozymes estimates a maximum dietary exposure of phosphoinositide phospholipase C enzyme preparation from all intended uses to be 0.002 mg TOS/kg body weight per day (mg TOS/kg bw/d) based on an average per capita consumption of vegetable oils and fats of 104 g per person per day.

Novozymes relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism. Additionally, Novozymes summarizes unpublished toxicological studies using the phosphoinositide phospholipase C enzyme liquid concentrate to corroborate safety of the intended uses. Novozymes states that the phosphoinositide phospholipase C enzyme is not mutagenic based on results from a bacterial reverse mutation assay, and on results from an *in vitro* micronucleus assay in cultured human lymphocytes. A 13-week oral toxicity study in rats using the phosphoinositide phospholipase C enzyme concentrate did not cause any treatment-related adverse effects up to the highest dose tested (equivalent to 505.8 mg TOS/kg bw/d). Based on the highest dose tested in the 13-week study and the estimated dietary exposure from the intended uses of the phosphoinositide phospholipase C enzyme preparation, Novozymes calculates a margin of safety to be 253,000. FDA notes the margin of safety is based on unpublished safety studies, and is corroborative with published information regarding enzyme preparations used in food processing.

Novozymes discusses potential food allergenicity of phosphoinositide phospholipase C enzyme. Novozymes states that naturally occurring food enzymes, if present in the final food, are unlikely to have allergenic potential because they are present in low concentrations and are susceptible to digestion in the gastrointestinal system. Additionally, Novozymes conducted a sequence homology search with a window of 80 amino acids from the peptide sequence of the phosphoinositide phospholipase C against known allergens stored in the FARRP allergen protein database and found no significant homology over 35% to known allergens. Novozymes did not find any significant homology between sequences of eight contiguous amino acids of phosphoinositide phospholipase C and known allergenic proteins. In addition, Novozymes reported that searches in the UNIPROT database did not identify any known toxins having homology

of greater than 20% to phosphoinositide phospholipase C peptide sequence. Novozymes 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 during manufacturing of enzyme-containing foods. Based on the totality of the information available, Novozymes concludes that it is unlikely that oral consumption of phosphoinositide phospholipase C enzyme will result in any allergenic responses.

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

Conclusions

Based on the information that Novozymes provided, as well as other information available to FDA, we have no questions at this time regarding Novozymes' conclusion that phosphoinositide phospholipase C enzyme preparation produced by *B. licheniformis* expressing a modified synthetic gene encoding a variant of the wild-type phosphoinositide phospholipase C from *Pseudomonas* sp. 62186 is GRAS under its intended conditions of use. This letter is not an affirmation that phosphoinositide phospholipase C enzyme preparation produced by *B. licheniformis* expressing a modified synthetic gene encoding a variant of the wild-type phosphoinositide phospholipase C from *Pseudomonas* sp. 62186 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 000728 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
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