



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

Re: GRAS Notice No. GRN 000689

Dear Ms. Oesterling:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000689. We received Novozymes North America, Inc. (Novozymes)'s GRAS notice on January 17, 2017, and filed it on March 3, 2017. We received an amendment containing additional safety information on June 23, 2017.

The subject of the notice is phospholipase C enzyme preparation produced by *Bacillus licheniformis* expressing a modified synthetic gene encoding a variant of the wild-type phospholipase C from *B. thuringiensis* (phospholipase C enzyme preparation) for use as an enzyme in the degumming of vegetable oils at a maximum level of 6.4 mg Total Organic Solids (TOS)/kg oil. The notice informs us of Novozymes' view that this use of 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 phospholipase C enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, phospholipase C is identified by the Enzyme Commission Number 3.1.4.3.¹ The accepted name is phospholipase C. The systematic name for this enzyme is phosphatidylcholine cholinephosphohydrolase. Phospholipase C hydrolyzes the major phospholipids present in oil, namely phosphatidylcholine and phosphatidylethanolamine, to their esters, phosphorylcholine and phosphorylethanolamine, respectively. The CAS Registry Number for phospholipase C is 9001-86-9. Novozymes states that the primary amino acid sequence of the expressed phospholipase C enzyme has been determined and it consists of 245

¹ EC No. 3.1.4.3 also includes lipophosphodiesterase I, lecithinase C, *Clostridium welchii* α -toxin, *Clostridium oedematiens* β - and γ -toxins, lipophosphodiesterase C, phosphatidase C, heat-labile hemolysin, and α -toxin; these enzymes differ in identity from the subject of GRN 000689.

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amino acids. Novozymes states that phospholipase C has a molecular weight of ~27 kDa. Novozymes confirmed the identity of the enzyme by mass spectrometry.

Novozymes states that the *B. licheniformis* production strain was constructed from the recipient strain *B. licheniformis* MaTa157.² Novozymes states that this recipient strain was previously modified at several chromosomal loci to inactivate genes encoding a number of proteases and genes 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 MaTa176 from MaTa157 by the targeted integration of an expression cassette carrying the modified synthetic gene encoding a variant of the wild-type phospholipase C gene³ from *B. thuringiensis*, a hybrid *Bacillus* promoter⁴, 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 enzyme production via Southern blot hybridization and free of any functional antibiotic resistance genes by genome sequence analysis.

Novozymes states that 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 the enzyme is secreted into the culture medium. The enzyme is recovered from the culture medium after an optional pretreatment step of pH adjustment and flocculation if required. This is followed by vacuum filtration or centrifugation of the supernatant containing the enzyme, which is then concentrated by ultrafiltration or evaporation. The concentrated enzyme solution is then filtered to ensure the removal of any production organisms. The enzyme solution is further concentrated by ultrafiltration or evaporation. The enzyme concentrate is stabilized by the addition of glycerol and formulated to an enzyme preparation with water, and preserved with 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 culture medium.

Novozymes states that the 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 Enzyme

² Novozymes states the MaTa157 was derived from *B. licheniformis* DSM9552, and has been deposited in Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany.

³ Novozymes states that the codon optimized phospholipase gene is a synthetic gene with a single amino acid residue difference compared to the wild-type phospholipase sequence from *B. thuringiensis*.

⁴ Novozymes states that the hybrid promoter carries elements from *B. licheniformis*, *B. amyloliquefaciens*, and *B. thuringiensis*.

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 phospholipase C enzyme concentrate to demonstrate consistency with the manufacturing specifications. Novozymes also confirms that a test for absence of any production organism in the final product is an established specification.

Novozymes intends to use phospholipase C enzyme preparation to hydrolyze phospholipids in the degumming of crude vegetable oils during edible oil refining process. The maximum level of phospholipase C enzyme preparation for the intended use corresponds to 6.4 mg TOS/kg of crude oil. Novozymes notes that the phospholipase C enzyme preparation will be diluted or removed during refining. To estimate dietary exposure to phospholipase C enzyme preparation, Novozymes assumes that the enzyme preparation will be used at the maximum intended levels, and that all of the enzyme preparation will remain in the final food. Based on these assumptions, Novozymes estimates a maximum dietary exposure of phospholipase C enzyme preparation from all intended uses to be 0.007 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.⁵

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 phospholipase C enzyme liquid concentrate to corroborate safety of the intended uses. Novozymes states that the 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 phospholipase C enzyme concentrate did not cause any treatment-related adverse effects up to the highest dose tested (equivalent to 714 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 phospholipase C enzyme preparation, Novozymes calculates a margin of safety to be 102000.⁶

Novozymes discusses potential food allergenicity of 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 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 phospholipase C and known allergenic proteins. In addition,

⁵ Novozymes assumes a mean bodyweight of 90 kg for males 20 years and older based on Centers for Disease Control's Anthropometric Reference Data for Children and Adults: United States, 2011–2014. We calculate a maximum dietary exposure of phospholipase C of 0.01 mg TOS/kg bw/d based on a mean bodyweight of 60 kg (http://www.fao.org/input/download/standards/6/cxg_003e.pdf).

⁶ Based on an estimated maximum dietary exposure of phospholipase C of 0.01 mg TOS/kg bw/d, we calculate a margin of safety of 71400.

Novozymes reported that the homology of phospholipase C peptide sequence to known toxins was low and random; this was assessed based on the <25% homology on the basis of the information present in the UNIPROT database. 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 of enzyme-containing foods during manufacturing. Based on the totality of the information available, Novozymes concludes that it is unlikely that oral consumption of phospholipase C enzyme will result in any allergenic responses.

Based on the data and information summarized above, Novozymes concludes that 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 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 phospholipase C enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing 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 phospholipase C enzyme preparation produced by *B. licheniformis* expressing the modified synthetic gene encoding a variant of the wild-type phospholipase C from *B. thuringiensis* (phospholipase C enzyme preparation) is GRAS under its intended conditions of use. This letter is not an affirmation that phospholipase C 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 000689 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams

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Dennis M. Keefe, Ph.D.

Director

Office of Food Additive Safety

Center for Food Safety

and Applied Nutrition

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