U.S. Food and Drug Administration
Department of Health and Human Services
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION

CFSAN/Office of Food Additive Safety
June 23, 2004

Agency Response Letter
GRAS Notice No. GRN 000142

Lori Gregg
Novozymes North America, Inc.
77 Perry Chapel Church Road
P.O. Box 576
Franklinton, NC 27525

Re: GRAS Notice No. GRN 000142

Dear Ms. Gregg:

The Food and Drug Administration (FDA) is responding to the notice, dated December 18, 2003, 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 December 24, 2003, filed it on December 29, 2003, and designated it as GRAS Notice No. GRN 000142. We received additional information regarding this notice on May 7, 2004.

The subject of the notice is phospholipase A1 (PLA1) enzyme preparation from *Aspergillus oryzae* expressing a gene encoding a PLA1 from *Fusarium venenatum*. For the purposes of this letter, this notified substance will be referred to as PLA1 enzyme preparation. The notice informs FDA of the view of Novozymes North America Inc. (Novozymes) that PLA1 enzyme preparation is GRAS, through scientific procedures, for use as an enzyme in cheese manufacturing at levels up to 5 grams of enzyme preparation per kilogram of milkfat. Novozymes estimates that this use of PLA1 enzyme preparation as a direct food ingredient would result in an estimated daily intake (EDI) of 0.7 milligrams per person per day of the total organic solids present in the PLA1 enzyme preparation.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component, which catalyzes the chemical reaction that is responsible for its technical effect, as well as substances used as stabilizers, preservatives or diluents. Enzyme preparations may also contain constituents derived from the source organism and constituents derived from the manufacturing process, e.g., components of the fermentation media or the residues of processing aids. Novozymes' notice provides information about each of these components of the PLA1 enzyme preparation.

Novozymes describes generally available information about the identity and technical effect of PLA1, as well as specific information about the identity and activity of the PLA1 enzyme preparation from *A. oryzae* that is the subject of GRN 000142. Phospholipases are classified as hydrolases and PLA1 specifically hydrolyzes the *sn*-1 ester bond of diacylphospholipids to form 2-acyl-1-lysophospholipid and a free fatty acid. Phospholipase A1 is also known as phosphatidylcholine 1-acylhydrolase and is identified by the following classification numbers: Enzyme Commission number 3.1.1.32 and Chemical Abstracts Service Registry Number 9043-29-2. Phospholipase A1 enzyme preparation is intended for use as an enzyme in cheesemaking to produce modified phospholipids in cheesemilk with improved emulsification properties.

Novozymes describes published information about the presence of PLA1 as a naturally-occurring component of animal and plant tissues. Novozymes notes that PLA1 has been found in cells and tissues of various organisms, including animal pancreas and small intestines. Novozymes notes that animal-derived pancreatic lipases have been affirmed as GRAS substances (21 CFR 184.1415). Novozymes concludes that PLA1, because it is found in cells and tissues that are consumed by man, should be digested like any other protein in food.

Novozymes describes the host microorganism, *A. oryzae* strain BECh2, that is used in the construction of the PLA1 production strain PFJo142 as a derivative of a well-known industrial production strain of *A. oryzae* (Ahlburg) Cohn. Novozymes obtained the strain, which is designated as IFO 4177(A 1560), from the Institute for Fermentation, Osaka, Japan. Novozymes considers *A. oryzae* to be nontoxigenic and nonpathogenic based on published criteria for the assessment of the safe use of microorganisms used in the manufacture of food ingredients.

Novozymes notes that the donor organism for the PLA1 gene, *F. venenatum*, is a saprophytic fungus found in soil, and is known to occur on several food crops (hops, potato, spinach, and corn). Novozymes also notes that *F. venenatum* is not considered to be a human pathogen. Novozymes acknowledges that *F. venenatum* is known to produce secondary metabolites such as trichothecences, culmorins, enniatins and fusarins. Batch analyses of the enzyme for secondary metabolites were provided and none were detected at specified detection limits. Novozymes also confirmed that no unintended *F. venenatum* coding sequences, including those related to secondary metabolite production, were introduced into the *A. oryzae* production strain.

Novozymes provides information about the components of the phospholipase A1 expression plasmid pPFJo142 that was introduced into the host *A. oryzae* strain BECh2 by plasmid transformation. Novozymes cites published scientific articles to support its view that all of the DNA sequences that were used in the construction of the production strain are well-known, well-characterized, and commonly used.

Novozymes assessed the identity and stability of the introduced DNA using Southern hybridization and concluded that the DNA is integrated into the *A. oryzae* chromosome as expected and is not prone to genetic transfer to other organisms. The resulting pPFJo142/BECh2 *A. oryzae* production strain meets the criteria for Good Industrial Large-Scale Practice published in the Organization for Economic Co-operation and Development's 1992 report entitled "Safety Considerations for Biotechnology."

Novozymes describes the manufacturing process for PLA1 enzyme preparation, which is

produced by a submerged, fed-batch pure culture fermentation of the *A. oryzae* production strain. Each fermentation is initiated with a lyophilized stock culture of the production organism. The enzyme is secreted into the fermentation broth. When the fermentation is completed, the broth is separated from the cells using vacuum drum filtration. The enzyme is concentrated by ultrafiltration and/or evaporation, followed by filtration. The resulting enzyme is preserved and stabilized. The stabilized concentrate is blended with water, glycerol and sucrose, and then preserved with sodium benzoate and potassium sorbate. Novozymes follows standard industry practices and uses a quality management system that complies with the requirements of ISO 9001. Novozymes cites several published sources to support the conclusion that the production and control methods used are generally accepted methods that are commonly used for the production of microbial enzyme preparations.

Novozymes states that the enzyme preparation complies with the general and additional requirements for enzyme preparations set forth in the Food Chemicals Codex (Fourth edition, Third supplement, 2001) and the specifications established by the Joint Food and Agricultural Organization/World Health Organization's (FAO/WHO) Expert Committee on Food Additives (General Specifications and Considerations for Enzyme Preparations Used in Food Processing, Compendium of Food Additive Specifications, FAO Food and Nutrition Paper 52, Addendum 9, 2001).

Novozymes cites publications in the toxicological and regulatory literature that support the conclusion that a wide variety of enzymes are used in food processing and that enzyme proteins do not generally raise safety concerns. Novozymes also notes that very few toxic agents have enzymatic properties. Novozymes states that phospholipases, in general, break down substrates into smaller units that do not have toxic properties and that are readily metabolized by the human body.

In the notice, Novozymes summarizes unpublished toxicological studies performed on the PLA1 enzyme preparation produced without the stabilization and standardization steps. These studies include two *in vitro* studies consisting of an Ames mutagenicity assay and a neutral red uptake in L929 monolayer culture cytotoxicity assay. Novozymes concludes that these studies showed no treatment related induction of gene mutation in bacteria or cytoxicity.

Novozymes provided information regarding the potential allergenicity of PLA1. Novozymes relates that there are no known cases of allergic responses to phospholipases in foods, reactions to food enzymes in general are very rare, and the level of exposure to this enzyme is very low. Based on these statements, Novozymes concludes that the risk of allergy due to ingestion of their PLA1 is negligible.

Based on the information provided by Novozymes, as well as other information available to FDA, the agency has no questions at this time regarding Novozymes' conclusion that PLA1 enzyme preparation from *A. oryzae* expressing the gene encoding a phospholipase A1 from *F. venenatum* 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 PLA1 enzyme preparation. As always, it is the continuing responsibility of Novozymes to ensure that food 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, as well as a

copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,
/s/
Laura M. Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

Food Ingredients and Packaging | Summary of all GRAS Notices

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