CFSAN/Office of Food Additive Safety
March 16, 2005

Agency Response Letter
GRAS Notice No. GRN 000158

Lori Gregg
Novozymes North America, Inc.
77 Perry Chapel Church Road
Street Address
Franklinton, NC 27525

Re: GRAS Notice No. GRN 000158

Dear Ms. Gregg:

The Food and Drug Administration (FDA) is responding to the notice, dated October 5, 2004, that you submitted on behalf of Novozymes North America, Inc. (Novozymes) 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 October 8, 2004, filed it on October 13, 2004, and designated it as GRAS Notice No. GRN 000158.

The subject of the notice is a lipase preparation produced by *Aspergillus niger* expressing a gene encoding a lipase from *Candida antarctica* (referred to as "lipase preparation" for purposes of this letter). The notice informs FDA of the view of Novozymes that the lipase preparation is GRAS, through scientific procedures, for use as a processing aid in the production of triglyceride products. The lipase preparation is used in food at the minimum levels necessary to achieve the intended technical effect.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component, which catalyzes the chemical reaction that is responsible for the technical effect of the enzyme preparation, as well as stabilizers, preservatives or diluents. Enzyme preparations may also contain constituents that are derived from the source organism, and constituents that are 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 lipase preparation from *A. niger*.

Novozymes describes the catalytic activity of the lipase as a triacylglycerol hydrolase and carboxylesterase, with a positional specificity depending upon the reactants. The systematic name of the enzyme is triacylglycerol acylhydrolase; the Enzyme Commission number is E.C.

3.1.1.3; and, the Chemical Abstract Services Registry number is 9001-62-1.

In assessing the safety of the enzyme itself, Novozymes states that enzymes generally do not raise safety concerns. Novozymes also discusses the history of safe use of lipases in food processing.(1)

Microbial lipases have been used in food production since 1952. Animal lipase is affirmed as GRAS (21 CFR 184.1415) based on its common use in food prior to 1958. Lipase enzyme preparation from *Rhizopus niveus* is affirmed as GRAS based on scientific procedures (21 CFR 184.1420). Esterase-lipase from *Mucor miehei* (now *Rhizomucor miehei*) is approved as a food additive (21 CFR 173.140). FDA filed a GRAS affirmation petition (petition no. GRP 7G0323) for immobilized esterase-lipase enzyme preparation derived from *M. miehei* in 1989 (54 FR 9565). In addition, Novozymes cites previous GRAS notices (GRN Nos. 000043 and 000075) that include lipase preparations from genetically engineered strains of *A. oryzae*.

In assessing the safety of the production organism, *A. niger*, Novozymes cites scientific review articles in support of its view that the safety of the production organism is the prime consideration in assessing the safety of an enzyme preparation intended for food use. Novozymes states that *A. niger* has a history of safe use in food and is considered to be safe for the production of enzymes or ingredients for use in food as evidenced by scientific publications and by several expert groups. Novozymes also cites various FDA regulations and GRAS notices for enzymes and ingredients derived from *A. niger*.

In assessing the safety of the donor strain, *C. antarctica*, Novozymes states that *C. antarctica* is not regarded as pathogenic or toxigenic based on scientific articles and criteria outlined in the National Institute of Health Guidelines for Research Involving Recombinant DNA Molecules, Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, April 2002.

In assessing the safety evaluation of food components derived from genetically engineered organisms, Novozymes states that the genetic modifications of the production strain are well characterized and specific and do not encode and express any known harmful or toxic substances. Novozymes also assessed the identity and stability of the integrated DNA using Southern blot hybridization and concluded that the DNA is integrated into the *A. niger* chromosome and is mitotically stable.

Novozymes describes the manufacturing process for the lipase preparation, which is manufactured using a submerged fed-batch pure culture fermentation of the bioengineered strain of *A. niger*. Each batch of the fermentation process is initiated with a lyophilized stock culture of the production organism and is controlled for identity, absence of foreign microorganisms, and enzyme-generating ability before use. The enzyme is recovered from the culture broth by pH adjustment and multiple filtration steps. Sodium benzoate and potassium sorbate are added for preservation and stabilization. The product is concentrated by ultrafiltration and/or evaporation, and then standardized with sorbitol and glycerol according to the product specifications.

Novozymes states that the lipase preparation conforms to the general and additional requirements for enzyme preparations as outlined in the monograph on enzyme preparations in the Food Chemicals Codex, 5th edition, 2003, 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).

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 the lipase preparation produced by *A. niger* expressing a gene encoding a lipase from *C. antarctica* 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 this lipase 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

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(1)Novozymes states that, in most cases, the enzyme preparation will be loaded to, and immobilized on, a carrier in the reactor prior to the enzyme catalyzed reaction. FDA notes that it is the responsibility of companies using the enzyme preparation in its immobilized form 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.