CFSAN/Office of Food Additive Safety
March 12, 2007

Agency Response Letter
GRAS Notice No. GRN 000214

Gary L. Yingling
Kirkpatrick & Lockhart Nicholson Graham LLP
1601 K Streeet
Washington, DC 20006-1600

Re: GRAS Notice No. GRN 000214

Dear Mr. Yingling:

The Food and Drug Administration (FDA) is responding to the notice, dated October 3, 2006, that you submitted on behalf of DSM Food Specialties (DSM) 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 5, 2006, filed it on October 11, 2006, and designated it as GRAS Notice No. GRN 000214.

The subject of the notice is asparaginase enzyme preparation from *Aspergillus niger* expressing a gene encoding an asparaginase from *A. niger* (*A. niger* asparaginase enzyme preparation). The notice informs FDA of the view of DSM that *A. niger* asparaginase enzyme preparation is GRAS, through scientific procedures, for use in reducing asparagine levels[(1)] in L-asparagine and carbohydrate-containing foods that are heated above 120 degrees Celsius (°C), specifically in (1) bread (such as tin bread, buns, rolls, French sticks or batards, variety breads like multigrain types of bread, raisin bread, biscuits, crackers); (2) other cereal-based products (cakes, Swiss rolls, Dutch honey cake, breakfast cereals); (3) potato-based products (French fries, potato chips); and (4) reaction flavors.

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 production organism and constituents derived from the manufacturing process, e.g., components of the fermentation media or the residues of processing aids. DSM's notice provides information about the enzyme component, the production microorganism, and the manufacturing process for *A. niger* asparaginase enzyme preparation. DSM provides general as well as specific information about the identity and technical effect of asparaginases as well as specific information about the identity and activity of the *A. niger* asparaginase enzyme preparation that is the subject of GRN 000214.

The L-asparaginase from *A. niger* is referred to as L-asparagine amidohydrolase in IUB nomenclature, with an EC number of 3.5.1.1 and a Chemical Abstracts Services Registration Number of 9015-68-3.

In assessing the safety of the production organism, DSM relies on 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. DSM states that the host organism has a long history of safe industrial use and concludes that an enzyme preparation derived from a recombinant microorganism will be safe if the host microorganism is nontoxigenic and nonpathogenic; the genetic information that is introduced into the host microorganism is well characterized; and the added DNA does not encode and express any known harmful or toxic substances.

DSM states that the production strain *A. niger* has been used in the construction of a variety of enzymes, including an enzyme that was the subject of GRN 000183. DSM further states that the gene encoding the asparaginase was derived from *A. niger* and that both donor and recipient belong to the same *A. niger* lineage. DSM provides the amino acid and nucleotide sequences for DSM's asparaginase and states that the *A. niger* asparaginase is fully functional and has significant sequence homology to other fungal asparaginases. DSM further states that the production organism does not contain antibiotic resistance genes, does not produce any known toxins, and is removed from the final product during the manufacturing process.

DSM describes the manufacturing process of the asparaginase enzyme preparation as submerged, aerobic, fed-batch pure culture. DSM uses physical and chemical control measures and performs microbiological analyses to confirm the identity of the production strain and to avoid contamination by other microorganisms.

DSM describes the recovery of *A. niger* asparaginase enzyme preparation. The fermentation is stopped by the addition of sodium benzoate; the enzyme is subsequently removed and concentrated using multiple filtration steps, then prepared as either a liquid or spray-dried enzyme preparation. The preparations comply with purity criteria for enzyme preparations in the Food Chemicals Codex (5th Edition) and with General Specifications for Enzyme Preparations used in Food Processing as proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the Compendium of Food Additive Specifications.

DSM states that for all proposed applications, the action of the *A. niger* asparaginase enzyme preparation takes place before heating the food. The enzyme is inactivated at temperatures above 70°C. No enzyme activity is expected to remain in the finished product because all intended applications involve heating above 120°C. DSM verified this expectation and measured the level of asparaginase in baked bread. DSM states that these experiments showed that no asparaginase activity is present in the finished product. Based on the use levels and specifications, DSM calculates the amount of total organic solidsy in the final food products to range between 0.2 to 562 milligrams per kilogram food.

DSM includes the results of toxicity studies. DSM concludes that the results of the toxicity and mutagenicity tests demonstrate the safety of DSM's asparaginase preparation and support the safe use of enzyme preparations produced by the production strain. In the notice, DSM states its intention to use the *A. niger* asparaginase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal

Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Based on the information provided by DSM, as well as other information available to FDA, the agency has no questions at this time regarding DSM's conclusion that *A. niger* asparaginase enzyme preparation 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 *A. niger* asparaginase enzyme preparation. As always, it is the continuing responsibility of DSM 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 responding to GRN 000214 as well as a copy of the information in this notice that conforms to the information in the proposed GRAS exemption claim (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,

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

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(1)DSM describes the intended effect of the asparaginase as the conversion of asparagine to aspartic acid to reduce the formation of acrylamide in specified products. FDA neither evaluated the efficacy of such treatments nor determined whether acrylamide levels detected by DSM in untreated foods represent a significant health concern.