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
May 11, 2006

Agency Response Letter
GRAS Notice No. GRN 000183

Mr. Gary Yingling
Kirkpatrick & Lockhart Nicholson Graham LLP
1601 K Street, N.W.
Washington, DC 20006-1600

Re: GRAS Notice No. GRN 000183

Dear Mr. Yingling:

The Food and Drug Administration (FDA) is responding to the notice, dated October 26, 2005, 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 28, 2005, filed it on November 2, 2005, and designated it as GRAS Notice No. GRN 000183.

The subject of the notice is phospholipase A2 enzyme preparation from *Aspergillus niger* expressing a gene encoding porcine phospholipase A2 (PLA2 (porcine) enzyme preparation from *A. niger*). The notice informs FDA of the view of DSM that PLA2 (porcine) enzyme preparation from *A. niger* is GRAS, through scientific procedures, for use as an enzyme for the hydrolysis of phospholipids in baked goods and egg yolk-based sauces and dressings at levels not to exceed good manufacturing practices.

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 PLA2 (porcine) enzyme preparation from *A. niger*.

DSM provides the following information: PLA2 is a member of the class of carboxylic ester hydrolases and catalyzes the hydrolysis of fatty acids at the sn2 position of phospholipids, resulting in formation of lysophospholipids. PLA2, its substrates (e.g., lecithin), and

endproducts of hydrolyis (e.g., lysolecithin) are normal constituents of food including wheat flour. PLA2 is a digestive enzyme in the pancreatic juice of mammals including humans. Commercial PLA2 enzymes are obtained from mammalian sources such as porcine pancreas and the bacterium *Streptomyces violaceoruber* (the subject of GRAS notice GRN 000145). PLA2 from porcine pancreas, from which DSM's enzyme was derived, has many years of use in foods for the hydrolysis of egg yolk phospholipids. Enzyme-modified lecithin or lysolecithin was affirmed as GRAS by FDA in 1996 (21 CFR 184.1063). Lysophospholipids act as surface active agents with emulsifying properties. The systematic name of PLA2 is phosphatidylcholine-2-acylhydrolase; alternative names are lecithinase A, phosphatidase, and phosphatidolipase. It is identified by Enzyme Commission (E.C.) No. 3.1.1.4 and Chemical Abstracts Service Registry Number (CAS Reg. No.) 9001-84-7.

In assessing the safety of the production organism, *A. niger*, 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 included a publication in its notice that discussed the safety of the host strain. DSM provided information, supporting that the production organism *A. niger* is not a human pathogen, has a long industrial use and that there is no evidence of toxin production by industrial *A. niger*. DSM also notes the GRAS status of lipase, lactase, glucose oxidase, carbohydrase, catalase, glucose oxidase, pectinase, protease, and pectin lyase enzyme preparations from *A. niger* affirmed by FDA and notes that enzymes from *A. niger* are accepted by other countries.

DSM states that the mRNA coding for PLA2 was obtained from porcine pancreatic tissues and used as a template for *in vitro* production of cDNA. DSM describes the components of the expression and selectable marker plasmids it transformed into its *A. niger* host strain. The transformed DNA contained only genes from *A. niger*, synthetic DNA, and cDNA synthesized from porcine mRNA for PLA2.

DSM describes its PLA2 (porcine) enzyme preparation from *A. niger* as produced by a controlled submerged, aerobic, fed batch fermentation of a selected pure culture of the *A. niger* production strain. Aseptically collected samples are taken to monitor cultures for growth of the organism and enzyme production. After appropriate growth and enzyme formation, the addition of sodium benzoate to the culture kills the production organism.

DSM describes the recovery of the PLA2 (porcine) enzyme preparation from *A. niger* by pH adjustment and multiple filtration steps, including ultrafiltration to concentrate PLA2. DSM uses this product, called the "UF concentrate," to produce a spray-dried granulated product standardized with flour and a liquid product purified by column chromatography with SP-Sepharose FF resin, diluted with water, with sodium benzoate added as a preservative. DSM states that the enzyme preparation is manufactured according to good manufacturing practices and that materials used in fermentation and recovery are food grade, safe, and suitable for their intended uses. Table 1 displays information about the dry and liquid enzyme preparations. The enzyme preparation complies with the general and additional requirements for enzyme preparations in the Food Chemical Codex, 5th edition, (2003) and conforms to the general specifications for enzyme preparations used in food processing provided by Joint Expert Committee on Food Additives of the FAO/WHO (JECFA, 2001). Specifications include limits on lead (under 5 mg/kg) and microbial limits.

Table 1. Properties of Dry and Liquid PLA2 (porcine) Enzyme Preparations from *A. niger*

Preparation	Description	Activity range (EYU[(1)])	Activity per mg Total Organic Solids (EYU per milligram TOS)
dry	off white granules (63-225 micrometers particle size)	10,000-25,000 (per gram)	25.3
liquid	pale yellow liquid	10,000-11,500 (per ml)	759

DSM provides estimates of the concentrations of PLA2 (porcine) enzyme preparation from *A. niger* in baked goods and in egg-yolk based sauces and dressings in Table 2.

Table 2. Use Levels of PLA2 (porcine) Enzyme Preparation from *A. niger* in Baked Goods and Egg-based Sauces and Dressings

Use	Activity level added (EYU/kg)	Level added (mg TOS/kg)
bread	2,500-10,000 (in flour)	35-360
fine bakery wares (e.g., pound cake, muffins)	10,000-29,000 (in egg yolk)	
egg-based sauces/dressings	10,000-20,000 (in egg yolk)	0.4- 0.7

DSM summarizes unpublished toxicological studies performed on PLA2 (porcine) enzyme preparation from *A. niger*, including acute oral toxicity studies in rats either given enriched enzyme preparation or EDTA-inactivated enzyme preparation and subacute (14-day) and subchronic (90-day) oral toxicity assay in rats given the UF concentrate by gavage. DSM also presents results from mutagenicity tests (*in vitro* reverse mutation assay with and without metabolic activation from rat liver- derived S9, an *in vitro* chromosome aberration test, and an in vivo mouse micronucleus test). DSM concludes that the enzyme preparation is nontoxic and nonmutagenic.

DSM discusses the possibility that enzymes, like other proteins, may elicit allergenic responses, but that few dietary proteins are food allergens. DSM notes that only small quantities of enzymes are used in food production, that enzymes like phospholipase A2 are similar to endogenous enzymes in humans and other forms of life, and that only isolated cases reports of allergies to enzymes have been reported, most attributed to occupational exposures rather than sensitization to food enzymes.

Standards of Identity

In the notice, DSM states its intention to use PLA2 (porcine) enzyme preparation from *A. niger* in several food categories, including foods for which standards of identity exist (e.g., mayonnaise, salad dressing), 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.>/p>

Conclusions

Conclusions 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 PLA2 (porcine) enzyme preparation from *A. niger* 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 PLA2 (porcine) enzyme preparation from *A. niger*. 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 000183, 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)One EYU (egg yolk unit) is defined as the amount of enzyme producing one micromole (μmole) of free fatty acid per minute under the conditions described for an egg yolk assay in the Food Chemicals Codex, 5th Edition.