May 1, 2013

Dr. Alberto Davidovich
DSM Nutritional Products, Inc.
45 Waterview Boulevard
Parsippany, New Jersey 07054

Re: GRAS Notice No. AGRN 000-014

Dear Dr. Davidovich:

The Food and Drug Administration (FDA) is responding to the notice, dated October 12, 2012 that you submitted on behalf of DSM Nutritional Products, Inc. ("DSM") under FDA's Center for Veterinary Medicine (CVM) Pilot Program for substances generally recognized as safe (GRAS) added to food for animals (See 75 FR 31800; June 4, 2010). FDA's Center for Veterinary Medicine received the notice on October 18, 2012, filed it on November 14, 2012 and designated it as GRAS Notice No. AGRN 000-014.

The subject of your notice is a 6-phytase enzyme produced by an *Aspergillus oryzae* strain expressing a synthetic gene coding for a 6-phytase from *Citrobacter braakii* ("phytase"). The notice informs FDA of the view of DSM that phytase is GRAS, through scientific procedures, to increase the digestibility of phytin-bound phosphorous or to increase phosphorous availability from phytate in poultry diets when fed at the rate of 250-4000 FYT/kg feed (phytase enzyme units). The intended target animal species includes only the following food-producing poultry: turkey, broiler chickens, and egg laying hens.

DSM provides information about the identity, specifications, method of manufacture, and conditions of use of phytase (CAS number 9001-89-2).

DSM provides information about the genetic engineering of the source organism, *Aspergillus oryzae*, and the manufacture. The notified substance is produced through a fermentation which is tightly controlled. Following fermentation, the biomass is purified and excipient materials are added. DSM indicates all materials added to the final formulations are of suitable quality for feed and in compliance with FDA regulations. Raw ingredients and final product specifications were also included. DSM includes analytical methodology to determine phytase activity of the final ingredient as well as the ingredient in pre-mixes and final feed. DSM also provides stability, packaging, and homogeneity information for the notified substance.

DSM provides finished product specifications: phytase activity (>50,000 FYT/g, >20,000 FYT/g, >10,000 FYT/g for the three formulations, respectively); heavy metals (not more than 30 ppm), lead (not more than 5 ppm), arsenic (not more than 3 ppm), total viable count (not more than 30/g), *E. coli* (not detected in 25g), and *Salmonella sp.* (not detected in 25g)

DSM provides information on an *Aspergillus oryzae* strain that was bioengineered to produce a phytase enzyme with the same amino acid sequence as the phytase produced by *Citrobacter*

braakii ATCC 51113. DSM provides a description of the safety of the host and gene donor organisms, *in silico* synthesis of the genes for the phytase enzyme, construction of the expression vectors, and methods used to introduce the circular expression vectors into the genome of the host organism. Two synthetic genes with different codon profiles, but encoding for the same amino acid sequence, are expressed in the source strain. A publication cited in the notification states that the two genes shared 85% identity at the DNA level and 70% and 73% identity to the wild type *Citrobacter* phytase gene. The notice contains information that demonstrates 100% similarity at the protein level between the deduced amino acid sequences for the two expression products and the phytase protein produced by *C. braakii* ATCC 51113. The protein expressed from the two synthetic genes in the source organism was verified by mass spectroscopy to be 100% identical to the *C. braakii* ATCC 51113 phytase. Based on information available in the literature, *A. oryzae* has a history of safe use in fermentation processes.

DSM provides published and unpublished information to support the intended use of the phytase to increase phosphorous availability when added at the rate of 250-4000 FYT/kg feed. Nine unpublished study reports were included to support functionality: three studies each in broiler chickens, layer chickens, and turkey. A published paper by Aureli et al. (2011) also supports the conditions of use.

To address human food safety, the notice states that the primary consideration is the safety of the production organism. DSM also states that the final product meets purity criteria for enzymes set by the Food Chemicals Codex (FCC) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). DSM references to toxicology tests discussed to address human food safety. Additionally, DSM states that the highest expected exposure of the target animals is expected to be low.

To address target animal safety, DSM summarized data that established safety of the production organism, summarized published *in vitro* and *in vivo* toxicology studies, and summarized published and unpublished tolerance studies in poultry that were fed up to 10 times the recommended dose measured in phytase units. Pivotal articles cited to support safety of the phytase for its intended use were Lichtenberg, et al. (2011) and Aureli, et al. (2011).

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 phytase 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 phytase. 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.

The Association of American Feed Control Officials (AAFCO) publishes a list of names and definitions for accepted feed ingredients. FDA recognizes these names as being the "common and usual" names for feed ingredients. FDA recognizes the name "phytase" as the common and usual name for 6-phytase produced by an *Aspergillus oryzae* strain expressing a synthetic gene coding for a 6-phytase from *Citrobacter braakii* included in animal food.

In addition, in our review of DSM's notice for phytase, FDA did not review whether food containing phytase would violate section 301(ll) of the Food, Drug, and Cosmetic Act (FDCA) [21 U.S.C. 331(ll)], or whether any of the exemptions in section 301(ll) apply to foods containing phytase. Section 301(ll) of the FDCA prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FDCA, 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. *See* section 301(ll) of the FDCA.

In accordance with the proposed 21 CFR 570.36(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 the proposed GRAS exemption claim (21 CFR 570.36(c)(1)), is available for public review and copying on the Center for Veterinary Medicine's internet website (http://www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/GenerallyRecognizedasSafeGRASNotifications/ucm243845.htm).

If you have any questions about this letter, please contact Dr. Andrea Krause at 240-276-9768 or by email at andrea.krause@fda.hhs.gov. Please reference AGRN-000014 in any future correspondence regarding this submission.

Sincerely,

/s/

Daniel G. McChesney, Ph.D.
Director
Office of Surveillance and Compliance
Center for Veterinary Medicine

References

Aureli, R, Umar Faruk, R, Cechova, I, Pedersen, PB, Elvig-Joergensen, WG, Fru, F, and J Broz. 2011. The Efficacy of a Novel Microbial 6-Phytase Expressed in Aspergillus oryzae on the Performance and Phosphorus Utilization in Broiler Chickens. International Journal of Poultry Science 10: 160-168.

Lichtenberg, J, Pedersen, PB, Elvig-Jorgensen, SG, Skov, LK, Olsem, CL, and LV Glistoe. 2011. Toxicological Studies On A Novel Phytase Expressed From Synthetic Genes in Aspergillus oryzae. Regulatory Toxicology and Pharmacology 60: 401-410.