November 2, 2023

Mr. Jonathan McDonough
Senior Regulatory Affairs Specialist
BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121

Re: Animal Generally Recognized as Safe Notice No. 61 – Phytase from a *Pseudomonas fluorescens* strain expressing an altered *app*A 6-phytase gene from *Escherichia coli* strain K12

Dear Mr. McDonough,

The Food and Drug Administration's (FDA or the agency) Center for Veterinary Medicine (CVM or we) refers to a generally recognized as safe (GRAS) notice, dated April 28, 2022 and received December 14, 2022, submitted by BASF Enzymes LLC (BASF or the notifier). The subject of the submission is phytase from a *Pseudomonas fluorescens* (*P. fluorescens*) strain expressing an altered *app*A 6-phytase gene from *Escherichia coli* strain K12 to be used to increase the availability of phytin-bound phosphorus in swine diets at a recommended supplementation level of a minimum of 500 U/kg to a maximum of 2000 U/kg in complete feed. The submission informs us of the notifier's conclusion that the subject of the submission is GRAS through scientific procedures. In an amendment dated January 31, 2023, the notifier submitted additional information on the utility and other information incorporated by reference from animal GRAS notice (AGRN) 55. Following an initial evaluation, you were notified in a letter dated February 10, 2023 that the GRAS notice was acceptable for filing, and the notice was designated as AGRN 61. We have completed our evaluation of AGRN 61.

To address the chemistry, manufacturing and controls of the notified substance, BASF refers to AGRN 55 for information regarding the identity, method of manufacture and specifications of the notified substance and market formulations containing the notified substance, referred to here as CIBENZA® PHYTAVERSE® L10 and CIBENZA® PHYTAVERSE® G10. One phytase unit (U) is defined as the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the reaction conditions specified by the International Standard procedure (ISO 30024:2009(E): Animal feeding stuffs – determination of phytase activity). The phytase enzyme is manufactured using a fed-batch fermentation process followed by recovery and formulation. The manufacturing is performed according to current Good Manufacturing Practices for animal foods and the OECD's criteria for Good Industrial Large-Scale Practice with appropriate quality controls procedures based on Hazard Analysis and Critical Control Points and risk mitigation principles. The raw materials used are standard ingredients used in the animal food/enzyme industry. The raw materials are either food grade and GRAS where available, or high-quality chemical or pharmaceutical grades. The notifier performed analytical testing for enzyme activity and other specifications. The notifier provides phytase enzyme product specification with test method and acceptance criteria: L10: Appearance (amber to brown liquid), pH 5.0-5.2, Specific Gravity (g/ml) 1.05-1.20, Sediment (% v/v) ≤ 0.5, Total Plate Count, Aerobic (CFU/g) ≤1000; G10: Appearance

(white to beige granules), Bulk Density-untapped (g/cm^3) ≥ 0.50, Particle Size (mesh) < 2% on 20 Mesh < 10% thru 140 mesh, Loss on Drying (%) ≤ 12, Total Plate Count, Aerobic (colony forming units CFU/g) ≤ 1000; and L10 and G10: Activity (U/g) NLT 10,000, Lead (mg/kg) ≤ 0.5, Total Coliform (MPN/g) ≤ 30, *E. coli* (/25g) Absent, *Salmonella* (/25g) Absent, Production Organism (CFU/g) Absent, Antibiotic Activity (Zone of Inhibition) Absent, Yeast and Mold (CFU/g) < 20, Mycotoxin – Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2, NMT 1.0 ppb each, Fumonisin B1, Fumonisin B2, Fumonisin B3, NMT 0.1 ppm each, Ochratoxin A NMT 2.0 ppb, Deoxynivalenol NMT 0.6 ppm, Acetyldeoxynivalenol NMT 0.8 ppm, Fusarenon X NMT 0.4 ppm, Nivalenol NMT 0.6 ppm, T-2 Toxin NMT 0.2 ppm, HT-2 Toxin NMT 0.2 ppm, Neosolaniol NMT 0.4 ppm, Diacetoxyscirpenol NMT 0.4 ppm, Zearalenone NMT 43.1 ppb, Sterigmatocystin NMT 200 ppb, PCB < 1 ppt, Dioxin< 1 ppt. Batch data provided for L10 and G10 phytase products demonstrated that the level of Arsenic is in the 0.030 mg/kg to < 0.25 mg/kg range, Cadmium is in the < 0.025 mg/kg to < 0.05 mg/kg range, and Mercury is in the < 0.025 mg/kg to < 0.066 mg/kg range. The notifier provided some stability and homogeneity information for notified phytase enzymes in market formulations (L10 and G10), premix, and feed (mash and pelleted). The market formulations are composed of the notified phytase enzyme and other ingredients that are suitable for use in animal food.

To address the utility of the notified substance and its intended use to increase the availability of phytin-bound phosphorus when included in complete diets for swine, the notifier includes a publication (Ren, et al., 2020) reporting two experimental studies conducted in swine evaluating the functionality of the notified substance. The study described as 'experiment one' which was conducted in swine fed corn soybean meal-based diets with reduced levels of phosphorus and inclusion of the notified phytase at 250 or 500 enzymatic activity units per kilogram of diet was considered pivotal in evaluating the intended use of the notified substance. Bone ash percentage was considered the pivotal parameter to reach a conclusion on the notified substance's functionality. A significant increase in bone ash percentage observed in experiment one when the notified substance was added at 500 enzymatic activity units per kilogram feed provides pivotal evidence to support the notifier's conclusion about the intended use of the notified substance.

To address pre-fermentation manufacturing processes, the notifier includes a description of genetic modifications that were performed during development of the genetically engineered strain, *P. fluorescens* BD50104, which will be used as the source organism to produce the notified substance. The notifier also addresses genetic stability, plasmid mobilization, potential new open reading frames, and absence of the antibiotic resistance markers that were used in the genetic engineering process.

To address the target animal safety of the notified substance, the notifier includes the following information to support the target animal safety conclusion of the intended use of the notified substance in swine feed: 1) Safety of the phytase enzyme; 2) Safety of the production organism: *Pseudomonas fluorescens* strain BD50104; 3) Safety of the donor organism: *Escherichia coli* K12; 4) Safety of the inserted genetic material; and 5) Safety studies included in the publication by Krygier et al., 2014, 2015.

Safety studies included a bacterial reverse mutation assay (Ames assay), chromosomal aberrations in cultured human peripheral blood lymphocytes assay, mouse micronucleus assay, and oral toxicity in rats (oral acute and 90-day studies). These studies were used to calculate the safety margin for the notified substance and the inducer isopropyl β-D-1-

thiogalactopyranoside (IPTG).

To address the human food safety of the notified substance, the notifier includes information to address the safety conclusion in humans that consume edible tissues of swine fed diets containing the notified substance at the inclusion rate of 500 to 2,000 units per kilogram of feed: 1) The lack of mutagenicity/genotoxicity of the notified substance and 2) The metabolic fate of the notified substance in animals is expected to be similar to that of any other protein, and therefore, it is not absorbed/deposited in edible tissues.

The Association of American Feed Control Officials publishes in their Official Publication a list of names and definitions for accepted feed ingredients. FDA recognizes these names as being the "common or usual" names for feed ingredients. FDA recognizes "phytase" as the common or usual name for this phytase from a *Pseudomonas fluorescens* strain expressing an altered *app*A 6-phytase gene from *Escherichia coli* strain K12 included in animal food.

Section 301(ll) of the FD&C Act

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, 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. In our evaluation of BASF Enzymes LLC's notice, concluding that the notified substance, phytase from a *P. fluorescens* strain expressing an altered *app*A 6-phytase gene from *Escherichia coli* strain K12 at a recommended supplementation level of a minimum of 500 U/kg to a maximum of 2000 U/kg in complete feed is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing the notified substance. Accordingly, our response should not be construed to be a statement that foods containing the notified substance if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusion

Based on the information contained in the notice and amendment, submitted on behalf of BASF Enzymes LLC, and other information available to FDA, we have no questions at this time regarding the notifier's conclusion that phytase from a *Pseudomonas fluorescens* strain expressing an altered *app*A 6-phytase gene from *Escherichia coli* strain K12 to be used to increase the availability of phytin-bound phosphorus in swine diets at a recommended supplementation level of a minimum of 500 U/kg to a maximum of 2000 U/kg in complete feed is GRAS. The agency has not, however, made its own determination regarding the GRAS status of the intended use of the notified substance in swine diets under Title 21 of the *Code of Federal Regulations* (21 CFR) part 570.35. Unless noted above, our evaluation did not address other provisions of the FD&C Act. As always, it is the continuing responsibility of BASF to ensure that animal food ingredients that it markets are safe and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with 21 CFR 570.275(b)(2), the text of this letter responding to AGRN 61 is accessible to the public on our website for the Current Animal Food GRAS Notices Inventory at https://www.fda.gov/animal-veterinary/generally-recognized-safe-gras-notification-program/current-animal-food-gras-notices-inventory.

If you have any questions about this letter, please contact Ms. Wasima Wahid at (240) 402-5857 or at wasima.wahid@fda.hhs.gov.

Sincerely,

/s/
Timothy Schell, Ph.D.
Director
Office of Surveillance and Compliance
Center for Veterinary Medicine

References:

Krygier, et al., 2014, "Safety evaluation of phytase 50104 enzyme preparation (also known as VR003), expressed in *Pseudomonas fluorescens*, intended for increasing digestibility of phytate in monogastrics". *Regulatory Toxicology and Pharmacology* 70: 545-554

Krygier, et al., 2015, "Corrigendum to safety evaluation of phytase 50104 enzyme preparation (also known as VR003), expressed in *Pseudomonas fluorescens*, intended for increasing digestibility of phytate in monogastrics". *Regulatory Toxicology and Pharmacology 71 (2)*: 352

Ren, et al., 2020, "Effects of a novel *E. coli* phytase expressed in *Pseudomonas fluorescens* on growth, bone mineralization, and nutrient digestibility in pigs fed corn–soybean meal diets." *Transl. Anim. Sci.* 4 (4)