U.S. FOOD & DRUG ADMINISTRATION

September 16, 2022

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. 55 – Phytase enzyme

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 December 7, 2020, submitted by BASF Enzymes, LLC (BASF or the notifier). The subject of the submission is 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 broiler 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. Following an initial evaluation, you were notified in a letter dated January 20, 2022 that the GRAS notice was acceptable for filing, and the notice was designated as animal GRAS notice number (AGRN) 55. During the evaluation, we received an amendment, dated July 11, 2022, in response to our request for more information. We have completed our evaluation of AGRN 55.

To address the Chemistry, Manufacturing and Controls of the notified substance, BASF provides information about the identity, method of manufacture and specifications of the notified substance and market formulations containing the notified substance, referred to here as L10 and 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 (cGMPs) for animal foods and the OECD's criteria for Good Industrial Large-Scale Practice (GILSP) with appropriate quality controls procedures based on Hazard Analysis and Critical Control Points (HACCP) 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 (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,

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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 enzyme preparations 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 L10 and G10 phytase enzymes in market formulations, premix, and feed (mash and pelleted). The market formulations are composed of the phytase enzyme and other ingredients that are suitable for use in animal food.

To address the utility of the notified substance, phytase from a *P. fluorescens* strain expressing an altered *app*A 6-phytase gene from *Escherichia coli* strain K12, and its intended use to increase the availability of phytin-bound phosphorus when included in complete diets for broilers, BASF includes one publication containing two studies (Pieniazek, et al., 2017) and a corroborative unpublished study. All studies were conducted in broiler chickens fed corn soybean meal diets, and the notified phytase was supplemented at levels of 250, 500 and 2,000 units per kilogram of diet. Bone ash was considered the pivotal parameter to reach a conclusion on the notified substance's functionality.

To address the molecular biology and pre-fermentation manufacturing processes, the notice 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, phytase 50104 enzyme. The notifier also addressed genetic stability, plasmid mobilization, potential new open reading frames and absence of 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 address the target animal safety of the intended use of the notified phytase 50104 in broiler 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 in the publication by Krygier, et al., 2014, 2015.

The safety studies include 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). The test article in the oral toxicity studies was the phytase 50104 enzyme (named VR003) produced by the genetically modified microorganism *P. fluorescens* strain BD50104 manufactured using a representative process for the commercial enzyme at scale. The 90-day oral study in rats includes four levels of the test article at 0, 500, 1,000, and 2,000 mg/kg. Parameters evaluated include body weight, food consumption, hematology/clinical chemistry, necropsy findings. The NOAEL was selected from this study to calculate the safety margin in broilers for the phytase 50104 and the inducer isopropyl **β-D-1-**thiogalactopyranoside (IPTG).

To address the human food safety of the notified substance, BASF includes the following information to address the safety conclusion in humans that consume edible tissues of broilers fed diets containing the notified phytase 50104 at the inclusion rate of 500 to 2,000 units per kilogram of feed: 1) The lack of mutagenicity/genotoxicity of the phytase 50104; and 2) The

metabolic fate of the phytase 50104 in broilers 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 from a *Pseudomonas fluorescens* strain expressing an altered *app*A 6-phytase gene from *Escherichia coli* strain K12" as the common or usual name for the notified substance.

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 *Pseudomonas 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 phytase from a *Pseudomonas fluorescens* strain expressing an altered *app*A 6-phytase gene from *Escherichia coli* strain K12 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 the amendments, submitted on behalf of BASF Enzymes LLC, as well as 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 broiler 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 broiler 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 55 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:

Pieniazek, et al., 2017, "Evaluation of increasing levels of a microbial phytase in phosphorus deficient broiler diets via live broiler performance, tibia bone ash, apparent metabolizable energy, and amino acid digestibility". *Poultry Science* 2017 Feb 1; (96): 370-382

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*, *RTP*, 70 (2014) 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 RTP* 70 (2014) 545-554; *Regulatory Toxicology and Pharmacology RTP* 71 Issue 2 (2015) 352