January 10, 2025

BASF Corporation
Attention: Andreas Kern, Ph.D.
Senior Regulatory Affairs Manager
100 Park Avenue
Florham Park, NJ 07932

Re: GRAS Notice AGRN 67 – Beta-mannanase from a *Thermothelomyces thermophilus* Strain Expressing an Altered *man1* gene from *Trichoderma reesei*

Dear Dr. Kern:

The Food and Drug Administration's (FDA, the Agency) Center for Veterinary Medicine (CVM or we) refers to a generally recognized as safe (GRAS) notice, dated March 11, 2024, submitted by BASF Corporation (BASF). The subject of the notice is beta-mannanase from a *Thermothelomyces thermophilus* strain expressing an altered *man1* gene from *Trichoderma reesei* (hereafter referred to as beta-mannanase or the notified substance) for the use of reducing the viscosity of intestinal digesta in poultry diets when included at a level of 800 Thermostable Mannanase Units (TMU) per kg of feed. The submission informs us of BASF's conclusion that the subject of the submission is GRAS through scientific procedures. You were notified in a letter dated April 18, 2024, that the GRAS notice, as amended, was acceptable for filing, and the notice was designated as animal GRAS notice number (AGRN) 67. On September 16, 2024, CVM received an amendment from BASF containing additional chemistry, manufacturing and controls, microbial safety, molecular biology, utility, and target animal safety information. We have completed our evaluation of AGRN 67 and have no questions at this time.

To address the identity, method of manufacture, and specifications of the notified substance, BASF provides information on enzyme identity, manufacturing processes and controls, analytical methods used to determine the mannanase activity, enzyme's pH and thermal profiles, and side activities. One mannanase unit, TMU, is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from locust bean gum (0.3 g/100 ml, final concentration in the reaction solution: 0.27 g/100 ml) in one minute under the assay conditions of 50.0 ± 0.1 °C and pH 3.5. The notified substance is produced from a fermentation of a genetically engineered *Thermothelomyces thermophilus* strain followed by cell inactivation, recovery, and formulation. The production strain is absent in the produced enzyme preparation. BASF provides the same specifications for both market formulations Natupulse® TS (beige to brownish fine powder) and Natupulse® TS L (clear, brownish liquid): enzyme activity ≥8000 TMU/g, lead ≤1.0 mg/kg, aflatoxin B1 <3.0 µg/kg, ochratoxin A <5.0 µg/kg, deoxynivalenol <500 µg/kg, zearalenone <50 µg/kg, *Salmonella* not detected in 25 g, *E. coli* <1 CFU/25g, coliforms < 10 CFU/g. BASF provides stability, enzyme thermotolerance under high temperatures and during pelleting process, and packaging information.

To address molecular biology, the notice includes a description of genetic modifications that were performed during development of the source strain, T. thermophilus DSM 33149, to

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produce the notified substance. BASF also addresses genetic stability, potential new open reading frames, and absence of antibiotic resistance markers that were used in the genetic engineering process.

BASF concludes that information demonstrating the physical or technical effect of the notified substance is not necessary based on BASF's conclusion that use of the notified substance to increase the digestibility of β-mannans and thereby reduce digesta viscosity when used in complete and balanced poultry diets does not impact target animal safety.

To address target animal safety of the intended use of the notified substance, BASF provided a description of enzyme identity, safety of the production organism and the donor organism, and the manufacturing process. Publicly available safety data included: a) results of a battery of toxicology studies, including a subchronic rat study using the notified beta-mannanase (Kern et al., 2020), and b) a broiler tolerance study that demonstrated at least a 100-fold safety factor for the intended use rate of the notified substance (Fickler et al., 2024).

To address identity and microbial safety, BASF provides a narrative based on scientific data and literature that addresses different aspects, including published literature, pathogenicity, genomic analysis, and toxin production, to support its conclusion that *Thermothelomyces thermophilus* strain DSM 33149 is safe as the source organism to produce the notified substance for use of reducing the viscosity of intestinal digesta in poultry diets.

To further address the human food safety of the intended use of the notified substance, BASF includes publicly available data and information to conclude that the notified substance is an enzyme, and because enzymes are proteins, the notified substance is expected to be digested in the gastrointestinal tract of poultry and converted into amino acids which are expected to be indistinguishable from the digestion of other proteins in the gastrointestinal tract. BASF also includes an *in vitro* battery of genotoxicity studies, and information to support that, under the intended conditions of use, there is reasonable certainty that a) residues of the notified substance, b) reaction products from the hydrolysis of β-mannans by the notified substance, and/or c) low molecular weight components of the formulated enzyme products will not remain in edible animal tissues.

Section 403(a) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Under section 403(a) of the FD&C Act, a food is misbranded if its labeling is false or misleading in any particular. BASF did not provide any information to demonstrate thatthe notified substance functions as intended because BASF concluded that the intended use would not be expected to impact safety. Therefore, we did not evaluate whether the notified substance would achieve the effect claimed for it. However, please note that if products containing the notified substance bear any claims on the label or in labeling regarding the function of the notified substance, these claims should be supported by appropriate data and information. FDA may take enforcement action if any claims on labels or labeling are found to be false or misleading.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (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's notice, as amended, concluding that beta-mannanase from a *Thermothelomyces thermophilus* strain expressing an altered *man1* gene from *Trichoderma reesei* for the use of reducing the viscosity of intestinal digesta in poultry diets when included at a level of 800 Thermostable Mannanase Units per kg of 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, as amended, submitted by BASF Corporation, and other information available to the FDA, we have no questions at this time regarding BASF's conclusion that beta-mannanase from a *Thermothelomyces thermophilus* strain expressing an altered *man1* gene from *Trichoderma reesei* is GRAS under the intended conditions of use. The Agency has not, however, made its own determination regarding the GRAS status of the intended use of the notified substance in animal food under 21 CFR 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 Corporation to ensure that animal food ingredients that BASF 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 67 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-notificationprogram/current-animal-food-gras-notices-inventory.

If you have any questions or comments, please contact Ms. Carissa Adams at 240-402-6283 or at carissa.adams@fda.hhs.gov.

Sincerely,

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

References

Kern A., D. Shanahan, R. Buesen, D. Geiger. 2020. Safety evaluation of a β-mannanase enzyme preparation produced with *Thermothelomyces thermophilus* expressing a protein-engineered β-mannanase gene. PLOS ONE 15:e0243647. https://doi.org/10.1371/journal.pone.0243647.

Fickler, A., M. Francesch, K. Kore, and AB. Mandal. 2024. Tolerance on supplementation of graded levels of novel β-mannanase (Natupulse® TS) in broiler chickens. Animal Nutrition and Feed Technology 24:193-201. https://doe.org/10.5958/0974-181X.2024.00014.3.