June 21, 2022

GRAS Notice No. AGRN 44

Kristi O. Smedley, Ph.D.
Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Road
Woodbridge, VA 22192

Dear Dr. Smedley,

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 29, 2020, submitted on behalf of your client, BioResource International, Inc. (the notifier). The subject of the submission is endo-1,4-β-xylanase, prepared from *Komagataella phaffii* (*K. phaffii*) expressing the gene encoding xylanase from *Orpinomyces sp.*, used for the hydrolysis of xylans at a use rate of 10,000 to 40,000 xylanase units per kilogram (XU/kg) in complete feed for poultry and swine. The submission informs us of the notifier's conclusion that the subject of the submission is GRAS through scientific procedures. Additionally, we received an amendment, dated February 18, 2021, providing more information for this submission. Following an initial evaluation, you were notified in a letter dated February 25, 2021 that the GRAS notice, as amended, was acceptable for filing, and the notice was designated as animal GRAS notice number (AGRN) 44. During the evaluation, we received amendments, dated October 7 and November 2, 2021 and January 18, 2022, in responses to requests for more information. We have completed our evaluation of AGRN 44.

BioResource International, Inc. provided information about the identity, method of manufacture, specifications, analytical methods, contaminants, and stability of the notified substance and the intended use. The notified substance is produced from a fermentation process of a genetically modified *K. phaffii* (*Pichia pastoris*) production strain. It is intended to be used for hydrolysis of xylans, a component of hemicellulose which are major components of plant cell wall, in poultry and swine feeds at a recommended use level of 10,000 to 40,000 XU/kg feed. One XU activity is defined as the amount of enzyme needed for the release of 1 nanomole of reducing sugars (xylose equivalents) per second from 0.5% beechwood xylan at 50 Celsius (°C) in 50 millimolar (mM) trisodium citrate buffer pH 6.0. The manufacturing process involves fermentation, downstream processing, and packaging. The xylanase enzyme is manufactured using a controlled batch fermentation reaction using a pure culture of the genetically modified *K. phaffii* production strain followed by recovery and formulation. The manufacturing is performed according to current Good Manufacturing Practices (cGMPs) using equipment specifically designed for the intended purpose of producing a high-quality feed grade finished product. The raw materials used are standard ingredients used in the enzyme industry. The notifier performs analytical testing for enzyme activity and other specifications. The notifier provided specifications for the market formulation of the xylanase enzyme in the original submission and replaced those specifications in the amendment submission. The notifier stated that the specifications for certain contaminants, such as, mercury, cadmium, certain mycotoxins, dioxins,

and genetically modified organisms that were removed in the amendment submission had results lower than the limit of detection. The notifier stated that each batch of finished product is tested for appearance, moisture, and xylanase activity; quarterly tested for total aerobic plate count, total yeast and filamentous fungi, *Escherichia. coli*, *Salmonella*, and coliforms; and annually tested for heavy metals, mycotoxins, dioxins, and absence of genetically modified organisms. The notifier stated that that genetically modified organisms are absent in the final enzyme product. The notifier provided xylanase enzyme marketed product specification with test method and acceptance criteria: Appearance (light grey powder), moisture (<3%), xylanase activity (≥150,000 XU/g), mycotoxins – Aflatoxin B1 (<0.5 ppb[1]), Aflatoxin B2 (<0.5 ppb), Aflatoxin G1 (<0.5 ppb), Aflatoxin G2 (<0.5 ppb), heavy metals – arsenic (<3 mg/kg), lead (<3 mg/kg[2]), microbial contamination – coliforms (<10 CFU/g[3]), *Escherichia coli* (< 10 CFU/g), *Salmonella sp.* (absence in 25 g), molds (<10 CFU/g). The notifier has provided stability and homogeneity information for the xylanase enzyme in market formulations and feed (mash and pelleted). The marketed product, is composed of the endo-1,4-β-xylanase, calcium carbonate, and starch. The notifier states that the xylanase enzyme product is added to animal feed directly and is not added through a premix.

To address the identity and microbial safety of the host organism, the notifier provides a narrative, including genomic analyses and scientific literature, evaluating the use of *K. phaffii* as a host organism in the development of a source organism strain used for the production of the notified substance.

To address the molecular biology of the notified substance, the notice includes a description of the genetic modifications that were performed during the development of the *K. phaffii* Xyl-2, which will be used as the source organism for the production of the modified xylanase enzyme. The notifier states that two copies of the pAKa-AXr plasmid were inserted into the genome of *K. phaffii* BG10, next to the *AOX1* gene. This plasmid contains two expression cassettes, one for a modified xylanase that is secreted from the yeast cells and the other one for the zeocin antibiotic resistance gene. The genetic modifications were characterized using whole genome sequencing of the host and source organisms. The notifier also addresses genetic stability, and highlights that the manufacturing process ensures that genomic deoxyribonucleic acid (DNA) from the source organism is not present in the market formulation, thus removing the zeocin antibiotic resistance gene from the final product.

To address the target animal safety of the notified substance, the notifier's determination is based on its determination of safety of the production organism, including the long history of safe use of *K. phaffii* in production of enzymes for use in food. Additionally, the notifier cites its well characterized genetic modifications to make the source strain, lack of toxigenic potential in the source strain, absence of production cells or DNA in the xylanase to support its conclusion of safety. Additionally, the notifier states that the enzyme is expected to degrade or become inactivated as the digesta moves through the gastrointestinal tract. To further support its conclusion of safety, the firm cites five journal articles that demonstrate safe consumption of the xylanase by broilers and pigs. In the publications, the xylanase use rate ranged between 10,000 to 40,000 XU/kg feed.

To address the human food safety of the notified substance and its intended use for hydrolysis of xylans in feed for poultry and swine, the notifier included a discussion of expected fate of the

[1] Parts per billion.
[2] Milligrams per kilogram.
[3] Colony forming units per gram.

xylanase enzyme, the safety of *K. phaffii* as the microorganism that produces the notified substance, and potential risks to humans who consume edible tissues from the target animals. The notifier states that *K. phaffii* is nontoxigenic and nonpathogenic and, based on the proteinaceous nature of enzymes, the xylanase is expected to be digested to its component amino acids within the gastrointestinal tract of poultry and swine consuming it as part of their diets. Based on analytical data derived from the xylanase preparation, the notifier expects no safety concerns for humans due to enzyme hydrolysis or the presence of heavy metals, mycotoxins, microbial contaminants, or from the production (source) organism. The notifier concludes that there are no concerns for human food safety due to consumption of eggs and meat from animals consuming feed containing the notified substance.

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 the name "Endo-1,4-β-xylanase enzyme" as the common or usual name for the notified substance.

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. The notifier did not provide any information to demonstrate that the notified substance functions as intended because BioResource International, Inc. concluded that the intended use would not be expected to impact safety. Therefore, we did not evaluate whether endo-1,4-β-xylanase from *K. phaffii* expressing the gene encoding xylanase from *Orpinomyces sp*. would achieve the effect claimed for it. However, please note that if products containing endo-1,4-β-xylanase from *K. phaffii* expressing the gene encoding xylanase from *Orpinomyces sp*. 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 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 BioResource International, Inc.'s notice, concluding that endo-1,4-β-xylanase, prepared from *K. phaffii* expressing the gene encoding xylanase from *Orpinomyces sp.*, 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 Endo-1,4-β-xylanase enzyme, 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 BioResource International, Inc., as well as other information available to FDA, we have no questions at this time regarding the notifier's conclusion that endo-1,4-β-xylanase, prepared from *K. phaffii* expressing the gene encoding xylanase from *Orpinomyces sp.*, used for the hydrolysis of xylans at a use rate of 10,000 to 40,000 XU/kg in complete feed for poultry and

swine is GRAS. The agency has not, however, made its own determination regarding the GRAS status of the intended use of the notifier substance in poultry and swine feed 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 BioResource International, Inc. 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 44 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. Chelsea Cerrito by telephone at (240) 402-6729 or by email at chelsea.cerrito@fda.hhs.gov. Refer to AGRN 44 in any future correspondence regarding this notice.

Sincerely,

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