June 23, 2022

GRAS Notice No. AGRN 50

James M. Ligon, Ph.D.
Agrivida, Inc.
1023 Christopher Drive
Chapel Hill, NC 27517

Dear Dr. Ligon:

The Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM or we) refers to a generally recognized as safe (GRAS) notice dated August 18, 2021, submitted by Agrivida, Inc. (Agrivida). The subject of the submission is ground grain obtained from a corn (*Zea mays*) variety that expresses an altered AC1 beta-glucanase gene obtained from an environmental DNA library (transformation event FG259) to increase digestibility of swine feeds containing soluble non-starch polysaccharides at a use rate of 50-500 glucanase activity units per kg of complete feed. The submission informs FDA of the Agrivida's conclusion that the subject of the submission is GRAS through scientific procedures. Following an initial evaluation, you were notified in a letter dated October 25, 2021 that the GRAS notice was acceptable for filing, and the notice was designated as animal GRAS notice number (AGRN) 50. During the evaluation we received an amendment, on March 30, 2022, in response to a request for more information. We have completed our evaluation of AGRN 50.

Agrivida incorporates the information from its previous GRAS notice (AGRN 31) about the identity, method of manufacture and specifications of the notified substance. The notified substance is ground grain from a corn (*Zea mays*) variety that expresses an altered AC1 beta-glucanase gene obtained from an environmental DNA library (transformation event FG259). The production of the notified substance is the same agronomic practices as is typically used for the production of traditional corn, including the application of chemical fertilizers and crop protection chemicals approved for use on maize. After harvesting, the crop is shelled to produce whole corn grain, which is then dried and milled. Agrivida provides specifications for the finished product which include: 150-300 units of beta-glucanase activity per gram of grain, *Escherichia coli* (not detected in 10 g), and *Salmonella sp.* (not detected in 25 g). The notice contained data to support use of the notified substance in pelleted swine feed if the pelleting temperature does not exceed 90 °C. Agrivida also provides AC1 glucanase stability, homogeneity, and packaging information for the notified substance.

To address the functionality of the notified substance, ground grain obtained from a corn (*Zea mays*) variety that expresses an altered AC1 beta-glucanase gene obtained from an environmental DNA library (transformation event FG259), and its intended use to "increase the digestibility of soluble non-starch polysaccharides in complete balanced swine diets thereby reducing the viscosity of intestinal digesta associated with feed containing soluble non-starch polysaccharides", Agrivida includes a publication (Lessard et al., 2021) describing a study evaluating the functionality of the notified glucanase. The study was conducted in swine fed corn soybean meal diets with two different levels of dried distillers grains solubles, and the

notified enzyme was supplemented at 150 and 450 units per kilogram of diet. Agrivida also incorporates by reference to its previous GRAS notice (AGRN 31) information pertaining to effects of the notified enzyme on viscosity of feed *in vitro* to support the functionality of the notified substance in swine. Agrivida also concludes that when the notified substance fails to function as intended, it does not impact safety of the target animal species as the notified substance will be added to swine feeds without any amendment to typical diet formulation and the animals would be fed a complete, nutritionally adequate diet.

To characterize the notified substance, Agrivida incorporates by reference information from its previous GRAS notice (AGRN 31) and includes a brief description of the genetic modifications that were performed during the development of the new corn variety that expresses a beta-glucanase in its grain. There are 12 amino acid changes in the beta-glucanase enzyme produced by event FG259 which Agrivida states was to address thermostability. Agrivida provides information pertaining to the molecular characterization of event FG259 and summarizes information that describes the transformation system, genetic elements of pAG4588 plasmid, the inserted DNA, genetic stability, and the absence of antimicrobial resistance markers in the new corn variety. Agrivida mainly references information in its previous notice when it addresses the information above.

Agrivida incorporates by reference safety information from GRAS notice AGRN 31 for the same bioengineered corn expressing the AC1 glucanase. Information referenced from GRAS notice AGRN 31 includes: 1) publicly available information on the safety of both the production host (corn) and the gene donor (an environmental library), and 2) the decision tree analysis (as described by Pariza and Johnson, 2001) used to determine the safety of the AC1 glucanase in animal food. In addition, Agrivida includes an updated BLAST[1] search on the global sequence of the AC1 amino acid sequence of the AC1 glucanase against the NCBI[2] Protein database.

To address the target animal safety of the notified substance, Agrivida includes a tolerance study in swine by Lessard et al., 2021, reporting the effects of the notified substance in swine diets at a level of up to 4X the intended use level on growth performance, hematology, clinical chemistry, and gross pathology. Agrivida also describes that exposure to other substances that could result from the enzymatic action of the AC1 glucanase would be to substances derived from the maize grain that are considered safe for food because the AC1 glucanase is contained within the grain of maize and the nutritional composition of the grain is not different from that of conventional maize grain.

Agrivida states that AC1 glucanase, like most proteins, will be metabolized during the animal's digestion into constituent amino acids and will not present a hazard to human health following consumption of swine feed diets containing the notified substance. Agivida adds that it has demonstrated using an *in vitro* system that the AC1 glucanase enzyme is sensitive to digestion in a simulated gastric environment. Agrivida concludes that meat derived from swine that consume feed containing the notified substance is safe for human consumption and does not present any human safety concerns because: 1) AC1 glucanase is expected to be digested in the gastro-intestinal tracts of swine and it is not expected to be absorbed intact into the blood of animals that consume it or to be deposited into swine tissues, including the meat, and 2) glucanases have been included in the feed of poultry and swine for decades without any adverse effects on human health or nutrition.

[1] Basic Local Alignment Search Tool

[2] National Center for Biotechnology Information

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. We question whether the notice adequately demonstrates that the notified glucanase achieves its intended effect. However, Agrivida concludes that the intended use of the notified substance would not be expected to impact safety when added to complete balanced diets that meet all nutrient requirements for swine. If products containing ground grain obtained from a corn (*Zea mays*) variety that expresses an altered AC1 beta-glucanase gene obtained from an environmental DNA library (transformation event FG259) bear any claims on the label or in labeling regarding the function of the notified substance, these claims must 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 Agrivida's notice, concluding that ground grain obtained from a corn (*Zea mays*) variety that expresses an altered AC1 beta-glucanase gene obtained from an environmental DNA library (transformation event FG259) that 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).

The Association of American Feed Control Officials publishes 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 "beta-glucanase" as the common or usual name for the beta-glucanase produced by a corn (*Zea mays*) variety that expresses an altered AC1 beta-glucanase gene obtained from an environmental DNA library (transformation event FG259).

Conclusion

Based on the information contained in the notice and the amendments submitted by Agrivida Inc. and other information available to the FDA, the agency has no questions regarding Agrivida's conclusion that ground grain obtained from a corn (*Zea mays*) variety that expresses an altered AC1 beta-glucanase gene obtained from an environmental DNA library (transformation event FG259) 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 ground grain obtained from a corn (*Zea mays*) variety that expresses an altered AC1 beta-glucanase gene obtained from an environmental DNA library (transformation event FG259) in animal food under Title 21 *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 Agrivida Inc. to ensure that animal food ingredients that you market are safe and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with 21 CFR 570.275(b)(1), the information in this notice described in 21 CFR 570.225(c)(2) through (c)(5) will be accessible to the public 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. Carissa Adams at (240) 402-6283 or by e-mail at carissa.adams@fda.hhs.gov. Refer to AGRN 50 in any future correspondence regarding this submission.

Sincerely,

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

References

Lessard, P. A., X. Li, J.N. Broomhead, M.H Parker, C. Bailey, and R.M. Rabb (2021). Properties of corn-expressed carbohydrase AC1 in swine diets and its effects on apparent ileal digestibility, performance, hematology, and serum chemistry. Heliyon **7**(8): E07696. Available at: https://doi.org/10.1016/j.heliyon.2021.e07696.