May 23, 2017

James M. Ligon, Ph.D.
Vice President, Regulatory Affairs and Stewardship
Agrivida, Inc.
1023 Christopher Drive
Chapel Hill, NC 27517

Re: GRAS Notice No. AGRN 21

Dear Dr. Ligon,

The Food and Drug Administration (FDA, we) completed our evaluation of AGRN 21. We received Agrivida Inc.'s ("Agrivida") notice on May 6, 2016 and additional information on June 6, 2016 to clarify target animal species and to provide information related to human food safety and self-limiting use level. This notice was filed on July 28, 2016 under CVM's GRAS Notification Pilot Program. Agrivida submitted an amendment on March 23, 2017 providing revisions to the molecular biology process, analytical method, intended use, and target animal safety sections of the notice.

The notified substance is ground grain obtained from a corn (*Zea mays)* variety that expresses an altered *appA* 6-phytase gene obtained from *Escherichia coli* strain K12. This notice informs us of Agrivida's view that the notified substance is GRAS, through scientific procedures, to increase the digestibility of phytin-bound phosphorous or to increase phosphorous availability from phytate in poultry diets when used at the rate of 75 g (gram) to 1.7 kg (kilogram) per ton of complete feed to provide 250-6000 phytase units (FTU)/kg complete feed.

As a part of the GRAS notice, Agrivida provides a report of a panel of individuals (the GRAS panel) who evaluated the data and information that are the basis for Agrivida's GRAS conclusion. Agrivida considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of the notified substance under its intended conditions of use. The conclusion of the GRAS panel, that the notified ground corn grain containing phytase is safe for its intended use, is based on the data and information provided by Agrivida including data and information compiled from the literature and results from unpublished studies.

Agrivida provides a description of the genetic modifications that were performed during the development of the genetically engineered corn *(Zea mays)* variety (transformation event PY203). The native *E. coli appA* phytase gene was altered using gene site-saturation mutagenesis. Southern blotting, polymerase chain reaction (PCR), nucleotide sequencing, and bioinformatics analyses were used to independently verify the genetic material that was introduced into the genetically engineered corn variety. These techniques were also used to verify that none of the nucleotide sequences in the backbone region of the vector, including the antimicrobial resistance markers, were incorporated into the genetically engineered corn variety. Southern blot and PCR analyses were used to demonstrate that the sequences inserted into the corn genome were genetically stable across several generations. Agrivida also conducted open reading frame analyses to demonstrate that nucleotide sequences around the junctions at the

sites of insertion would not lead to the production of unintended proteins that would raise animal food safety concerns.

Agrivida provides information about the method of manufacture and specifications of the notified substance. The production of the notified ground corn grain containing phytase is the same agronomic practices as are typically used for the production of traditional corn. 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: 4000 - 7000 units of phytase activity per gram of grain, *Escherichia coli* (not detected in 10 g), and *Salmonella sp.* (not detected in 25 g). Agrivida also provides phytase activity stability, homogeneity, and packaging information for the notified substance. We note that information provided in the notice regarding phytase thermostability during the pelleting process includes some inconsistent stability results for pelleting temperatures above 80°C.

To address the characterization and identity of the expressed phytase in the notified substance, Agrivida provides the Enzyme Commission number, its molecular weight, immunoreactivity, intactness, enzymatic activity, and glycosylation status. In addition, Agrivida provides amino acid sequence data and reports that the enzyme has 92 % identity with the *E. coli* K12 *appA* phytase protein. Agrivida states that expressed phytase enzyme is altered to have improved thermotolerance and increased sensitivity to digestion in the gastric environment and provides the pH and temperature optima of the expressed phytase.

To address the functionality of the expressed phytase, Agrivida provides four unpublished studies, where pivotal and supporting parameters showed significant responses to demonstrate the ability of the enzyme as a phytase to increase the availability of phosphorus in typical poultry diets. Agrivida also cites publications on the functionality of another corn-expressed *E. coli* phytase that is related to the phytase expressed in the notified substance.

To address the target animal safety, Agrivida provides publically available pivotal information 1) on the safety of both the production host (corn) and the gene donor (*Escherichia coli* K12 strain); 2) to indicate that as expected for other proteins, the expressed phytase enzyme will be digested into amino acids and small peptides in the gastrointestinal tract of animals and will not represent a safety concern in poultry fed with the notified substance at the proposed use rate. As corroborative information, Agrivida provides the results of two unpublished tolerance studies in chickens showing a lack of adverse effects when the notified substance is used at levels up to 4X and 7X the intended use rate. In addition, Agrivida cites other published studies on an investigational genetically engineered corn variety that expresses a phytase to support the target animal safety conclusion.

To address human food safety, Agrivida states that the expressed phytase, as with most proteins, will be metabolized during animal digestion into constituent amino acids and will not present a hazard to human health following consumption of poultry consuming feed containing the notified substance. Agrivida adds that they have demonstrated that expressed phytase is sensitive to digestion in a simulated gastric environment. Agrivida concludes that meat derived from animals that consume feed containing the notified substance is safe for human consumption and does not present any human safety concerns.

The Association of American Feed Control Officials (AAFCO) publishes a list of names and definitions for accepted feed ingredients. FDA recognizes these names as being the "common and usual" names for feed ingredients. FDA recognizes the name "phytase" as the common and usual name for 6-phytase produced by a corn (*Zea mays*) variety that expresses an altered *appA* 6-phytase gene obtained from *E. coli* strain K12.

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 your notice concluding that ground grain obtained from a corn (*Zea mays)* variety that expresses an altered *appA* 6-phytase gene obtained from *Escherichia coli* strain K12 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 ground grain obtained from a corn (*Zea mays)* variety that expresses an altered *appA* 6-phytase gene obtained from *Escherichia coli* strain K12. Accordingly, our response should not be construed to be a statement that foods containing ground grain obtained from a corn (*Zea mays)* variety that expresses an altered *appA* 6-phytase gene obtained from *Escherichia coli* strain K12, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information contained in the notice and amendment submitted by Agrivida, as well as other information available to the Agency, we have no questions at this time regarding Agrivida's conclusion that ground grain obtained from a corn (*Zea mays*) variety expressing an altered *appA* 6-phytase gene obtained from *Escherichia coli* strain K12 is GRAS under its intended conditions of use. Our evaluation only pertains to the transformation event PY203 described in this notice. This letter is not an affirmation that the notified substance is GRAS under 21 CFR 570.35. Unless noted above, our evaluation did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant 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 http://www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/GenerallyRecognizedasSafeGRASNotifications/ucm243845.htm.

If you have any questions concerning this letter, please contact Dr. Lei Tang via e-mail at lei.tang@fda.hhs.gov or by phone at 240-402-5922. Please refer to AGRN 21 in any future correspondences.

Sincerely,

/s/

Daniel G. McChesney, Ph.D.
Director
Office of Surveillance and Compliance
Center for Veterinary Medicine