April 4, 2012

Raymond A. Matulka, Ph.D.
Burdock Group
801 N. Orange Ave. Suite 710
Orlando, Florida 32801

Re: GRAS Notice No. AGRN 000-008

Dear Dr. Matulka:

The Food and Drug Administration (FDA) is responding to the notice, dated May 27, 2011, that you submitted under FDA's Center for Veterinary Medicine (CVM) Pilot Program for substances generally recognized as safe (GRAS) added to food for animals (See 75 FR 31800; June 4, 2010). FDA's Center for Veterinary Medicine received the notice on June 2, 2011, filed it on June 10, 2011, and designated it as GRAS Notice No. AGRN 000-008.

The subject of the notice is *Bacillus cereus* variant *toyoi* (*B. cereus*) strain. The notice informs FDA of the view of Rubinum S.A. Animal Health that *Bacillus cereus* variant *toyoi* strain is GRAS, through scientific procedures, for use as an ingredient in animal feed for consumption by swine (fattening pigs, sows and gilts, and piglets), fattening cattle and calves, chicken broilers and turkeys, fattening rabbits and breeding does to help maintain the animal's gut microflora.

As discussed more fully below, the notice does not provide a sufficient basis for a determination that *Bacillus cereus* variant *toyoi* is GRAS under the conditions of its intended use in animal food.

Data and information that Rubinum S.A. Animal Health presents to support its GRAS determination

Rubinum S.A. Animal Health (Rubinum) provides information regarding the common name of the ingredient, conditions of use, and specifications. Rubinum also provides publicly available information regarding manufacturing methods, identity, information that *Bacillus cereus* variant *toyoi* strain can be differentiated from other *Bacillus cereus* strains, analytical methods, and unpublished stability data. The ingredient is manufactured by a bacterial fermentation process to form spores. Immediately following cultivation, the spores are mixed with corn flour and calcium carbonate at concentrations up to 2.0×10^9 CFU *Bacillus cereus* variant *toyoi* /kg feed.

As part of their GRAS notice submission, Rubinum provides the report of a panel of individuals (Rubinum's GRAS panel) who evaluated the data and information that are the basis for Rubinum's GRAS determination. Rubinum considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to animal food. The conclusions of Rubinum's GRAS panel, that the substance is safe for its intended use, were based on published and unpublished toxicity data. Rubinum's GRAS Panel

evaluated data from studies that were publicly available and from studies that were not publicly available.

Rubinum discusses published data on the following topics: enterotoxicity, feeding trials (rabbits, pigs, chickens, turkeys, and cattle), acute toxicity studies in rodents, subchronic toxicity studies, chronic toxicity studies, genotoxicity studies, ocular irritation, and a human clinical trial. Additionally, the notifier discusses published articles that described safe oral administration of the *Bacillus cereus* variant *toyoi* strain to pregnant and lactating sows and their weaned pigs, rabbits, turkeys, broiler chickens, and Japanese quail. Rubinum also describes data from an unpublished study report that demonstrated safe use of the notified substance when fed to dairy calves in milk replacer. The notifier also discusses both unpublished and published data that were used to determine bacterial safety. Collectively, these data were interpreted by the notifier and their GRAS panel to indicate an absence of toxicity in several species, over a range of relevant amounts, routes, and durations of exposure and supported Rubinum's determination that the *Bacillus cereus* variant *toyoi* strain is GRAS under the conditions of its intended use.

Regarding human food safety, Rubinum provides published toxicology studies and primary research studies as well as literature review documents, including the opinions of the Scientific Committee on Animal Nutrition (SCAN) of the European Commission (currently the European Food Safety Authority (EFSA)), stating that the *Bacillus cereus* variant *toyoi* strain is safe for the intended use as described in published reports EC, 2001, EFSA, 2007, EFSA, 2008.

Rubinum provides both published and unpublished data and information to demonstrate intended use. Rubinum supplied several articles, primarily in swine, to demonstrate that the administration of the *Bacillus cereus* variant *toyoi* strain at the appropriate use rate would support/maintain the gastrointestinal microflora. These articles varied in the target animal species and class, the test diets, and in the methodologies used to show the maintenance of the gut microflora.

FDA's evaluation of the data and information in Rubinum S.A. Animal Health notice

FDA has evaluated the information that Rubinum S.A. Animal Health discusses in its GRAS notice as well as other data and information that are available to the agency.

FDA has the following comments regarding manufacturing chemistry:

1. Your notice indicated only that the raw materials used in the fermentation of *B. cereus* variant *toyoi* spores were suitable for general use in food, or are appropriate for use as processing aids and are of food quality. You did not provide any raw material specifications, especially for calcium carbonate or corn flour which are added to the *B. cereus* variant *toyoi* spores.

2. EFSA reported that homogeneity was demonstrated in premixtures and feeds for pigs, poultry (chickens and turkeys for fattening), and rabbits; however, the data that EFSA used is not publicly available. Homogeneity is an important aspect of the manufacture of animal feeds. While, unpublished information may be used as corroborative, the pivotal information should be available in the published literature. Therefore, your notice does not adequately address homogeneity for

Bacillus cereus variant *toyoi* spores in premixtures and feeds for the intended animal species.

3. Similarly, stability is an important aspect of the manufacture of animal feeds. While you provided unpublished stability data which may be used as corroborative, the pivotal information should be available in the published literature. Therefore, your notice does not adequately address stability of the *Bacillus cereus* variant *toyoi* spores in the mash and pelleting process for the intended animal species.

FDA has the following comments regarding demonstration of intended use:

4. The information and data in the submitted notice are difficult to interpret due to the different methodologies used to generate the data, which are additionally confounded with the effects of animal class, age, and diet. Several cited authors note that the observed changes in GI microflora were impacted by animal age, hygiene, and the effect of weaning (Thelen (2004) and Jadamus (2000)). We also note that the type of change associated with *B. cereus* treatment varies with sampling location, either within the gastrointestinal tract or from the feces. The proposed claim is that the *B. cereus* helps to maintain/support the gut microflora. However, the microbial classes examined in the available articles represent only a fraction of the gut microbiotia, even when just culture techniques are considered and thus, it is not clear that the claim would be substantiated even if the data were more satisfactory.[1]

5. We note that no articles or information were submitted to address functional ruminants. The data for calves are not published and do not reflect the composition of the gut microflora as only *Lactobacillu*s and *E. coli* are reported. Similar comments pertain to the studies supplied to support use in poultry and rabbits. In several of the articles cited in the notice, there are reported treatment effects, yet the notice failed to address how these differences impact or substantiate the proposed intended use. In addition, these studies did not utilize diets that would be typical of animal husbandry practices in the United States. Thus, the notice does not include a reasonable demonstration that the administration of this *B. cereus* strain at the intended use levels helps maintain/support the gut microflora in the target animals.

We have the following administrative recommendations regarding the notice:

6. The notice should include consecutive page numbers throughout the entire notice.

[1] Leser, T., J. Amenuvor, T. Jensen, R. Lindecrona, M Boye, and K. Møller. 2002. Culture-Independent Analysis of Gut Bacteria: the Pig Gastrointestinal Tract Microbiota Revisited. App. Envir. Microbiol. 68:673-690. Robinson, I., S. Whipp, J. Bucklin, and M. Allison. 1984. Characterization of predominant bacteria from the colons of normal and dysenteric pigs. Appl. Environ. Microbiol. 48:964–969.

Conclusions

FDA has evaluated the data and information in AGRN 000-008 as well as other available information. The notice does not provide a sufficient basis for a determination that *Bacillus cereus* variant *toyoi* is GRAS under the conditions of its intended use in animal food.

In accordance with the Federal Register notice announcing the CVM Pilot Program, a copy of the text of this letter responding to AGRN 000-008, and a copy of the information in this notice that conforms to the information described in your GRAS exemption claim is available for public review and copying via the FDA home page at http://www.fda.gov. To view or obtain an electronic copy of this information, follow the hyperlinks from the "Animal & Veterinary" topic to the "Products" section to the "Animal Food & Feeds" to the "Generally Recognized as Safe (GRAS) Notifications" page where the Animal Food GRAS Inventory is listed.

If you have any questions about this letter, please contact Dr. M. Thomas Hendricks at 240-453-6869 or by email at thomas.hendricks@fda.hhs.gov. Please reference AGRN 000-008 in any future correspondence regarding this submission. If Rubinum S.A. Animal Health wishes to have FDA consider any new information that Rubinum S.A. Animal Health submits regarding *Bacillus cereus* variant *toyoi*, the appropriate mechanism would be for the notifier to submit, in accordance with proposed 21 CFR 570.36, a complete GRAS notice. FDA would assign a new file number to a new notice regarding *Bacillus cereus* variant *toyoi*.

Sincerely,

Sharon A. Benz, Ph.D., PAS
Director
Division of Animal Feeds
Center for Veterinary Medicine