U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR VETERINARY MEDICINE

July 1, 2020

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

Re: GRAS Notice No. AGRN 33

Dear Dr. Smedley:

The Food and Drug Administration (FDA, we) completed our evaluation of AGRN 33. We received KnipBio Inc.'s ("KnipBio") notice on August 16, 2019 and filed it on September 20, 2019. KnipBio submitted amendments to the notice on September 13, 2019 and May 5, 2020. KnipBio submitted amendments to update its statement on the basis of safety and to address issues concerning target animal safety (i.e. specification for formaldehyde content, an updated specification table and product labeling).

The notified substance is Dried *Methylobacterium extorquens* biomass. The notice informs us of KnipBio's view that Dried *Methylobacterium extorquens* biomass is GRAS, through scientific procedures, for use as a protein source in food for aquaculture crustacean species when used at an intended use rate of up to 6% of the diet.

KnipBio previously submitted GRAS notice AGRN 26, for which the notified substance is Dried *Methylobacterium extorquens* biomass. In GRAS Notice AGRN 26, the notifier informs FDA that the notified Dried *Methylobacterium extorquens* biomass is GRAS, through scientific procedures, for use as a protein source in food for finfish when used at an intended use rate of up to 10% of the diet. On February 11, 2019, CVM issued a response letter indicating that CVM had no questions at that time regarding the notifier's conclusion that the notified Dried *Methylobacterium extorquens* biomass is GRAS under its conditions of intended use as a protein source in food for finfish when used at an intended use rate of up to 10% of the diet.

The notifier provides by reference to AGRN 26 information on identity and manufacture. Dried *Methylobacterium extorquens* biomass is produced from genetically engineered *Methylobacterium extorquens* strain KB203 through fermentation using starting raw materials suitable for use in animal food and process controls. Following the fermentation, the biomass is centrifuged, and the resulting slurry is then spray dried. The notifier provided information regarding the common name of the ingredient, conditions of use, raw material specifications, final ingredient specifications, descriptions of the manufacture and the packaging, analytical methods for the determination of the contents of polyhydroxybutyrates (PHB), methanol, formaldehyde, and other test parameters, and stability information.

The notifier provides by reference to AGRN 26 a summary of the molecular techniques used to develop and characterize a genetically engineered *Methylobacterium extorquens*. The molecular techniques used to delete a specified operon and genes in another loci are described in the scientific literature and a United States patent. The genetic modifications were characterized using several techniques including polymerase chain reaction amplification across the deleted regions and Sanger sequencing of the amplicons, whole genome sequencing of the several strains leading up to and including the source organism, and appropriate changes in phenotype.

KnipBio provides a finished ingredient specification that consists of tests, acceptance criteria and methods: Appearance (fine powder), Color (light pink or reddish), Crude protein (>50%), poly-D-β-hydroxybutyrate (<25%), Moisture (<7%), Methanol (<0.3mg/g), Formaldehyde (<0.002mg/g), Lead (<0.05ppm), Total coliform (<5cfu/g). We note that ash can be a significant component of the notified substance

To address target animal safety of the intended use of Dried *Methylobacterium extorquens* biomass, KnipBio argues that safety data from Pacific white shrimp can be considered representative for addressing target animal safety of the intended use of the notified substance in crustacean diets. To address safety, the notifier presents both published and non-publicly available data. Data from the published article by Tlusty et al. (2017) are used by the notifier to support safe use of the notified substance in crustacean species. In this article, the pivotal target animal safety data are from a study in which the *Methylobacterium extorquens* biomass was fed to shrimp as a replacement for fish meal at use levels up to 12.6%. There were no observed reductions in shrimp weight gain and specific growth rate, when the notified substance was fed at an inclusion level of 6.3% of the diet. The notifier also includes corroborative unpublished data in shrimp from studies conducted at Auburn University to support the intended use rate of up to 6% of the diet.

The notifier also stated that the final commercial label for Dried *Methylobacterium extorquens* biomass would include a label statement about the maximum level of formaldehyde, i.e., below 0.002mg/g and the Use Directions would indicate the final feed must be pelleted to eliminate any viable *Methylobacterium extorquens* cells.

To address human food safety of the intended use of Dried *Methylobacterium extorquens* biomass, KnipBio provides literature references that were contained in the previous GRAS notice AGRN 26 and three new references which corroborate the human food safety of the intended use for crustacean species. KnipBio states that the notified substance will largely consist of protein and amino acids, which when ingested by the target species will be metabolized and incorporated into proteins and other molecules within the gut of the target species. All components of the notified substance will be digested in the gut of the target species like other protein ingredients. KnipBio states that the notified substance is expected to contain levels of PHB no greater than 25%, and that such levels will be diluted at least sixteen-fold in the diets of aquaculture crustacean species. The firm provides evidence that microorganisms capable of degrading PHB into short chain fatty acids can be found in the guts of many fish and crustacean species, and so it is expected that there will be no residual concentrations of PHB in the tissues of crustacean species to which the notified substance has been fed.

To address the intended use of the notified substance, the notifier provides empirical data and information, both published and unpublished, generated using Pacific white shrimp as a representative target aquaculture crustacean species, to support the notified intended use as a source of protein. Body weight gain was the parameter used to evaluate the intended use of the notified substance as a source of protein in aquaculture crustacean feed at the intended use rate of up to 6% of the feed.

Results of the published study by Tlusty et al. (2017) provide pivotal evidence to support the intended use of the notified substance as a source of protein in aquaculture crustacean feeds when added at levels up to 6%. Additionally, corroborative evidence from a series of unpublished trials provides secondary information to support the notified intended use.

Based on the totality of the data and information described above, KnipBio concludes that Dried *Methylobacterium extorquens* biomass produced from genetically engineered *Methylobacterium extorquens* strain KB203 through fermentation is GRAS under the conditions of its intended use for both the target animal and for humans consuming human food derived from these animals.

The Association of American Feed Control Officials (AAFCO) publishes a list of names and definitions for accepted animal food ingredients. FDA recognizes these names as being the "common or usual" names for animal food ingredients. FDA recognizes the name "Dried *Methylobacterium extorquens* biomass" as the common or usual name for the notified substance.

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 KnipBio's notice concluding that Dried *Methylobacterium extorquens* biomass is GRAS under its conditions of intended use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing Dried *Methylobacterium extorquens* biomass. Accordingly, our response should not be construed to be a statement that foods containing Dried *Methylobacterium extorquens* biomass, 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 amendments submitted by KnipBio and other information available to FDA, we have no questions at this time regarding KnipBio's conclusion that Dried *Methylobacterium extorquens* biomass is GRAS under the conditions of its intended use as a protein source in food for aquaculture crustacean species at an intended use rate of up to 6% of the diet. The agency has not, however, made its own determination regarding the GRAS status of the intended use of the notified Dried *Methylobacterium extorquens* biomass in food for aquaculture crustacean species 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 KnipBio to ensure that animal food ingredients that the firm 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 33 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 Dr. Louis Carlacci at 240-402-2921 or by email at louis.carlacci@fda.hhs.gov. Please reference AGRN 33 in any future correspondence regarding this submission.

Sincerely,

/s/

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

References

Tlusty, M., Rhyne, A., Szczebak, J.T., Bourque, B., Bowen, J.L., Burr, G., Marx, C.J. and Feinberg, L., 2017. A transdisciplinary approach to the initial validation of a single cell protein as an alternative protein source for use in aquafeeds. PeerJ 5:e3170; DOI 10.7717/peerj.3170.