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

February 11, 2019

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

Re: GRAS Notice No. AGRN 26

Dear Dr. Smedley:

The Food and Drug Administration (FDA, we) completed our evaluation of AGRN 26. We received KnipBio Inc.'s (KnipBio) notice on November 28, 2017, and filed it on February 7, 2018. KnipBio provided correspondence dated January 29, 2018, designating Dr. Kristi O. Smedley of Center for Regulatory Services, Inc. as an authorized representative for the GRAS notice. KnipBio submitted amendments to the notice on August 9, 2018, September 27, 2018, and December 3, 2018. KnipBio submitted amendments containing data and information on manufacturing chemistry (i.e., identity of certain metabolites, identity of starting raw materials, quality testing, validation of analytical methods), target animal safety (i.e., exposure calculation, spillover effects, organism viability, additional safety narrative including discussions on new references and content of a contaminant), metabolic pathways and demonstration of utility for the target animal.

The subject of the notice 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 species when used at an intended use rate of up to 10% of the diet.

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. The organism is grown in a mineral salts solution and methanol is added as the carbon source for growth. 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 and formaldehyde, and other test parameters, and stability information.

The notifier provides a summary of the molecular techniques used to develop and characterize a genetically engineered *M. 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 consisted 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), Lead (<0.05ppm), and 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 provides published data to support safety of the intended substance for use as a protein source to replace fishmeal at a use rate of up to 10% of the diet for finfish. This includes empirical data and information, both published and unpublished, generated using representative target aquaculture species, the characteristics and composition of the material in fish diets, and other information available to the agency.

The notifier provides the published article, Tlusty et al. (2017). Based on this article, inclusion of the dried *Methylobacterium extorquens* biomass at 25% in the diet of smallmouth grunt for 41 days did not significantly affect mortality, length, weight, condition factor, specific growth rate, or fish protein content when compared to that of the control. The notifier provides the published article, Hardy et al. (2018). Based on this article, inclusion of the dried *Methylobacterium extorquens* biomass at 10% in the diet of rainbow trout for 12 weeks did not significantly affect mortality, body weight, growth rate, feed consumption, or body composition when compared to data from control fish.

To address safety of the dried *Methylobacterium extorquens* biomass for use in diets for crustacean species, the notifier presents both published and non-publicly available data. Data from the published Tlusty et al. (2017) article are used by the notifier to support safe use of the notified substance in crustaceans. In this article, data are included from a study in which the *Methylobacterium extorquens* biomass was fed to shrimp as a replacement for fish meal in the diet at use levels of up to 12.6%. There was an observed reduction in shrimp weight gain and specific growth of shrimp at the 12.6% inclusion level. The notifier included corroborative unpublished data in shrimp from studies conducted at Auburn University.

The label for Dried *Methylobacterium extorquens* biomass shall state that the maximum level of formaldehyde is below 0.00004% w/v and the Use Directions shall specify that the final animal food must be pelleted.

To address human food safety of the intended use of Dried *Methylobacterium extorquens* biomass, 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 fish gut. 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 tenfold in the fish diet. The firm provides evidence that microorganisms

capable of degrading PHB into short chain fatty acids can be found in the gut of many fish species, and so it is expected that there will be no residual concentrations of PHB in the tissue of fish 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 representative target aquaculture species, to support the notified intend use. Body weight gain and digestibility are the parameters used to evaluate the intended use of the notified substance as a source of protein in aquaculture animal food at the intended use rate of up to 10% of the diet.

The results of studies published by Tlusty et al. (2017) and Hardy et al. (2018) that evaluated the use of the notified substance in diets for smallmouth grunt, Atlantic salmon, and rainbow trout, are provided to address the notified intended use of Dried *Methylobacterium extorquens* biomass in the diets for finfish as a source of protein at levels of up to 10% of the diet. Data for Pacific white shrimp from the published Tlusty et al. (2017) study, which are corroborated by unpublished studies, address the notified intended use of Dried *Methylobacterium extorquens* biomass in the diets for aquaculture crustaceans as a source of protein at levels of up to 10% of the diet.

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.

FDA's Evaluation of the data and information in KnipBio's notice and other data and information available to FDA

Based on the information contained in the notice and amendments submitted by KnipBio, Inc. and other information available to the agency, we have no questions at this time on KnipBio's conclusion that the use of Dried *Methylobacterium extorquens* biomass is safe for the target animals, finfish, under the conditions of its intended use as a protein source in food for finfish at an intended use rate of up to 10% of the diet. However, we have questions regarding KnipBio's conclusion that the use of Dried *Methylobacterium extorquens* biomass is safe for the target animals, crustaceans, under the conditions of its intended use as a protein source in food for crustaceans at an intended use rate of up to 10% of the diet for the following reasons:

> No information was provided to support safety in crustaceans at the proposed 10% inclusion level. There was an observed reduction in shrimp weight gain and specific growth of shrimp at the 12.6% inclusion level in the Tlusty et al. (2017) article and mixed results were observed in the unpublished studies provided.

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 intended conditions of 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 intended conditions of use in food for finfish. However, the notice does not provide a sufficient basis for a conclusion that Dried *Methylobacterium extorquens* biomass is GRAS under the conditions of its intended use in food for crustaceans. 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 animal food 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 26 is accessible to the public on our website for the Current Animal Food GRAS Notices Inventory at https://www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/GenerallyRecognizedasSafeGRASNotifications/ucm243845.htm.

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 26 in any future correspondence regarding this submission.

Sincerely,

/s/

Jeanette B. Murphy, M.S.
Acting Director
Office of Surveillance and Compliance
Center for Veterinary Medicine

References

Hardy, R.W., Patro, B., Pujol-Baxley, C., Marx, C.J., Feinberg, L., 2018. Partial replacement of soybean meal with *Methylobacterium extorquens* single-cell protein feeds for rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture Research, 49:2218-2224.

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.