August 13, 2020

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

Re: GRAS Notice No. AGRN 34

Dear Dr. Smedley:

The Food and Drug Administration (FDA, we) completed our evaluation of AGRN 34. We received C J CheilJedang Corporation's ("CheilJedang") notice on November 13, 2019 (including additional information dated November 19, 2019) and filed it on November 26, 2019. CheilJedang submitted an amendment to the notice on July 13, 2020 to clarify the composition, information on starting materials, the product specification, the analysis of the contents of biogenic amines, and an analytical method citation.

The notified substance is Dried L-Threonine Fermentation Product. The notice informs the United States Food and Drug Administration (FDA) of CheilJedang's view that the notified Dried L-Threonine Fermentation Product is GRAS, through scientific procedures, for use as a source of the nutrient L-threonine in the diets of livestock and poultry, at an intended use rate dependent on current good feeding practices for the target species.

To address the identity, method of manufacture, and specifications of Dried L-Threonine Fermentation Product, CheilJedang describes the common name of the ingredient, conditions of use, descriptions of manufacture and packaging, composition, analytical methods used to determine the contents of threonine and other constituents, the method used to ensure inactivation of the production organism, and stability information. Dried L-Threonine Fermentation Product is manufactured by fermentation of the genetically engineered strain *Corynebacterium glutamicum* KCCM80178. At the end of the fermentation, the broth is sterilized and the production organism is inactivated by lowering the pH and applying heat. The sterilized liquid is concentrated with steam prior to granulation, drying and particle sizing.

The notice includes a description of the genetic modifications that were performed during the development of the genetically engineered strain, *Corynebacterium glutamicum* KCCM 80178, which will be used as the source organism for the production of the Dried L-Threonine Fermentation Product. CheilJedang used homologous recombination to insert nucleotide sequences for genes involved in the L-threonine biosynthetic pathway. The genetic modifications were characterized using several techniques, including PCR amplification of the loci, Sanger sequencing of the amplicons, whole genome sequencing of the source organism, and appropriate

changes in phenotype (secretion of L-threonine). CheilJedang also addresses the potential for production of putative proteins, genetic stability, and the absence of the antimicrobial resistance marker that was used in the genetic engineering process.

CheilJedang provides a finished ingredient specification which include: L-threonine (≥75%), Moisture (≤5%), Ash (≤3%).

CheilJedang's target animal safety conclusion on the intended use of Dried L-Threonine Fermentation Product is based as follows: a) The safety of the production organism (*Corynebacterium glutamicum*) and donor organisms (*Corynebacterium glutamicum* ATCC 13032, and *Escherichia coli* K12); b) The safety of the genetic modifications used to genetically engineer the production strain *Corynebacterium glutamicum* KCCM 80178; c) The safety of the amino acid L-threonine; d) The safety of Dried L-Threonine Fermentation Product; and e) The safety assessment of impurities and/or potential contaminants in Dried L-Threonine Fermentation Product.

To address the safety of the production organism (*Corynebacterium glutamicum* ATCC 13032) and donor organisms (*Corynebacterium glutamicum* ATCC 13032, and *Escherichia coli* K12), the notifier provides publicly available information documenting their safe use in the industrial production of amino acids for animal food use.

To address the safety of the genetic modifications introduced to the production strain *Corynebacterium glutamicum* KCCM 80178, CheilJedang describes these genetic modifications and provides information on the lack of potential spillover effects on cell metabolism of the production strain *Corynebacterium glutamicum* KCCM 80178. The spillover information includes the following data and information: a) Flux Balance Analysis of the parent and production strains, *Corynebacterium glutamicum* ATCC 13032 and *Corynebacterium glutamicum* KCCM 80178, respectively; and b) A comparison of the levels of extracellular amino acids and organic acids in the fermentation media from parent and production strains, and the levels of biogenic amines in the Dried L-Threonine Fermentation Product.

To address the safety of L-threonine, CheilJedang cites publicly available information to support the supplemental use of L-threonine in animal food. To address the safety of the Dried L-Threonine Fermentation Product, CheilJedang includes the results of one unpublished acute toxicity study in rats and one unpublished Bacterial Reverse Mutation Assay. CheilJedang also includes information showing that viable *Corynebacterium glutamicum* KCCM 80178 is not detected in the Dried L-Threonine Fermentation Product, and that the dietary contribution of amino acids (other than L-threonine), total minerals and heavy metals associated with the maximum inclusion level of 0.5% Dried L-Threonine Fermentation Product is not significant and do not raise safety concerns in the target species.

To address human food safety of the intended use of Dried L-Threonine Fermentation Product, CheilJedang states that Dried L-Threonine Fermentation Product derived from the genetically engineered *Corynebacterium glutamicum* will be indistinguishable from other threonine sources, as will be the potential impurities, which are all normal components of animal feed. Threonine in animal feed results in the incorporation of threonine in tissue protein, excess threonine beyond the requirement of the animal will be excreted. Consequently, no free threonine occurs or

accumulates in target animal tissues and the only form of threonine that humans will be exposed to from its use in animal feed is in the form of protein that will be digested, absorbed, and metabolized consistent with human nutrient needs. CheilJedang concludes that the absence of residual threonine, or any other constituents of the notified substance, in the tissues of animals consuming any form of threonine in its diet will, therefore, not result in a subsequent human exposure or food safety issue.

To address the intended use of the notified substance, CheilJedang provides published empirical data and information generated through a bioavailability study in poultry using the notified substance to support the intended use. Body weight gain in broiler chickens was the parameter used to evaluate the intended use of the notified substance as reported in Wensley et al., 2019. Secondary supporting evidence for use in swine, and thus, other livestock species, is available based on arguments using data and information present in articles by Htoo et al., 2016, Oliveria et al., 2019, and Wensley et al., 2019 that addressed the bioavailabilities of other free essential amino acids present in related *Corynebacterium glutamicum* fermentation products.

Based on the totality of the data and information described above, CheilJedang concludes that Dried L-Threonine Fermentation Product produced from the genetically engineered strain *Corynebacterium glutamicum* KCCM 80178 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 feed ingredients. FDA recognizes these names as being the "common or usual names" for feed ingredients. FDA recognizes the name "Dried L-Threonine Fermentation Product" as the common or usual name for the Dried L-Threonine Fermentation Product (≥75% L-threonine) produced from the genetically engineered strain *Corynebacterium glutamicum* KCCM 80178.

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 CheilJedang's notice concluding that Dried L-Threonine Fermentation Product 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 L-Threonine Fermentation Product. Accordingly, our response should not be construed to be a statement that foods containing Dried L-Threonine Fermentation Product, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusion

Based on the information contained in the notice including an amendment submitted by CheilJedang and other information available to FDA, we have no questions at this time regarding CheilJedang's conclusion that Dried L-Threonine Fermentation Product is GRAS under the

conditions of its intended use as a source of the nutrient L-threonine in the diets of livestock and poultry, at an intended use rate dependent on current good feeding practices for the target species. The agency has not, however, made its own determination regarding the GRAS status of the intended use of the notified Dried L-Threonine Fermentation Product in food for livestock and poultry 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 CheilJedang 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 34 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 34 in any future correspondence regarding this submission.

Sincerely,

/s/

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

References

Htoo, J. K., Oliveira, J.P., Albino, L.F.T, Hannas, M.I., Barbosa, N.A.A., and Rostagno, H. S. 2016. Bioavailability of L-lysine HCl and L-lysine sulfate as lysine sources for growing pigs. J. Animal Science 94:253.

Wensley, M.R., Woodward, J.C., DeRouchey, J.M., Dritz, S.S., Tokach, M.D., Goodband, R.D., Walters, H.G., Leopold, B.A., Coufal, C.D., Haydon, K.D. and Lee, J.T. 2019. Effects of amino acid biomass or feed grade amino acids on growth performance of growing swine and poultry. Translational Animal Science, https://doi.org/10.1093/tas/txz163.

Oliveira, M.S.F., Htoo, J.K., Gonzalez-Vega, J.C. and Stein, H,H. 2019. Bioavailability of valine in spray-dried L-valine biomass is not different from that in crystalline L-valine when fed to weanling pigs. Journal of Animal Science. 97(10) :4227.