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

July 24, 2024

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

Re: Animal Generally Recognized as Safe Notice No. 64 - Dried L-Tryptophan Fermentation Product

Dear Dr. Smedley:

The Food and Drug Administration's Center for Veterinary Medicine (we) refers to a generally recognized as safe (GRAS) notice dated November 1, 2023, received on November 8, 2023, submitted on behalf of your client, CJ CheilJedang Corporation (the notifier). The subject of the submission is Dried L-Tryptophan Fermentation Product as a source of L-tryptophan in swine feed with a maximum usage being 0.37% of the feed. The notice informs us of the notifier's conclusion that the subject of the submission is GRAS through scientific procedures. Following an initial evaluation, you were notified in a letter dated November 30, 2023 that the GRAS notice was acceptable for filing, and the notice was designated as AGRN 64. On March 27, 2024, CVM received an amendment from the notifier containing additional chemistry, manufacturing, and controls information. We have completed our evaluation of AGRN 64 and have no questions at this time.

To address the identity, method of manufacture, and specifications of the notified substance, the notifier provides information for the method of manufacture, composition, analytical methods used to determine the contents of L-tryptophan and other constituents in the notified substance, and method to inactivate the production organism. The notified substance is Dried L-Tryptophan Fermentation Product produced by a genetically engineered *Corynebacterium glutamicum (C. glutamicum)* strain, KCCM 80346. At the end of the fermentation, the production organism is inactivated by applying heat. The sterilized liquid is concentrated prior to granulation, drying and particle sizing. The notifier provides specifications for the finished product as follows: L-tryptophan (≥60% on dry matter basis), Moisture (≤5%), Ash (≤10%), and absent of viable cells of production strain *C. glutamicum* KCCM 80346. The notifier also provides stability and packaging information for the notified substance.

To address target animal safety of the intended use of the notified substance, the notifier provides publicly available information on the following: the safety of L-tryptophan, the safety of the Dried L-Tryptophan Fermentation Product, and the safety assessment of impurities and/or potential contaminants in the Dried L-Tryptophan Fermentation Product. The notifier also provides the results of an unpublished sub-chronic toxicity study in rats and a bacterial reverse mutation assay. The notifier also provided information showing the lack of viable cells of the production strain *C. glutamicum* KCCM 80364 in the notified substance. The notifier also included the estimated dietary exposure of swine to amino acids (other than L-tryptophan), total minerals and heavy metals resulting from the inclusion of Dried L-Tryptophan Fermentation Product in swine feeds at levels consistent with good feeding practices.

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To address the human food safety of the intended use of the notified substance, the notifier assayed for the presence of potential Eosinophilia-Myalgia Syndrome (EMS)-related substances in Dried L-Tryptophan Fermentation Product using liquid chromatography with tandem mass spectrometry (LC-MS/MS). 1'-ethylidenebistryptophan (EBT), 3-phenylaminoalanine (PAA), 2-(2-hydroxyindoline)-Tryptophan (HIT) and (S)-2-amino-3-(2-(E)-dec-l-en-l-yl)-1H-indol-3-yl) propanoic acid (AAA) were not detected from any sample tested. 2(3-indoylmethyl)-L-tryptophan (IMT), 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-b]-indole-2-carboxylic acid (PIC), (S)-2-amino-3-(5-hydroxy-1H-indol-3-yl) propanoic acid (HTP) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) were detected, and total amount of those substances was below 800 mg/kg. The notifier concludes that since the amount of Dried L-Tryptophan Fermentation Product is a maximum of 0.37% of the diet (providing 0.22% L-tryptophan), the effect of potential EMS-related substance is not a concern, as these detected compounds already exist in highly purified L-tryptophan and commercial complete feed products at similar levels.

The notifier also reports on a 26-week study in pigs fed diets containing basal feed, basal feed top dressed with L-tryptophan (min. 98%) or top dressed with Dried L-Tryptophan Fermentation Product (min. 60%) (Lee et al, 2023). When the edible tissue and organs from animals fed basal feed, L-tryptophan, or Dried L-Tryptophan Fermentation Product were analyzed, IMT, HTP, and MTCA were not detected. Only 3a-hydroxy- 1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-b]-indole-2-carboxylic acid (PIC) was detected in all samples, but the notifier concludes that the difference between experimental groups was not significant. PIC was also detected in commercially available meat products.

The notifier adds that the FDA has no maximum limits in regulation, guidance, or policy (formal or informal) of potential EMS-related compounds in L-tryptophan dietary supplements, L-tryptophan as a human food ingredient, or for any other amino acid or food ingredient, nor does the FDA's Center for Food Safety and Applied Nutrition provide such guidance when assessing the safety of dietary supplements or human food ingredients or require the reporting of potentially EMS-related compounds. EMS or similar symptoms have not been reported in animals or humans consuming edible tissues from animals previously fed L-tryptophan. The notifier concludes that Dried L-Tryptophan Fermentation Product is safe for use in animal feeds and for humans consuming animal products previously fed the notified substance.

To address the utility of the notified substance, the notifier provides a published pivotal study showing the effects of the notified substance on the growth performance of swine. The experimental results show that the growth performance of pigs fed the notified substance is comparable to pigs fed crystalline tryptophan, suggesting that tryptophan is equally absorbed and utilized by the pigs regardless of the source.

The notice includes a description of the genetic modifications that were performed during the development of a biotechnology derived strain, *C. glutamicum* KCCM 80346, which will be used as the source organism for the production of the notified substance. In order to enhance L-tryptophan production, the notifier used homologous recombination to insert or delete various genes of intertwined metabolic pathways that consume the key precursors of L-tryptophan biosynthesis. The genetic modifications were characterized using several techniques, including polymerase chain reaction amplification of the locus, Sanger sequencing, and whole genome sequencing of the source organism. The notifier also addresses genetic stability and the absence of the antimicrobial resistance marker that was used in the biotechnological modification process.

The Association of American Feed Control Officials publishes in their Official Publication 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-Tryptophan Fermentation Product" 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 CJ CheilJedang Corporation's notice, as amended, concluding that Dried L-Tryptophan Fermentation Product as a source of L-tryptophan in swine feed 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 L-Tryptophan Fermentation Product. Accordingly, our response should not be construed to be a statement that foods containing Dried L-Tryptophan 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, as amended, submitted by CJ CheilJedang Corporation, and other information available to the FDA, we have no questions at this time regarding the notifier's conclusion that Dried L-Tryptophan Fermentation Product is GRAS under the intended conditions of use. The Agency has not, however, made its own determination regarding the GRAS status of the intended use of the notified substance 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 CJ CheilJedang Corporation to ensure that animal food ingredients that the notifier 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 64 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 Ms. Megan Hall at 301-796-3801 or at megan.hall@fda.hhs.gov.

Sincerely,

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

Reference:

Lee, D-H., Kim, Y. H., Baek, M., Heo, I-K, and Shin, Y. (2023). Simultaneous determination of L-tryptophan impurities in meat products. Amino Acids, 55, 173-182.