U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Alan B. Richards, PhD
Vanguard Regulatory Services, Inc.
1311 Iris Circle
Broomfield, CO 80020

Re: GRAS Notice No. GRN 001192

Dear Dr. Richards:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001192. We received the notice you submitted on behalf of Mitsubishi Chemical Corporation (MCC) on May 15, 2024 and filed it on July 30, 2024. MCC submitted amendments to the notice on December 27, 2024, February 17, 2025, and March 4, 2025, containing additional information on enzyme identity, manufacturing, specifications and analytical methods, and the safety narrative.

The subject of the notice is tannase enzyme preparation produced by *Aspergillus oryzae* (tannase enzyme preparation) for use as an enzyme at up to 25 mg Total Organic Solids (TOS)/L in tea-based beverages, juices, juice drinks, soft drinks, flavored water, jelly, and botanical extracts. The notice informs us of MCC's view that this use of tannase enzyme preparation is GRAS through scientific procedures.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component that catalyzes the chemical reaction as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. MCC's notice provides information about the components in the tannase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, tannase is identified by the Enzyme Commission Number 3.1.1.20,[1] and the Chemical Abstracts Service Number 9025-71-2. MCC states that tannase consists of two subunits with primary sequence of 570 amino acids and a molecular weight of 63 kDa. MCC notes that the native tannase consists of four pairs of the two subunits, forming a hetero-octamer with an approximate molecular weight of 300 kDa.

MCC states that the *A. oryzae* production organism is a non-pathogenic and non-

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/1/1/20.html

toxigenic fungus with a history of safe use in food production. The "NBRC 110971" production strain was not subjected to genetic engineering.[2]

MCC states that tannase enzyme preparation is manufactured by controlled fermentation of a pure culture of the *A. oryzae* production strain. The enzyme is secreted into the fermentation medium. After fermentation, the medium containing the enzyme is separated from the biomass, recovered, and concentrated by a series of filtration and ultrafiltration steps. The tannase enzyme concentrate is spray-dried and formulated with glucose resulting in a white to dark brown powder. MCC states that the entire process is performed in accordance with good manufacturing practices and that all raw materials and processing aids used in the manufacturing process are food grade and approved as a food additive, are the subject of an effective food contact notification, or are GRAS for their intended use. MCC also states that the tannase enzyme preparation does not contain any major food allergens.

MCC has established food-grade specifications including a limit for lead (≤0.5 mg/kg) and states that the tannase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 12th edition), and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). MCC provides results from analyses of three non-consecutive batches of tannase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism in the final product.

MCC intends to use tannase enzyme preparation at a maximum level of 25 mg TOS/L in tea-based beverages, juices, juice drinks, soft drinks, flavored water, jelly, and botanical extracts. Tannase hydrolyses the depside bonds in tannins (gallotannins and ellagitannins). MCC notes that the tannase enzyme is inactivated during food production. Using data from the 2015-2018 National Health and Nutrition Examination Survey, MCC estimates dietary exposure to tannase enzyme preparation from the use in food and drinks with the assumption that enzyme will remains present in the final food to be 0.14 mg TOS/kg bw/day at the mean and 0.31 mg TOS/kg bw/day at the 90th percentile for the U.S. population aged 2 years and older.

MCC relies on published information that discusses the safety of the *A. oryzae* production organism, the safety of microbial enzyme preparations used in food processing, and the safety of the tannase enzyme. MCC discusses results from published toxicity studies with MCC's tannase enzyme preparation, including a reverse mutation assay, a chromosomal aberration assay, and a 90-day repeated dose oral toxicity study in rats.[3] MCC concludes that the tannase enzyme preparation is not mutagenic or clastogenic. MCC states that no test-article related adverse effects were observed in the 90-day oral repeat dose toxicity study in rats up to the highest dose tested (915 mg

[2] MCC states that the production strain was been deposited at the National Institute of Technology and Evaluation, Biologic Resource Center (NBRC, Tokyo, Japan) in February 2015 as NBRC 110971.

[3] FDA notes that the toxicological studies with the MCC tannase enzyme preparation that were summarized in the notification were published in the *Japanese Journal of Medicine and Pharmaceutical Science*, and thus provide corroborative evidence in support of MCC's safety conclusion.

TOS/kg bw/d). In an amendment dated December 27, 2024, MCC summarizes published toxicological studies from another tannase enzyme preparation from *A. oryzae* and concludes that the tannase preparation was not mutagenic nor were there any treatment related adverse effects noted in the 90-day subchronic toxicity study in rats.[4] MCC discusses similarities between their tannase enzyme preparation and the tannase enzyme preparation that was used in the Lane et al., 1997 studies, and includes a sequence comparison of the enzymes. MCC concludes that the two tannase enzymes share greater than 99% sequence identity, and the slight differences are not expected to pose a safety concern. Additionally, MCC states that a literature search through March 2024 did not identify any information that would contradict a general recognition of safety of the tannase enzyme preparation.

MCC discusses publicly available literature, as well as the conclusions of several organizations and working groups, about the low risk of allergenicity posed by enzymes from their intended use, to address potential allergenicity due to tannase. Based on bioinformatic analyses, using criteria recommended by FAO/WHO (FAO/WHO, 2001; Codex Alimentarius, 2009; JECFA, 2016), MCC reports that no sequence homology of *A. oryzae* tannase to known allergens that would raise allergenicity concerns were identified. Based on the totality of the information available, MCC concludes that it is unlikely that oral consumption of tannase will result in allergenic responses from its intended uses.

Based on the data and information summarized above, MCC concludes that tannase enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, MCC states its intention to use tannase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

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 MCC's notice concluding that tannase enzyme preparation 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 tannase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing tannase enzyme preparation, if introduced or delivered

[4] Lane et al., 1997. Safety evaluation of tannase enzyme preparation derived from *Aspergillus oryzae*. Food Chem Toxicol 35: 207-212.

for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that MCC provided, as well as other information available to FDA, we have no questions at this time regarding MCC's conclusion that tannase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that tannase enzyme preparation is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 001192 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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Susan Carlson, Ph.D.
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