APPLICATION TO AMEND STANDARD 1.3.3 OF THE AUSTRALIA AND NEW ZEALAND FOOD STANDARDS CODE TO INCLUDE *ASPERGILLUS NIGER* AS A SOURCE ORGANISM FOR GLUTAMINASE

Executive Summary

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DATE:
04 January 2021

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Shin Nihon Chemical Co., Ltd. (Shin Nihon) is requesting an amendment to Standard 1.3.3 of the Food Standards Code (the Code) the include glutaminase (EC 3.5.1.2) derived from non-genetically modified *Aspergillus niger* for use as a processing aid in the manufacture of glutamic acid-rich food ingredients. Currently, Schedule 18 lists glutaminase from *Bacillus amyloliquefaciens* for use as a processing aid in the production of certain seasoning ingredients (*e.g.,* yeast extract, hydrolysed vegetable proteins and hydrolysed animal proteins) or food products used as seasonings (*e.g.,* soy sauce, miso, vinegar, fish sauce, etc.). Therefore, Shin Nihon is requesting to amend the Code to include a different source organism, *A. niger*, as a source of glutaminase.

Shin Nihon's glutaminase is manufactured in accordance with current Good Manufacturing Practice and HACCP. The enzyme is produced using food-grade materials and using quality-controlled fermentation and purification/recovery processes. Shin Nihon maintains a master cell bank and working cell bank from which the production strain is derived. The production strain is non-genetically modified and is selected based on its ability to produce high quantities of glutaminase, its viability, and lack of mycotoxin and secondary metabolite production. The production organism, *A. niger*, is well characterised and recognised as a safe and suitable source organism for the production of food ingredients. The production strain is non-pathogenic and non-toxigenic, and does not produce antibiotic activity.

The glutaminase food enzyme is produced as an ultra-filtered concentrate that meets the purity and microbial requirements established for enzyme preparations by JECFA and the Food Chemicals Codex. The results of 3 non-consecutive production batches of glutaminase from *A. niger* demonstrate that the manufacturing process produces a consistent product that conforms to the product specifications. In addition, the same production batches, as well as the production strain, were confirmed to be absent of mycotoxins and secondary metabolites.

Glutaminase derived from various microbial sources, including *Bacillus amyloliquefaciens*, *B. subtilis*, and *Candida* sp. are permitted for use as a processing aid in food processing (*e.g.,* in the production of protein hydrolysates and yeast extracts), or as a food additive (uses not specified) in Australia and New Zealand, China, France, South Korea, and Japan. The Association of Manufacturers and Formulators of Enzyme Products (AMFEP) lists glutaminase from non-genetically modified *B. amyloliquefaciens* or *B. subtilis* for use in food processing.

Glutaminase catalyses the hydrolytic deamination of L-glutamine to L-glutamate and free ammonia. Therefore, the intended uses of the enzyme in food processing increases the inherent L-glutamate content of food ingredients, such as yeast extracts and protein hydrolysates, that can, in turn, be added to finished foods such as fish products, gravies and sauces, plant protein products, snack foods, and soups and soup mixes. Increasing the L-glutamate content imparts or enhances the flavour profile of these foods. Based on the intended uses of glutaminase from non-genetically modified *A. niger*, the theoretical maximum daily intake of the enzyme was estimated to be 0.8 mg total organic solids (TOS)/kg body weight/day in the general population, as calculated using the Budget Method.

Shin Nihon's glutaminase from non-genetically modified *A. niger* was subject to toxicological testing. These tests involved an evaluation of genotoxicity in a bacterial reverse mutation test, mammalian chromosomal aberration test, *in vivo* Comet assays, as well as systemic toxicity in a 90-day repeated-dose oral toxicity study in rats. All tests were performed in compliance with the Organisation of Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (GLP) and appropriate OECD Test Guidelines using a representative batch of the ultra-filtered enzyme concentrate.

No mutagenic effects were reported in the bacterial reverse mutation test. Conversely, a significant increase in structural chromosome aberration in cultured mammalian cells were reported at doses greater than µg TOS/mL, suggesting the potential for clastogenic effects. The genotoxic potential of glutaminase from *A. niger* was investigated in two *in vivo* alkaline comet assays at doses up to 2,570 mg TOS/kg body weight. The test included activated and inactivated glutaminase at the highest dose to eliminate the effects of enzyme activity. No genotoxic findings were reported on the basis of the Comet assay, and therefore, glutaminase from non-genetically modified *A. niger* was concluded to be non-genotoxic. In the 90-day study, no adverse findings on any study parameter were reported at doses up to 2,570 mg TOS/kg body weight/day. The NOAEL was concluded to be 2,570 mg TOS/kg body weight/day, the highest dose tested, based on the results of this study.

The potential allergenicity of the food enzyme was considered in a search of the scientific literature as well as a sequence homology search in accordance with the methodology described by FAO/WHO and Codex Alimentarius. No significant matches to known allergens were reported, and no scientific reports that suggest glutaminase would produce an allergenic response following consumption were identified. Considering that the food enzyme would be inactivated and denatured under normal food processing conditions, the use of glutaminase from non-genetically modified *A. niger* in food processing is not expected to pose an allergenic risk to consumers.

The available data on glutaminase from non-genetically modified *A. niger* as manufactured by Shin Nihon supports the conclusion that its use as a processing aid in food processing does not present a significant risk to human health and is safe. The production organism is non-pathogenic and non-toxigenic, and is suitable for use in food production. Therefore, amendment of the Code to include non-genetically modified *Aspergillus niger* as a source of glutaminase does not present a safety concern and is justified.