Petition to Amend Standard 1.3.3 of the Australia New Zealand Food Standards Code to Include Glutaminase from *Bacillus amyloliquefaciens* as a Processing Aid

- Executive Summary -

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EXECUTIVE SUMMARY

Amano Enzyme Inc. (referred to Amano Enzyme hereafter) is proposing to amend Standard 1.3.3 of the Australia New Zealand Food Standards Code to include glutaminase derived from *Bacillus amyloliquefaciens* as an enzyme of microbial origin. Glutaminase (L-glutamine aminohydrolase, EC 3.5.1.2, CAS number 9001-47-2) catalyses the hydrolysis of L-glutamine to L-glutamic acid. The glutamic acid produced by this reaction is a source of glutamate, which is an important component of the quality and taste of the foods in which glutaminase is added. The enzyme preparation marketed by Amano Enzyme (trade name Glutaminase SD-C100S) comprises 9% of the glutaminase enzyme derived from *B. amyloliquefaciens* and 91% sodium chloride (NaCl). Glutaminase SD-C100S is proposed for use as a processing aid in the production of certain seasoning ingredients (*e.g.*, yeast extracts, hydrolysed vegetable protein, and hydrolysed animal protein) or foods used for seasoning (*e.g.*, soy sauce, miso, vinegar, fish sauce, *etc.*) at levels of 0.2%, which corresponds to 0.018% of the glutaminase concentrate. Glutaminase SD-C100S will be used to increase the glutamate content in these products, which will reduce or completely avert the need for the addition of external sources of glutamate, such as monosodium glutamate.

The glutaminase enzyme is derived from a non-genetically modified strain of *B. amyloliquefaciens* (strain GT2). *Bacillus amyloliquefaciens* GT2 is obtained by subjecting the parent strain, *B. amyloliquefaciens* NP, to a conventional mutation process involving treatment with N- methyl-N'-nitro-N-nitrosoguanidine. The production process of the glutaminase enzyme comprises a cultivation step with *Bacillus amyloliquefaciens* GT2, followed by several filtration and purification steps to result in a glutaminase concentrate. The enzyme concentrate is then diluted with NaCl to produce the final enzyme preparation (Glutaminase SD-C100S) intended for use in the manufacture of food/food ingredients. All of the raw materials used in the manufacture of the glutaminase enzyme are safe and suitable for use. Glutaminase SD-C100S is produced under Good Manufacturing Practice and ISO-9000 quality management systems. Production controls are in place to monitor the strain during the fermentation and ensure the avoidance of genetic drift. Furthermore, the product specifications along with extensive batch analysis of Glutaminase SD-C100S demonstrate the purity of the enzyme preparation, including the absence of microbiological, mycotoxins, and heavy metal contaminants, as well as the lack of antibiotic activity.

Glutaminase SD-C100S is stable for at least 12 months when maintained at a pH of 5.0 to 9.0 and a temperature of up to 50°C. Glutaminase SD-C100S is inactivated when exposed to temperature greater than 65°C and a pH of greater than 9.0, and Amano Enzyme recommends that an inactivation step be included when Glutaminase SD-C100S is used as a processing aid in the manufacture of foods/food ingredients. Amano Enzyme has also determined that Glutaminase SD-C100S does not exert significant secondary activity with non-target food substances.

The safety of glutaminase derived from *B. amyloliquefaciens* can be supported by its history of use in Japan and Europe, as well as toxicity studies (unpublished) that have been conducted with the glutaminase concentrate. Glutaminase is on the "List of Existing Food Additives" in Japan (MHLW, 2014), and glutaminase derived from *B. amyloliquefaciens* GT2 has been approved for use as a processing aid in the production of protein hydrolysates and yeast extracts in France (AFSSA, 2009). The glutaminase concentrate from *B. amyloliquefaciens* GT2 was not mutagenic or genotoxic when tested using the Ames Assay (van Delft, 1998) or the *in vitro* chromosomal aberration assay (van Delft and de Vogel, 1998). In a 13-week oral toxicity study conducted in Wistar rats (20/sex/group), the No-Observed-Adverse-Effect Level (NOAEL) was determined as 0.6% of glutaminase concentrate in the diet, corresponding to approximately 388 and 450 mg/kg body weight/day in male and female rats, respectively (Appel, 1999). Some reductions in growth were observed at the highest dose tested (2.0% of glutaminase concentrate), though a corresponding decrease in food consumption was also observed without any other signs of toxicity observed, suggesting that the effect may have been related to reduced palatability of the diet (Appel, 1999).

As further support for the safety of glutaminase derived from *B. amyloliquefaciens*, the source organism and its closely related species *Bacillus subtilis* have a long history of use in food production globally. For example, both *B. amyloliquefaciens* and *B. subtilis* are used as a source organism for food products and processing aids in Australia/New Zealand (FSANZ, 2014), and carbohydrase and protease enzyme preparations derived from these species have been deemed Generally Recognized as Safe for use as food ingredients in the United States (U.S. FDA, 1999). Therefore, the source organism of glutaminase (*i.e.*, *B. amyloliquefaciens*) does not pose any risk for pathogenicity or toxigenicity. Glutaminase derived from *B. amyloliquefaciens* also does not pose any allergenicity concerns, given the long history of use of the source organism. Additionally, a bioinformatic search indicated that the amino acid sequence of glutaminase does not match that of any allergens in the Allergen Database for Food Safety. It is notable that since 1992, no adverse effects have been reported in workers exposed to *B. amyloliquefaciens* GT2.

The estimated daily intake (EDI) of Glutaminase SD-C100S was calculated using the following data and assumptions:

- the mean consumption of foods in which Glutaminase SD-C100S is proposed for use;
- the intended level of use (0.2%);
- the anticipated residual Total Organic Solids (TOS) content of foods; and
- the assumption that the enzyme was not inactivated during food production.

Based on these factors the maximum anticipated level of exposure to TOS resulting from the proposed food uses for Glutaminase SD-C100S was 0.17 kg body weight/day. When compared to the NOAEL derived from the 13 week toxicity study, the safety margin (NOAEL/EDI) was calculated to be at least 2,282 for all population groups. As such, no safety concerns are anticipated with the proposed use of Glutaminase SD-C100S as a processing aid in Australia/New Zealand.