EXECUTIVE SUMMARY

Advanced Enzyme Technologies Ltd. is proposing to amend Schedule 18 of the Australia New Zealand Food Standards Code to include a processing aid maltogenic alpha amylase (E.C. 3.2.1. 133) from *Geobacillus stearothermophilus*, which is produced with a genetically modified strain of *Escherichia coli* BLASC. The processing aid maltogenic alpha amylase, produced with a genetically modified strain of *Bacillus subtilis* is already listed in the Schedule 18 (Australia New Zealand Food Standards Code – Schedule 18 – Processing aids, Sept 2020). This application is to amend Schedule 18 to include a new microbial source, i.e. *E. coli* BLASC as a production microorganism for the processing aid.

The application is submitted for assessment by the general procedure.

Maltogenic alpha amylase (E.C. 3.2.1.133, CAS number: 160611-47-2) is an enzyme catalysing the hydrolysis of (1→4)-alpha-D-glucosidic linkages in starch polysaccharides to produce maltose (dimer of glucose units) and maltotriose (trimer of glucose units) as main hydrolysis products.

Maltogenic alpha amylase is used as a processing aid in baking, brewing and starch processing applications. In baking process, the selective hydrolysis of starch by the maltogenic alpha amylase prevents retrogradation of starch in baked products, which improves their shelf life. Maltogenic alpha amylase is denatured and inactivated due to high temperature during baking process and no residual enzyme activity remains in the finished product.

In starch processing, the saccharification process is added by the application of maltogenic alpha amylase. Maltogenic alpha amylase hydrolyses 1,4-oligosachharide links to predominantly yield maltose/ glucose syrup. Post saccharification process, the heating steps cause denaturation of the maltogenic amylase and no residual enzyme activity remains in the product.

In brewing process, maltogenic alpha amylase hydrolyses the starch containing substrates to produce simple sugars that support yeast growth during fermentation, resulting in better yields of alcohol. The increase in temperature following the mashing process results in inactivation of the maltogenic alpha amylase; further, the enzyme is also denatured in the consecutive lautering or mash filtration and wort boiling steps.

In these food processing applications, the maltogenic alpha amylase has technological function during food processing and not in the final food. Maltogenic alpha amylase is used as a processing aid, the enzyme is either not present in the final food or present in insignificant quantities having no function or in denatured form.

Safety of the strain and the enzyme product has been thoroughly assessed

- The production organism has a history of safe use for the food-grade enzyme production and is not known to produce any toxic metabolites.

- The genetic modifications in the production strain are well-characterised, safe and the recombinant DNA is stable in the production organism and unlikely to pose a safety concern.
- The enzyme preparation complies with international specifications ensuring absence of contamination by toxic substances or noxious microorganisms
- Sequence homology assessment to known allergens and toxins shows that oral intake of the maltogenic alpha amylase does not pose allergenic or toxic concern.
- *In vitro* mutagenic studies showed no evidence of genotoxic potential of the enzyme preparation.
- An oral feeding study in rats for 13-weeks showed that all dose levels were generally well tolerated and no evidence of toxicity.
- Theoretical Maximum Daily Intake was calculated using the Budget Method and Food Consumption Database. Based on a worst-case scenario, Theoretical Maximum Daily Intake (TMDI) was calculated as 1.0593 mg TOS/kg body weight/day. This still offers a 791 fold margin of safety.

European Food Safety Authority (EFSA) has conducted a safety assessment on the maltogenic alpha amylase from *Geobacillus sterothermophilus* produced with the strain *E. coli* BLASC and found no safety concern under intended conditions of use (EFSA Journal, 2019).

The processing aid maltogenic alpha amylase, produced using another production microorganism, has already been evaluated by FSANZ and included in Schedule 18. [Permitted enzymes (section 1.3.3—6)—Enzymes of microbial origin of Schedule 18, 2016; Page no. 8]

Based on the results of safety studies conducted and scientific literatures, maltogenic alpha amylase from *E. coli* BLASC has been demonstrated as safe for its intended applications and at the proposed usage levels. No safety concerns are anticipated with the proposed use of maltogenic alpha amylase as processing aid in Australia/ New Zealand.