Executive Summary: Application for the Authorization of D-psicose 3-epimerase from Genetically Modified *Escherichia coli* Strain K-12 in Australia and New Zealand

Tate & Lyle Solutions USA LLC ("Tate & Lyle") is submitting an application for the authorisation and inclusion of -psicose 3-epimerase (Enzyme Commission [EC]) 5.1.3.30) from a genetically modified (GM) strain of *Escherichia coli* strain K-12 in Standard 1.3.3 of the Food Standards Code. The enzyme is intended for use as a processing aid for the production of D-allulose (also known as D-psicose). The recipient organism, *E. coli* strain K-12 W3310-TKO, is a GM derivative of *E. coli* strain K-12 W3110, one of the most widely used and well-characterized laboratory strains of *E. coli.* Strain K-12 W3110 is, in turn, derived from the wild-type *E. coli* strain K-12. The recipient organism was genetically modified to express a synthetic gene from *Desmospora* sp. encoding the D-psicose 3-epimerase enzyme. The gene was codon-optimized for expression by the *E. coli* production organism. The principal enzymatic activity of Tate & Lyle's enzyme is D-psicose 3-epimerase.

Tate & Lyle's D-psicose 3-epimerase is manufactured in accordance with cGMP and FSSC 22000. The enzyme is produced using food-grade materials and using quality-controlled fermentation and purification/recovery processes. The production strain has been deposited in a recognised culture collection. The production organism from which the enzyme is derived is a GM strain of *E. coli* strain K-12 modified to express a synthetic and codon-optimized gene encoding D-psicose 3-epimerase. *E. coli* strain K-12 is well-characterized and has a long history of safe use in the production of enzymes used in food processing, and there have not been any known reports of pathogenicity and/or virulence.

The product specifications of the enzyme are compliant with the purity requirements for enzyme preparations established by the Food Chemicals Codex and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Analytical data on 3 non-consecutive production-scale batches of the immobilised enzyme demonstrate that the manufacturing process produces a consistent product that conforms to the product specifications.

D-psicose 3-epimerase catalyses the reversible epimerization of D-fructose at the C3 position to produce D-allulose. The enzyme acts by tautomerizing the proton at the C3 position of D-fructose, resulting in the formation of an enediol intermediate. The intermediate is then selectively re-protonated at C3, forming D-allulose. D-psicose 3-epimerase is a metal-dependent enzyme that requires a divalent metal ion cofactor to exhibit activity. D-psicose 3-epimerase does not have any other subsidiary or side activities.

Tate & Lyle's D-psicose 3-epimerase derived from GM *E. coli* strain K-12, the subject of this application, was evaluated by the EFSA Panel on Food Enzymes for use in the processing of fructose to produce allulose. Based on the absence of viable cells and residual DNA from the production organism in the enzyme, toxicological testing requirements were waived. The Panel reviewed the available data on the enzyme and concluded that it does not give rise to safety concerns under the intended conditions of use.

The dietary exposure to the enzyme was not estimated based on analytical data demonstrating the absence of enzyme total organic solids in the final allulose product.

The potential toxicity of the D-psicose 3-epimerase enzyme was addressed the extensive digestion of the enzyme predicted in digestion models and the lack of similarity of the amino acid sequence to known protein toxins. Toxicological studies were not conducted using the D-psicose 3-epimerase from GM *E. coli* strain K-12 as dietary exposure to the immobilised food enzyme is not expected.

The potential allergenicity of the D-psicose 3-epimerase from GM *E. coli* strain K-12 was assessed by investigating sequence homology between the enzyme and known food allergens using the methodology described by FAO/WHO, Codex Alimentarius, and EFSA GMO Panel. No significant results suggestive of allergenic cross-reactivity of the D-psicose to known allergens were identified. Considering that the enzyme would be denatured if carried over into the final allulose product under the conditions of food processing, it is not expected to pose an allergic risk to consumers.

The totality of the data provided in this application supports the conclusion that Tate & Lyle's D-psicose 3-epimerase from GM *E. coli* strain K-12 expressing a synthetic and codon-optimized gene from *Desmospora* sp. is safe for use as a processing aid in the production of D-allulose and does not present a risk to human health. The enzyme is compliant with the purity requirements for enzyme preparations established by the Food Chemicals Codex and JECFA. The production organism is non-toxic, non-pathogenic, and is suitable for use in food production. Therefore, the authorisation of D-psicose 3-epimerase from GM *E. coli* strain K-12 *e* in Australia and New Zealand does not present a safety concern.