EXECUTIVE SUMMARY:

DuPont Nutrition & Biosciences (N&B) is seeking approval for a "Alpha-amylase (EC 3.2.1.1)" enzyme for use as processing aid in brewing application and for use in potable alcohol production. The enzyme is designated as "Alpha-amylase" throughout the dossier.

The enzyme Alpha-amylase is derived from a selected non-pathogenic, non-toxigenic strain of *Trichoderma reesei* which is genetically modified to overexpress the alpha-amylase gene from *Aspergillus kawachii.*

The enzyme is intended for use in brewed beverages and potable alcohol production. In brewing, Alpha-amylase is typically added in to the cereal cooker or in the mashing step and is thus denatured already in the consecutive lautering or mash filtration step. In the potable alcohol production industry, the Alpha-amylase is added in the pre-treatment, liquefaction and/or pre-saccharification step.

In Brewing and Potable alcohol production, Alpha-amylase increase extraction and saccharification of starch maximizing the conversion of starchy substrate to fermentable carbohydrates.

In all of these applications, Alpha-amylase will be used as a processing aid where the enzyme is either not present in the final food or present in insignificant quantities having no function or technical effect in the final food.

To assess the safety of the Alpha-amylase for use in these applications, Dupont N&B vigorously applied the criteria identified in the guidelines as laid down by Food Standards Australia New Zealand (FSANZ) and U.S. Food and Drug Administration (FDA) utilizing enzyme toxicology/safety data, the safe history of use of enzyme preparations from *T. reesei* and of other Alpha-amylase enzymes in food, the history of safe use of the *T. reesei* production organism for the production of enzymes used in food, an allergenicity evaluation, and a comprehensive survey of the scientific literature.

In addition, different endpoints of toxicity were investigated, and the results are evaluated and assessed in this document. In genotoxicity studies, Alpha-amylase is not mutagenic, clastogenic. Daily oral administration of Alpha-amylase up to and including a dose level of 184 mg total protein/kg bw/day or 229.6 mg TOS/kg bw/day does not result in any manifestation of systemic, hematologic, or histopathologic adverse effects.

Based on a worst-case scenario that a person is consuming Alpha-amylase in a brewed beverage, the calculated Theoretical Maximum Daily Intake (TMDI) will be 0.39 mg TOS/kg body weight/day. This still offers a 589 fold margin of safety.

Based on the results of safety studies and other evidence, Alpha-amylase has been demonstrated as safe for its intended applications and at the proposed usage levels. Approval of this application would provide manufacturers and/or consumers with benefits of facilitating the brewing and potable alcohol production processes by imparting processing efficiencies and/or more consistent product quality. Moreover, the applications lead to more effective production processes, resulting in better production economy and environmental benefits such as the use of less raw materials and the production of less waste. process, lowering the manufacturing cost, and improving quality of final foods.