EXECUTIVE SUMMARY

DuPont Industrial Bioscience (IB) is seeking approval for an Aspergillopepsin I enzyme product for use in processing of all food raw materials which naturally contain proteins. The enzyme is herein designated as Acid Fungal Protease (AFP).

AFP is derived from a selected non-pathogenic, non-toxigenic strain of *Trichoderma reesei* which is genetically modified to overexpress a native *T. reesei* protease enzyme, Aspergillopepsin I.

AFP will replace other proteases currently marketed for the intended uses. AFP will be used in potable alcohol production and protein processing.

In all of these applications, AFP 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 AFP for use in potable alcohol production and protein processing, DuPont IB vigorously applied the criteria identified in the guidelines utilizing enzyme safety data, the safe history of use of other enzyme preparations from *T. reesei* and of other proteases in food, the safe history of use of the production organism for the production of other enzymes used in food, and a comprehensive survey of the scientific literature.

The safety of the food enzyme from *T. reesei* has been assessed using toxicology studies conducted on earlier strains of the DuPont *T. reesei* Safe Strain Lineage. The most suitable standard package of toxicological tests from the Safe Strain Lineage was identified to support the safety of the food enzyme object of the current dossier. The toxicological tests showed the following results:

- Ames test: no mutagenic activity under the given test conditions
- Chromosomal aberrations: no clastogenic activity under the given test conditions
- 90-day oral toxicity on rats: The No Observed Adverse Effect Level (NOAEL) is 1000 mg TOS/kg bw/day, which is the high dose in the study

Based on a conservative assumption and a highly exaggerated value consumption data, the NOAEL still offers a 340 fold Margin of Safety.

Based on the results of safety studies and other evidence, AFP has been demonstrated as safe for its intended applications and at the proposed usage levels. Approval of this application would provide manufacturers with benefits of facilitating the process and lower the manufacturing cost in potable alcohol production and protein processing.