



EXECUTIVE SUMMARY:

DuPont Industrial Bioscience (IB) is seeking approval for a pullulanase enzyme product for use in converting pullulan and amylopectin in the processing of food raw material which naturally contains the substrate. The enzyme is herein designated as Pullulanase.

The Pullulanase preparation which is the subject of this submission is derived from a pure culture of a non-pathogenic, non-toxicogenic, and asporogenic strain of *Bacillus licheniformis*, BMP139. BMP139 is a genetically modified strain that has been constructed to express a pullulanase gene derived from a non-pathogenic and non-toxicogenic microorganism *Bacillus deramificans*.

The food enzyme object of the dossier is typically used in brewing and starch processing,

In all of these applications, Pullulanase 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 Pullulanase for use in brewing and starch processing, DuPont IB vigorously applied the criteria identified in the guidelines laid out by Food Standards Australia New Zealand (FSANZ) and U.S. Food and Drug Administration (FDA) utilizing enzyme safety data, the safe history of use of other enzyme preparations from *B. licheniformis* and of other pullulanases 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.

To assess the safety of Pullulanase produced by *B. licheniformis* BMP 139 in foods, DuPont IB investigated different endpoints of toxicity through studies conducted at BioReliance (Maryland) and ClinTrials (Canada). These studies are evaluated and assessed in this document. The toxicology studies included:

- Ames mutagenicity studies
- *In vitro* Chromosomal Aberration Study
- Sub-chronic 90-day toxicity study in the rat

Based on conservative assumptions and a highly exaggerated value consumption data, the NOAEL still offers a 4837× fold Margin of Safety.

Based on the results of safety studies and other evidence, Pullulanase 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 process and starch processing, potentially lowering the manufacturing cost, and improving quality of final foods.