EXECUTIVE SUMMARY:

IFF Health and Biosciences (IFF H&B) is seeking approval for a "Chymosin (EC 3.4.23.4)" enzyme for use as processing aid in dairy application. The enzyme is designated as "Chymosin" throughout the dossier.

The enzyme Chymosin is derived from a selected non-pathogenic, non-toxigenic strain of *Trichoderma reesei* which is genetically modified to overexpress the Chymosin gene from *Bos taurus.*

The enzyme is intended for use in dairy applications primarily in the manufacture of cheese and cheese products, fermented and rennetted milk products. In the applications Chymosin performs the technological function of clotting milk by highly specific cleavage activity of a single bond in κ-chain of casein.

In all these applications, Chymosin 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 Chymosin for use in these applications, IFF H&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) utilising enzyme toxicology/safety data, the safe history of use of enzyme preparations from *T. reesei* and of other Chymosin 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.

The safety of the food enzyme from *T. reesei* has been assessed using toxicology studies conducted on earlier strains of the IFF H&B *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: Under the conditions of this study, the no-observed-adverse-effect-level (NOAEL) was established at the high dose 700 mg total organic solids (TOS)/kg body weight/day.

Based on a worst-case scenario that a person is consuming Chymosin in dairy based food application, the calculated Theoretical Maximum Daily Intake (TMDI) will be 0.075 mg TOS/kg body weight/day. This still offers a 9,333-fold margin of safety.

Based on the results of safety studies and other evidence, Chymosin 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 coagulation of casein, lowering the manufacturing cost, and improving quality of dairy based foods.