Executive Summary

Inzymes ApS 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 *Thermothelomyces heterothallica* 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. 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, Inzymes ApS applied the criteria identified in the guidelines as laid down by Food Standards Australia New Zealand (FSANZ), European Food Safety Agency (EFSA) and U.S. Food and Drug Administration (FDA).

Enzyme toxicology studies were performed and safety data were obtained. A history of safe use of chymosin for cheese production as well as a history of safe use of food enzymes produced by the host strain *Thermothelomyces heterothallica* has been included in this application as well as an extensive allergenicity evaluation, and a comprehensive survey of the scientific literature.

The safety of food enzymes produced by *Th. heterothallica* has been assessed using toxicology studies on the bovine chymosin product, including *in vitro* genotoxicity studies and sub-chronic oral gavage toxicity studies on rodents.

No mutagenic activity was identified using Ames and Micronucleus tests. A 90 days oral toxicity study showed no adverse effects at the highest dosage applied.

The no-observed adverse-effect-level (NOAEL) was established at the high dose of 100 mg total organic solids (TOS)/kg body weight/day.

Using the EFSA calculator FEE Cheese[i] tool it was calculated that the highest theoretical intake of chymosin would be in a high consumption (95% percentile) group of toddlers with a daily intake estimated to be 0,064 mg/kg bw/ day.

A NOAEL level of 100 mg TOS/kg body weight/day and a calculated Theoretical Maximum Daily Intake (TMDI) of 0,064 mg TOS/kg bw/ day gives a 1563 times safety margin.

Based on the results of safety studies, a long history of safe use 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.

The present application seeks to amend schedule 18 - Processing Aids of the Australia New Zealand Food Standards Code (the Code) to approve a Chymosin (EC 3.4.23.4) enzyme preparation from

Thermothelomyces heterothallica, produced by Inzymes ApS, for use as a processing aid in the production of dairy products.

[i] Feim - Default (europa.eu) https://r4eu.efsa.europa.eu/app/feim