

## **EXECUTIVE SUMMARY:**

IFF Health & Biosciences (IFF) is seeking approval for a “Subtilisin (EC 3.4.21.62)” enzyme for use as processing aid in protein processing. The enzyme is designated as “Subtilisin” throughout the dossier, and in some documentation referred to as ‘Alkaline Protease’.

The enzyme Subtilisin is derived from a selected non-pathogenic, non-toxigenic strain of *Bacillus subtilis* which is genetically modified to overexpress the Subtilisin gene from *Bacillus clausii* (formerly taxonomically classified as *Bacillus lentus*).

The enzyme is intended for use in the enzymatic cleavage of protein bonds. This functionality is of benefit in the processing of all foods and food ingredients which naturally contain proteins and peptides, these food groups include, but not exclusively, dairy processing, egg processing, meat and fish processing, and protein processing.

When applied in food protein processing, Subtilisin will be used as a processing aid where the enzyme is either not present in the final food or present in insignificant quantities, thus having no function or technical effect in the final food.

To assess the safety of the Subtilisin for use in protein processing, IFF 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 *B.subtilis* and of other subtilisin enzymes in food, the history of safe use of the *B.subtilis* production organism for the production of enzymes used in food, an allergenicity evaluation, and a comprehensive survey of the scientific literature.

In addition, toxicity was investigated, in a 90-day oral toxicity study and the results are evaluated and assessed in this document. Daily oral administration of Subtilisin up to and including a dose level of 420 mg total protein/kg bw/day or 480.6 mg TOS/kg bw/day did not result in any manifestation of systemic, hematologic, or histopathologic adverse effects.

Based on a worst-case scenario that a person is consuming Subtilisin from processed protein products, the calculated Theoretical Maximum Daily Intake (TMDI) will be 2.73 mg TOS/kg body weight/day. This still offers a 175-fold margin of safety.

Based on the results of safety studies and other evidence, Subtilisin 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 in protein processing to facilitate the production of peptides (protein hydrolysis) with better functional properties such as solubility, emulsification, gelling and foaming, thus improving quality of final foods.