

## Executive Summary: Application for the Authorisation of Steviol Glycosides Produced by Enzymatic Conversion in Australia and New Zealand

Adorvia Biotechnology Co., Ltd (“Adorvia”) is submitting an application to amend the Code to authorise steviol glycosides with a high Rebaudioside (Reb) M or Reb D content produced by enzymatic conversion. The food additive is produced using sucrose synthase,  $\beta$ -1,3-glycosyltransferase, and  $\beta$ -1,2-glycosyltransferase obtained from genetically modified strains that are derivatives of *Escherichia coli* BL21 (DE3). These enzymes convert the naturally occurring steviol glycosides within an extract of *Stevia rebaudiana* Bertoni leaves to Reb M and Reb D. The production process is consistent with the process described in Annex 3 for enzyme-modified steviol glycosides by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The final product is a steviol glycoside preparation containing high amounts of Reb M or Reb D that meets JECFA’s purity specifications and is similar to other steviol glycosides already permitted for use in a range of conventional food and beverage products in Australia and New Zealand under Schedule 15.

The steviol glycosides with a high Reb M or Reb D content is produced from highly purified Reb A (total steviol glycoside  $\geq 95\%$ ) through *in vitro* multi-enzyme cascade reaction. In the first stage, genetically modified (GM) strains of *E. coli* BL21 (DE3) are fermented to produce 3 enzymes, which are then isolated for use in the enzymatic conversion process. In the second stage, the purified Reb A powder and sugar (glucose donor) are solubilised in water and mixed with the enzymes to generate a mixture of steviol glycosides containing more than  $>50\%$  Reb M. The enzymes are then utilised to produce steviol glycosides with a high Reb M or Reb D content, with total steviol glycoside content  $\geq 95\%$ . All materials and processing aids used in the manufacture of Adorvia’s steviol glycosides with high Reb M or Reb D content produced by enzymatic conversion are food-grade and comply with relevant internationally recognised standards. Analytical data on 5 non-consecutive production-scale batches of steviol glycosides with a high Reb M and 5 batches with high Reb D content demonstrate that the manufacturing process produces a consistent product that conforms to the product specifications.

Adorvia intends to market the food additive for use as an intense sweetener under the same conditions of use as those presently authorised for steviol glycosides. Since the food additive is intended to directly replace other steviol glycosides, the intake levels are expected to be the same as those already present in the market. Therefore, a separate intake assessment for Adorvia’s steviol glycosides was not conducted for this application.

Steviol glycosides are approved for use as food additives and/or sweeteners in a number of jurisdictions, including Australia/New Zealand, the European Union, United States (U.S.), Canada, Asia, Central/South America, and Africa. In the U.S., over 80 Generally Recognized as Safe (GRAS) notices have been submitted to the U.S. Food and Drug Administration (FDA) for review to date. In all cases, the FDA has consistently raised no safety concerns regarding these substances. The scientific conclusions regarding the safety of steviol glycosides are primarily based on the fact that all steviol glycosides and rebaudiosides share a common metabolic pathway following ingestion. Steviol glycosides (including rebaudioside M and D) are hydrolysed in the large intestine to steviol, which is then absorbed into the systemic circulation and conjugated with glucuronic acid to form steviol glucuronide. FSANZ has evaluated the safety of steviol glycosides and specific rebaudiosides produced using various manufacturing approaches, including enzymatic conversion, across multiple applications (e.g., A1157, A1172, A1176, A1183, and A1268).

Overall, the data provided supports the conclusion that the use of steviol glycosides with a high Reb M or Reb D content produced *via* enzymatic bioconversion in food and beverages intended for human consumption at the current use-levels permitted for steviol glycosides in Australia and New Zealand does not present a significant risk to human health. Batch analyses demonstrate that the final product is absent of impurities that can be carried over from the manufacturing process, such as protein and genetically modified materials. Therefore, the new manufacturing process used by Adorvia produces a high-purity product ( $\geq 95\%$  steviol glycosides) that does not present a safety concern.