APPLICATION FOR THE APPROVAL OF STEVIOL GLYCOSIDES FROM *YARROWIA LIPOLYTICA* UNDER THE AUSTRALIA AND NEW ZEALAND FOOD STANDARDS CODE – STANDARD 1.3.1 – FOOD ADDITIVES

Executive Summary

JOINT SUBMISSION BY:

[redacted] & [redacted]

WHICH COMPRISE THE GENERAL PARTNERSHIP:

[redacted]

DATE:
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EXECUTIVE SUMMARY

The applicant is submitting this application to Food Standards Australia New Zealand (FSANZ) concerning steviol glycosides and is seeking approval of a purified steviol glycoside mixture for use as a sweetener that is produced by fermentation of simple sugars using a *Yarrowia lipolytica* production strain. This product, Reb MD, is primarily comprised of rebaudioside M and may contain a mixture of the following additional glycosides in various concentrations, which are present in the leaves of the *Stevia rebaudiana* plant: rebaudiosides A, B, C, D, E, F, stevioside, steviolbioside, rubusoside, and dulcoside A. Although the distribution of individual steviol glycosides present in Reb MD will vary depending on the production process and final product formulation, the Reb MD subject to this application contains not less than 95% total steviol glycosides, determined as the sum of the aforementioned steviol glycosides. Reb MD, therefore, meets the general purity specification parameters for Steviol Glycosides from Fermentation as defined in Schedule 3 of the *Australia New Zealand Food Standards Code*, but *Y. lipolytica* is not listed as a source organism for a prescribed steviol glycoside.

The Reb MD ingredient, similar to other already permitted steviol glycoside preparations for use in food and beverages in Australia and New Zealand, would be used as high-intensity sweeteners for the replacement of sucrose in reduced-calorie or no-sugar-added products. Reb MD will be used in the same approved food uses and at the same use levels as other steviol glycosides already approved for many food applications within Australia and New Zealand. The use of Reb MD as an alternative sweetener to major individual steviol glycosides presents an improved sensory profile, and therefore, a better sweetness quality for consumers.

Reb MD is manufactured in accordance with current Good Manufacturing Practice (cGMP) and is produced by fermentation of simple sugars using a strain of *Y. lipolytica*. The resulting fermentation broth undergoes heat treatment to both stop fermentation and kill the yeast cells and is then followed by a combination of centrifugation, microfiltration, or clarification. The resulting filtrate undergoes typical purification processes used for steviol glycosides extracted from *S. rebaudiana* Bertoni leaves, producing a high-purity final steviol glycoside product, Reb MD, containing no less than 95% total steviol glycosides.

Product specifications for the physical, chemical, and microbiological parameters for Reb MD were established based on those most recently issued for Steviol Glycosides from Fermentation by FSANZ and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) publication the *Compendium of Food Additive Specifications*. Batch analysis of 5 non-consecutive lots of Reb MD verified that the manufacturing process produces a consistent product that meets the defined specifications. Compositional analyses of these same 5 lots of Reb MD demonstrate that the final product contains a mixture of individual glycosides in any combination, primarily rebaudiosides D and M, and that the total steviol glycoside content is consistently not less than 95%. Additionally, since Reb MD is produced from *Y. lipolytica*, the absence of protein following purification has been confirmed using the bicinchoninic acid (BCA) protein assay and the absence of residual DNA has been confirmed using polymerase chain reaction (PCR) analysis.

Steviol glycosides are approved for use in a number of global jurisdictions as sweeteners/food additives, including Asia, Australia/New Zealand, Canada, Central/South America, the European Union, and the United States (U.S.). Specifically, Reb MD produced by *Y. lipolytica* has GRAS status for use as a general-purpose sweetener in a variety of foods and beverages. The U.S. Food and Drug Administration (FDA) reviewed the GRAS notification (GRN 000882) describing the production of Reb MD produced by *Y. lipolytica* and responded on 02 February 2020 with a "no questions" letter regarding the GRAS status of Reb MD[1].

The safety of steviol glycosides has been extensively evaluated and is supported by conclusions from several scientific bodies and regulatory agencies, including the U.S. FDA, JECFA, FSANZ, European Commission's Scientific Committee on Food (SCF), European Food Safety Authority (EFSA), and Health Canada. The data available for these evaluations, which are summarised within the application, included a comprehensive examination on the comparative metabolism and pharmacokinetics of steviol glycosides in animals and humans, acute, short-, and long-term toxicity and carcinogenicity studies, reproductive and developmental toxicology studies, *in vitro* and *in vivo* mutagenicity/genotoxicity studies, and human studies. Based on the data from these studies, JECFA calculated an acceptable daily intake (ADI) for steviol glycosides of up to 4 mg/kg, expressed as steviol equivalents. Furthermore, several studies available in the public domain conducted with stevia extracts have demonstrated the shared metabolic fate of all steviol glycosides. Briefly, following ingestion, steviol glycosides are hydrolysed to steviol by members of the *Bacteroidaceae* family residing in the colon. The common metabolite steviol is absorbed from the lower gastrointestinal tract, conjugated to glucuronic acid, and excreted primarily *via* the urine in humans. Because of this shared metabolic fate, the safety database that exists for individual steviol glycosides can be extended to include all glycosylated derivates of the aglycone steviol, which includes Reb MD. An updated search of the scientific literature was conducted subsequent to the most recent steviol glycoside safety evaluation conducted by FSANZ to identify any new studies in the public domain related to steviol glycoside safety. The studies identified in the literature search update further corroborated the safety of steviol glycosides.

Reb MD is intended for use as an intense sweetener in the same approved food-uses and at the same use levels as other steviol glycosides currently in the Australian/New Zealand marketplace. Considering that Reb MD is intended to be a direct replacement for other steviol glycosides, the expected intakes of Reb MD would be similar to the intakes from other steviol glycosides that are currently on the market in Australia and New Zealand. Based on the foregoing, a separate intake assessment for Reb MD was not performed for the purposes of this application. In addition, since steviol glycoside use levels are expressed as steviol equivalents, specific use levels for each individual glycoside are not required. The use levels encompass all individual glycosides and are based on the total content of steviol in the final food or beverage product resulting from the addition of any steviol glycoside preparation meeting the appropriate specifications.

The sum of the data and information provided in this application support the safe use of Reb MD as a sweetener in food and beverages intended for human consumption in Australia and New Zealand. Reb MD is produced by fermentation of simple sugars using a *Y. lipolytica* production strain and is a high-purity steviol glycoside product containing no less than 95% steviol glycosides. The individual steviol glycosides present in Reb MD are identical, and therefore substantially equivalent, to steviol glycosides extracted from the leaves of *S. rebaudiana*. The extensive safety database that exists for steviol glycosides extracted from the plant may then be applied to establish the safety of Reb MD; an assertation that is supported by conclusions from several scientific bodies and regulatory agencies, including the U.S. FDA, JECFA, FSANZ, SCF, EFSA, and Health Canada. The weight of the scientific

[1] U.S. FDA (2020). *Agency Response Letter GRAS Notice No. GRN 882 [Rebaudioside M, Wayzata (MN): Cargill, Inc.]* Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=882 [Page Last Updated: Nov. 24, 2020].

evidence presented in this application indicates that consumption of Reb MD, through its use as a sweetener in foods, does not present a significant risk to human health and is safe for the intended use.