# Application to Amend the Specifications for Rebaudioside I Under Australia and New Zealand Food Standards Code – Standard 1.3.1 – Food Additives

# Executive Summary

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# EXECUTIVE SUMMARY

Sweegen uses a novel multi-step biosynthesis pathway process to manufacture high-purity rebaudioside I (≥95% purity) using enzymes uridine diphosphate (UDP)-glucosyltransferase and sucrose synthase that facilitate the transfer of glucose molecules to purified stevia leaf extract *via* glycosidic bonds. These enzymes are produced by a strain of *Pichia pastoris*, which has Qualified Presumption of Safety status for use in enzyme production. Currently, Sweegen's rebaudioside I produced *via* enzymatic bioconversion of purified stevia leaf extract does not comply with Schedule 3 of the *Australia New Zealand Food Standards Code* (The Code) which outlines specifications for "steviol glycosides from *Stevia rebaudiana* Bertoni" (S3—35), which includes rebaudioside; however, this specification "relates to a steviol glycosides preparation obtained from the leaves of the *Stevia rebaudiana* Bertoni plant". As a result, Sweegen is seeking to amend The Code to encompass the acceptability and permissibility of this new manufacturing methodology as another means to safely and effectively produce rebaudioside I.

Consistent with the already permitted food uses of steviol glycosides, rebaudioside I is intended for use as a low-calorie, high-intensity sweetener that provides technological advantages and benefits to consumers, and is suitable for use by individuals with diabetes as well as others who follow a low-glycaemic diet. According to a sensory panel, Sweegen's rebaudioside I was determined to be 167 times sweeter than sucrose.

Sweegen's rebaudioside I contains no less than 95% rebaudioside I and the product specifications (physical, chemical, and microbiological) are consistent with the specifications in Schedule 3 of The Code for "steviol glycosides from *Stevia rebaudiana* Bertoni" (S3—35), and comply with the assay and impurity specifications in the FAO JECFA Monograph 23 (Framework for) steviol glycosides. The results of 5 non-consecutive batches of a representative commercial lots of rebaudioside I produced *via* enzymatic bioconversion of purified stevia leaf extract demonstrate that the manufacturing process produces a consistent product that conforms to the product specifications. In addition, protein and pesticide residue analyses conducted on 3 to 5 representative batches of the final rebaudioside I product demonstrate that the product is of high purity and does not contain any residual impurities from the manufacturing process.

Rebaudioside I is produced in accordance with current Good Manufacturing Practices and meets appropriate food-grade specifications. The production process consists of two stages, involving fermentation, extraction and purification. In the first stage, the *P. pastoris* production strain expressing UDP-glucosyltransferase and sucrose synthase enzymes undergoes fermentation to generate the enzymes required for the bioconversion. The strain carries the UGT-A fusion enzyme (*i.e.*, glucosyltransferase fused with sucrose synthase). This same UGT-A fusion enzyme sourced from the same *P. pastoris* strain listed in Schedule 18 as a permitted processing aid for the conversion of purified stevia leaf extract to produce rebaudiosides M, D, and E. Following the fermentation step, the UGT-A fusion enzyme is isolated from the production microorganism. In the second stage, the UGT-A fusion enzyme is mixed with stevia extract (≥95% steviol glycosides) to generate rebaudioside I, which then undergoes a series of purification and isolation steps to produce the final high-purity rebaudioside I (≥95%).

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 50 Generally Recognized as Safe (GRAS) notices for steviol glycosides have been submitted to the U.S. Food and Drug Administration (FDA) for review. These notices include submissions for stevia leaf extract, glucosylated steviol glycosides, steviol glycosides manufactured using GM yeast, and steviol glycosides manufactured *via* enzymatic bioconversion, all with a total steviol glycoside content of no less than 95%. With the exception of the most recent GRAS notifications that are currently pending review, the FDA has raised no objections to the GRAS status of steviol glycoside products for use as general purpose sweeteners in foods. Of note, GRN 911 submitted

by Blue California[1] for rebaudioside I produced *via* enzymatic bioconversion of purified stevia leaf extract, is the same product that is the subject of this application. The U.S. FDA responded to GRN 911 with a "no questions" letter regarding the GRAS status of rebaudioside I produced *via* enzymatic bioconversion for use as a tabletop sweetener and as a general purpose non-nutritive sweetener in foods. Likewise, Health Canada has no objections to the use of Sweegen's steviol glycosides, including rebaudioside I, manufactured using enzymatic bioconversion (*i.e.,* using UDP-glucosyltransferase and sucrose synthase derived from strains of *P. pastoris*), provided that the products are used in accordance with the permitted uses of steviol glycosides as set out in Item S.1.2 of the *List of Permitted Sweeteners* and meet the current specifications for steviol glycosides set by JECFA.

The safety conclusions for steviol glycosides in general, including rebaudioside I, are based on the fact that all steviol glycosides share a common metabolic fate following ingestion. Steviol glycosides, including rebaudioside I, are hydrolysed to steviol in the large intestine, which is subsequently absorbed and conjugated with glucuronic acid to form steviol glucuronide that is excreted primarily *via* the urine in humans. On this basis, safety studies conducted on specific steviol glycosides can be used as surrogates for other individual steviol glycosides, including rebaudioside I, due to the shared metabolic fate. In 2019, Food Standards Australia New Zealand (FSANZ) received an application to expand the definition of steviol glycosides to include rebaudioside E produced by enzymatic bioconversion using UDP-glucosyltransferase and sucrose synthase enzymes, and the safety of all steviol glycosides was reviewed by FSANZ at this time. Therefore, for this specification amendment for rebaudioside I, only safety studies conducted with steviol glycosides that were published in 2019 through 2020 were reviewed and discussed. The findings reported in the new toxicokinetic/metabolic, toxicological, and human studies identified were found to corroborate the safety of steviol glycosides.

The safety of steviol glycosides has been reviewed by several scientific and regulatory authorities including the FDA, JECFA, FSANZ, European Commission's Scientific Committee on Food, European Food Safety Authority (EFSA), and Health Canada. The recent opinions/reports issued since the last steviol glycoside safety evaluation by FSANZ were summarised, including the most recent safety evaluation by JECFA at their 87$^{th}$ meeting in 2019, which included a new specification monograph for enzyme modified steviol glycosides (*i.e.,* steviol glycosides produced *via* enzymatic bioconversion of purified stevia leaf extract), new GRAS notices submitted to the U.S. FDA (including GRN 911 for rebaudioside I produced *via* enzymatic bioconversion of purified stevia leaf extract), and the 2019 Scientific Opinion published by EFSA on rebaudioside M produced by enzymatic conversion using the same UDP-glucosyltransferase and sucrose synthase enzymes as rebaudioside I. These recent opinions support that there are no safety concerns with the enzymatic bioconversion manufacturing process and that the current acceptable daily intake (ADI) of 0 to 4 mg/kg body weight for steviol glycosides, expressed as steviol, applies.

Rebaudioside I is proposed for use as a low-calorie intense sweetener and as an alternative to existing steviol glycoside products and is intended for use in the same food categories at the same use-levels currently permitted for steviol glycosides as outlined in *The Code* under Schedule 15. As such, the intakes of rebaudioside I will be the same as for steviol glycosides, which are already available in the Australian/New Zealand marketplace, as it is intended to be a direct replacement for other steviol glycosides. Accordingly, a separate intake assessment for rebaudioside I was not performed. It should be noted that use-levels for steviol glycosides are expressed as steviol equivalents, and as such, are not specified for any specific steviol glycoside; rather, the use-levels are based on the total content of the aglycone, steviol, in the final food product resulting from the addition of any steviol glycoside product meeting the appropriate specifications.

[1] All rights of Blue California have been granted to SweeGen, Inc. in regard to steviol glycosides.

Overall, the data provided supports the conclusion that the use of rebaudioside I produced *via* enzymatic bioconversion of purified stevia leaf extract 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 and is safe. 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 Sweegen produces a high-purity rebaudioside I product (≥95% purity), does not present a safety concern, and its use is justified.