Application to Amend the Specifications for Steviol Glycosides, Under Australia and New Zealand Food Standards Code – Standard 1.3.1 – Food Additives, to Include Rebaudioside D Manufactured by Enzymatic Bioconversion of Stevia Leaf Extract

Executive Summary

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EXECUTIVE SUMMARY

SweeGen, Inc. (SweeGen) has developed a novel multi-step biosynthesis pathway process to manufacture high-purity rebaudioside D (≥95% purity) using enzymes uridine 5'-diphospho(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.* Currently, SweeGen's rebaudioside D 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) that outlines specifications for "steviol glycosides from *Stevia rebaudiana* Bertoni" (S3—35), which includes rebaudioside D. As a result, SweeGen is seeking to amend Standard 1.3.1 and related Schedules for rebaudioside D (steviol glycosides) to include a new manufacturing process.

Consistent with the already permitted food uses of steviol glycosides, rebaudioside D 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 of 13 participants, SweeGen's rebaudioside D was determined to be 200 times sweeter than sucrose.

SweeGen's rebaudioside D contains not less than 95% rebaudioside D 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 20 for "steviol glycosides from *Stevia rebaudiana* Bertoni". The results of 5 non-consecutive batches of representative commercial lots of rebaudioside D demonstrate that the manufacturing process produces a consistent product that conforms to the product specifications. In addition, protein and pesticide residue analyses conducted on the same 5 batches of the final rebaudioside D product demonstrate that it is of high purity and does not contain any impurities from the manufacturing process.

Rebaudioside D 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, a strain of *P. pastoris* expressing UDP-glucosyltransferase and sucrose synthase enzymes undergoes fermentation to generate the enzymes required for the bioconversion (*i.e.,* UGT-A fusion enzyme). 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 D. The resulting rebaudioside D undergoes a series of purification and isolation steps to produce the final high-purity rebaudioside D product (≥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 have been submitted to the U.S. Food and Drug Administration (FDA) for review to date. These notices include submissions for purified individual steviol glycosides, mixtures of steviol glycosides, and glucosylated steviol glycosides, 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 No. 715 submitted by Blue California[1] for rebaudioside D produced *via* enzymatic bioconversion of purified stevia leaf extract, is the same product that is the subject of this application. The FDA responded with no questions to the GRAS status of rebaudioside D produced *via* enzymatic

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bioconversion for use as a table top sweetener and as a general purpose non-nutritive sweetener in foods.

The scientific conclusions regarding the safety of rebaudioside D are primarily based on the fact that rebaudioside D and all other steviol glycosides share a common metabolic pathway following ingestion. Steviol glycosides (including rebaudioside 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. This phase II metabolite is excreted primarily in the urine in humans. In 2016, Food Standards Australia New Zealand (FSANZ) received an application to expand the definition of steviol glycosides to include all steviol glycosides present in the *S. rebaudiana* leaf and the safety of all steviol glycosides was reviewed by FSANZ at this time. Therefore, for this specification amendment for rebaudioside D, only safety studies conducted with steviol glycosides that were published in 2016, 2017, and 2018 to date 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 U.S. 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 82nd meeting in 2016, new GRAS notices submitted to the U.S. FDA (including GRN No. 715 submitted by Blue California[2] for rebaudioside D produced *via* enzymatic bioconversion of purified stevia leaf extract), and the recent expansion of the steviol glycoside definition in 2017 by Health Canada to include all steviol glycosides in the *S. rebaudiana* Bertoni plant. These recent opinions support the current acceptable daily intake (ADI) of 0 to 4 mg/kg body weight for steviol glycosides, expressed as steviol.

Rebaudioside D is proposed for use as a low-calorie intense sweetener and as an alternative to existing steviol glycosides, 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 D 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 D 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.

Overall, the data provided supports the conclusion that the use of rebaudioside D 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 D product (≥95% purity), and does not present a safety concern and is justified.

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