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 E Manufactured by Enzymatic Bioconversion of Stevia Leaf Extract

PREPARED FOR:
Standards Management Officer
Food Standards Australia New Zealand
Ground Floor, Boeing House
55 Blackall Street
Barton ACT 2600
Australia

PREPARED BY:
Blue California
[redacted]

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A. GENERAL REQUIREMENTS

In accordance with Section 3.1.1 – General Requirements of the Food Standards Australia New Zealand (FSANZ) *Application Handbook* (FSANZ, 2016) the following general information must be provided:

1. Format of the application;
2. Applicant details;
3. Purpose of the application;
4. Justification for the application;
5. Information to support the application;
6. Assessment procedure;
7. Confidential commercial information;
8. Other Confidential information;
9. Exclusive capturable commercial benefit;
10. International and other national standards;
11. Statutory declaration; and,
12. Checklist.

Each point is addressed in turn in Section A that follows.

A.1 Format of the Application

1. Information Related to Changes to Standard 1.3.1 – Food Additives

This application for an amendment to Standard 1.3.1 and related Schedules is prepared pursuant to Section 3.3.1 – Food Additives of the FSANZ *Application Handbook* (FSANZ, 2016) which requires the following structured format to assess an application for a new food additive:

A. General information on the application;

B. Technical information on the food additive;

C. Information on the safety of the food additive; and,

D. Information on dietary exposure to the food additive.

The application is presented in this format. At the start of each section (A to D) the information that must be addressed therein is specified in more detail. Additionally, an executive summary for the application is provided as a separate electronic document to this application. The application has been prepared in English and submitted electronically, as required by the FSANZ *Application Handbook* (FSANZ, 2016).

A.2 Applicant Details

Blue California is a science-based developer, producer, and distributer of non-caloric, non-GMO, high-quality sweeteners for the food, flavour, and beverage industries.

[redacted]
Manager - Technical and Regulatory Affairs
Blue California
30111 Tomas, Rancho Santa Margarita,
California, 92688
USA

[redacted]

In addition, Dr. Ashley Roberts, Senior Vice President of the Food & Nutrition Group at Intertek Scientific & Regulatory Consultancy is involved in the preparation, submission, and stewardship of this application. His contact details are listed below:

[redacted]

A.3 Purpose of the Application

Blue California is submitting this application to FSANZ concerning rebaudioside E that is produced using a new methodology and is therefore seeking the amendment of Standard 1.3.1 and related Schedules for steviol glycosides. Blue California uses a novel multi-step biosynthesis pathway process to manufacture high purity rebaudioside E (≥85% rebaudioside E; ≥95% steviol glycosides) using enzymes uridine diphosphate (UDP)-glucosyltransferase and sucrose synthase that facilitate the transfer of glucose to purified stevia leaf extract *via* glycosidic bonds. These enzymes are made by a strain of *Pichia pastoris* (*P. pastoris*).

Schedule 3 of the *Australia New Zealand Food Standards Code* (The Code) contains specifications for "steviol glycosides from *Stevia rebaudiana* Bertoni" (S3—35), which includes rebaudioside E. This specification "relates to a steviol glycosides preparation obtained from the leaves of the *Stevia rebaudiana* Bertoni plant." Although rebaudioside E produced by enzymatic bioconversion is chemically identical to rebaudioside E extracted from the leaves of *Stevia rebaudiana* (*S. rebaudiana*) Bertoni, the rebaudioside E for which this application is being made by Blue California does not comply with specification S3—35 based on the source (*i.e.,* it is produced by enzymatic bioconversion *versus* extraction from the leaf).

This application, therefore, aims to amend The Code to encompass the acceptability and permissibility of Blue California's new manufacturing methodology as another means to safely and effectively produce rebaudioside E. This application does not intend to change the purity specification (≥95% steviol glycosides) or propose an extension for the use of rebaudioside E in additional food products nor does it propose to increase the permitted quantities of rebaudioside E in permitted food products.

A.4 Justification of the Application

A.4.1 Technological Function for the Food Additive

Steviol glycosides extracted from the leaves of *S. rebaudiana* Bertoni, including rebaudioside E, are already permitted for use as high-intensity sweeteners in Australia and New Zealand for the replacement of sucrose in reduced-calorie or no-sugar-added products. The primary reason for developing alternative methods to the traditional extraction methods for steviol glycosides is that not all glycosides are naturally produced to the same degree in the leaves of *S. rebaudiana* Bertoni. For example, stevioside is a major glycoside present in the leaves of the plant, constituting about 5 to 10% in dry leaves (JECFA, 1999), whereas rebaudioside E is a minor glycoside that is present at much lower levels. Some of the minor glycosides, such as rebaudioside E, have more favourable sensory characteristics when compared to the major glycosides (*i.e.,* stevioside, rebaudioside A) and have taste profiles that are more reflective of sucrose, and hence the development of the new technology to produce a glycoside with preferential sensory characteristics for product development.

A.4.2 Costs and Benefits for Industry, Consumers, and Government Associated with Use of the Food Additive

Since rebaudioside E provides improved sensory characteristics over the major steviol glycosides (*i.e.,* stevioside, rebaudioside A), but is naturally present in much lower quantities within the S*. rebaudiana* Bertoni leaf, it is in the interest of industry to develop alternative production methods that yield higher quantities of rebaudioside E than traditional leaf extraction. Blue California's production methods involve the use of UDP-glucosyltransferase and sucrose synthase enzymes to convert purified stevia leaf extract to a high purity rebaudioside E (≥85% rebaudioside E; ≥95% steviol glycosides). Therefore, it is expected that Blue California's high purity rebaudioside E will present an attractive alternative as a sweetener for food manufacturers. Blue California anticipates that food manufacturers may incorporate their rebaudioside E into products after importation into Australia and New Zealand. In addition,

globally-positioned companies may also import their own finished products containing Blue California's rebaudioside E.

The benefits to the consumer would mirror those for other steviol glycosides currently permitted for use in Australia and New Zealand. Blue California's rebaudioside E, like other steviol glycosides, would be used in foods and beverages to replace sugar, which will benefit consumers seeking products that have reduced caloric content. In addition, this would also include consumers with specific medical conditions that require reduced sugar intake, such as those with diabetes, as the consumption of steviol glycosides does not interfere with glucose homeostasis (EFSA, 2010).

Since Blue California does not intend to propose an extension for the use of rebaudioside E in additional food products nor do they wish to propose to increase the permitted quantities of rebaudioside E in permitted food products, there is no perceived benefit or added cost to the government.

A.5 Information to Support the Application

Technical information specific to Blue California's manufacturing methodology for rebaudioside E is presented in detail in Section B, including information regarding the production of UDP-glucosyltransferase and sucrose synthase enzymes (*i.e.,* UGT-A fusion enzyme) from a strain of *P. pastoris* and their use as processing aids. These same enzymes are already permitted for use as processing aids in the production of rebaudioside M by enzymatic conversion of stevia leaf extract as described in S3–35 of The Code (*i.e.,* "UDP-glucosyltransferase and sucrose synthase sourced from a *Pichia pastoris* strain expressing UGT-A"), and therefore, the safety of their use, including the source microorganism utilised to produce them, has been previously reviewed by FSANZ.

FSANZ reviewed an application to amend the specifications for rebaudioside M to include a new manufacturing process (*i.e.,* rebaudioside M produced by enzymatic bioconversion using UDP-glucosyltransferase and sucrose synthase enzymes) in 2018, and as such reviewed the safety of steviol glycosides (FSANZ, 2018). Since the safety of rebaudioside E and steviol glycosides in general have been previously reviewed and established by FSANZ, Section C provides a short summary of steviol glycoside safety and focuses on presenting: a) new safety publications present in the scientific literature which have not previously been evaluated by FSANZ; and b) recent opinions released by regulatory agencies and/or scientific bodies (*i.e.,* Joint FAO/WHO Expert Committee on Food Additives [JECFA]).

A.6 Assessment Procedure

Blue California considers the most appropriate procedure to be adopted in assessing the application to be the General Procedure – Level 2. It is anticipated that this application will involve amending *Standard 1.3.1 – Food Additives* of The Code to modify the specifications outlined in Schedule 3 for steviol glycosides from *S. rebaudiana* Bertoni (section S3–35). Blue California also requests that the evaluation be expedited.

A.7 Confidential Commercial Information (CCI)

Blue California requests that certain proprietary information required for Section B.5 (Manufacturing Process) be considered confidential commercial information (CCI). Non-confidential general summaries of proprietary manufacturing information are provided within this application, and all details considered CCI have been removed and are presented in Appendix A. Blue California requests that all information presented in Appendix A remain confidential as it holds significant commercial value to the company, including proprietary details on the manufacture of the final rebaudioside E product.

A.8 Other Confidential Information

Blue California requests that the identity of the companies that perform analysis testing (*i.e.,* stability, residue, *etc.*) are to remain confidential. More specifically, Blue California wishes not to disclose the companies by name but is amicable with the general disclosure of the companies' location (*i.e.,* "a lab in Europe").

A.9 Exclusive Capturable Commercial Benefit (ECCB)

Blue California is currently not the only manufacturer of rebaudioside E. Therefore, as there are other manufacturers of rebaudioside E, the application would not confer exclusive capturable commercial benefit (ECCB) in accordance with Section 8 of the FSANZ Act, as there are other companies who would likely benefit from approval of this application.

A.10 International and Other National Standards

A.10.1 The Joint FAO/WHO Expert Committee on Food Additives (JECFA)

The JECFA Committee recently re-evaluated the safety, dietary intake, and specifications for steviol glycosides at its 82nd meeting in 2016. The safety of steviol glycosides as well as the acceptable daily intake (ADI) of 0 to 4 mg/kg body weight, expressed as steviol, were confirmed. Details of a new manufacturing process for rebaudioside A utilising a strain of *Yarrowia lipolytica* (*Y. lipolytica*) that was genetically modified to overexpress the steviol glycoside biosynthetic pathway were submitted to and reviewed by the Committee. As a result, the Committee issued a new specification monograph for "Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica*" (JECFA, 2016). The purity of rebaudioside A from genetically modified *Y. lipolytica* must be no less than 95% total steviol glycosides on the dried basis. The Committee also reviewed data demonstrating the shared metabolism of all steviol glycosides and issued new specifications for "Steviol Glycosides from *Stevia rebaudiana* Bertoni" (JECFA, 2010), expanding the definition of steviol glycosides to "a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties (glucose, rhamnose, xylose, fructose, arabinose, galactose and deoxyglucose) in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni". The purity of steviol glycosides from *S. rebaudiana* Bertoni must be no less than 95% total steviol glycosides on the dried basis.

A.10.2 United States

In the United States (U.S.), steviol glycosides are Generally Recognised as Safe (GRAS) for use as general purpose sweeteners in foods, and to date, over 50 GRAS notices have been submitted to the U.S. Food and Drug Administration (FDA) for review. 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 currently pending review, the U.S. FDA has raised no objections to the GRAS status of steviol glycoside products for use as general purpose sweeteners in foods. Of particular relevance to this submission, GRN No. 823 pertaining to the GRAS conclusions of rebaudioside E produced by enzymatic bioconversion of purified stevia leaf extract was submitted by Blue California (U.S. FDA, 2017a,b, 2019a). The Notice is currently under review by the Agency. The rebaudioside E as described in GRN No. 823 is identical to the rebaudioside E as described herein. The manufacturing process utilized to generate the rebaudioside E (*i.e.,* by enzymatic bioconversion) is similar to the production processes described in GRN No. 667 and 715 for rebaudiosides M and D produced by enzymatic bioconversion, respectively, that were submitted by Blue California (U.S. FDA, 2017a,b, 2019a). GRN 667 and 715 pertaining to the GRAS conclusions of rebaudioside M and D produced by enzymatic bioconversion of purified stevia leaf

extract, respectively, for use as table top sweeteners and as general purpose non-nutritive sweeteners in foods were filed without objection by the Agency.

A.10.3 Other Jurisdictions

Steviol glycosides are approved for use in a number of other jurisdictions, including the European Union, Canada, Asia, Central/South America, Africa, and the Middle East (PureCircle Stevia Institute, 2019). In the European Union, commercially available steviol glycoside products must comply with the specifications for steviol glycosides (E 960) adopted by the European Commission in 2012 and recently updated in 2016 (EU, 2012, 2016). Presently, the specifications stipulate that steviol glycoside products must contain no less than 95% of 11 named steviol glycosides: stevioside, rebaudiosides A, B, C, D, E, F and M, steviolbioside, rubusoside, and dulcoside. Health Canada has approved the use of steviol glycosides for use as food additives in Canada, and recently expanded the definition in the list of permitted sweeteners to include all the steviol glycosides in the *S. rebaudiana* Bertoni plant (Health Canada, 2017a).

In several Asian countries including Japan, China, Hong Kong, Indonesia, Malaysia, Myanmar, Pakistan, Philippines, Singapore, Taiwan, Thailand, and Vietnam, steviol glycosides are approved food additives/sweetening agents. For example, the Ministry of Health and Welfare in Japan has authorised the use of 3 types of stevia extracts, including α-glucosyltransferase-treated stevia, powdered stevia, and stevia extract (Japan Food Chemical Research Foundation, 2014). Purified stevioside (crude extract, 50% purity, and ≥90% purity) and *S. rebaudiana* leaf extracts are also accepted for general use as sweeteners in foods and beverages in Japan (Marie, 1991; Das *et al.*, 1992; Ferlow, 2005). The Food Safety and Standards Authority of India (FSSAI) has approved the use of steviol glycosides as a non-nutritive sweetener in a variety of food and beverage categories (FSSAI, 2015; MOHFW, 2016). In several Central/South American countries (*e.g.,* Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Mexico, Paraguay, Peru, Uruguay, Venezuela, Honduras) stevioside, *S. rebaudiana* leaves, and highly refined stevia extracts are permitted for use as low-calorie sweeteners. Steviol glycosides are also approved as food additives in the Middle East (Belarus, Bahrain, Iran, Jordan, Kazakhstan, Kuwait, Lebanon, Omar, Qatar, Saudi Arabia, Turkey, Uzbekistan, Yemen), Africa (Algeria, Cape Verde, Egypt, Equatorial Guinea, Gambia, Ghana, Guinea Bissau, Guinea Conakry, Kenya, Liberia, Libya, Morocco, Mauritania, Nigeria, Sierra Leone, South Africa, Tunisia), Switzerland, and Russia.

A.11 Statutory Declaration

Signed Statutory Declarations for Australia and New Zealand are provided in Appendix B.

A.12 Checklist

Completed checklists relating to the information required for submission with this application based on the relevant guidelines in the FSANZ *Application Handbook* are provided in Appendix C.

B. TECHNICAL INFORMATION ON THE FOOD ADDITIVE

In accordance with Section 3.3.1 – Food Additives of the FSANZ *Application Handbook* (FSANZ, 2016) the following technical information must be provided:

1. Nature and technological purpose of the food additive;
2. Information to enable identification of the additive;
3. Information on the chemical and physical properties of the additive;
4. Information on the impurity profile;
5. Manufacturing process;
6. Specifications for identity and purity;
7. Information for food labelling;
8. Analytical method for detection; and,
9. Potential additional purposes of the food additive when added to food.

These points are addressed in the Section that follows.

B.1 Nature and Technological Purpose of Rebaudioside E

B.1.1 Technological Purpose

Blue California's rebaudioside E is produced by enzymatic bioconversion of purified stevia leaf extract and the final product is a highly purified preparation containing no less than 85% rebaudioside E and no less than 95% total steviol glycosides. As per the technological purposes listed in Schedule 14 – Technological purposes performed by substances used as food additives, Blue California's rebaudioside E fulfils the function as an intense sweetener and a flavour enhancer, consistent with rebaudioside E and steviol glycoside preparations already approved for use in Australia and New Zealand. Blue California does not intend for this application to extend the use of rebaudioside E or steviol glycosides in general to foods for which its use levels have not already been permitted; Blue California intends to use their rebaudioside E in the current food categories and at use levels currently permitted for steviol glycosides. Likewise, Blue California does not intend to propose additional or different food matrices to which the addition of steviol glycosides has not already been approved.

B.1.2 Sweetness Potency

The sweetness equivalency to sucrose of Blue California's rebaudioside E produced *via* enzymatic bioconversion of purified stevia leaf extract was evaluated by a sensory panel. Serial dilutions of sucrose (1.0, 3.0, and 6.0%) were prepared in bottled water at room temperature. The rebaudioside E solution at a concentration of 300 ppm was prepared in bottled water at room temperature. Participants (n=13) consumed the rebaudioside E solution and results were evaluated against the serially diluted sucrose samples starting with the lowest to the highest concentration. Results were averaged and converted to sweetness equivalency compared to sucrose. The results were consistent among all participants. Based on the results, rebaudioside E was determined to be 137 times sweeter than sucrose (full study report provided in Appendix D).

B.1.3 Stability

Extensive stability testing has been conducted on steviol glycosides and at the 68th meeting of the JECFA Committee it was concluded that "steviol glycosides are thermally and hydrolytically stable for food use, including acidic beverages, under normal conditions of processing/storage" (JECFA, 2007). At the 82nd meeting in 2016, the Committee reviewed additional stability data and concluded, "the stability of steviol glycosides extract preparations established by JECFA at the 68th meeting can be extended to include steviol glycoside extract preparations containing higher levels of new glycosides added to the definition appearing in commercial products, mainly rebaudioside D and rebaudioside M" (FAO, 2016). Oehme *et al.* (2017) evaluated the structural stability of 3 commercial batches each of the dried stevia leaves, the first aqueous infusion of the ground stevia, and a high-purity stevia leaf extract (≥95% steviol glycosides), to determine whether the manufacturing process adversely impacts steviol glycoside composition. Changes in steviol glycosides were analysed by high-performance liquid chromatography (HPLC)-UV and HPLC-ESI-MS/MS. The authors reported that all 9 steviol glycosides defined by JECFA were detected in all samples, demonstrating that processing does not chemically alter or modify the steviol glycoside content.

To confirm that these conclusions apply to Blue California's rebaudioside E produced *via* enzymatic bioconversion of purified stevia leaf extract, a 6-month accelerated stability study was conducted on 5 representative batches of the final rebaudioside E product (Lot No. 160202-1601, 160921-1602, 160921-1603, 20170665-04, 20131005). The samples were stored at 40±2°C at a relative humidity of 75±5%. Rebaudioside E was observed to be stable over the course of the accelerated stability study, based on appearance, moisture content, and percent rebaudioside E content measured by HPLC compared to baseline (Table B.1.3-1).

Table B.1.3-1 Accelerated Storage Stability Data for Blue California's Rebaudioside E

Duration (months)	Appearance	Manufacturing Lot No.													
		160202-1601			160921-1602			160921-1603			20170665-04			20131005		
		Moisture (%)	Reb E (HPLC, %)	Reb D (HPLC, %)	Moisture (%)	Reb E (HPLC, %)	Reb D (HPLC, %)	Moisture (%)	Reb E (HPLC, %)	Reb D (HPLC, %)	Moisture (%)	Reb E (HPLC, %)	Reb D (HPLC, %)	Moisture (%)	Reb E (HPLC, %)	Reb D (HPLC, %)
0	White powder	3.46	85.75	8.52	2.78	87.44	7.62	2.33	86.73	8.46	3.27	87.16	8.28	3.06	86.65	8.43
1	White powder	3.41	85.73	8.43	3.01	87.46	6.63	2.58	86.72	8.21	3.32	87.13	8.79	3.17	86.63	8.88
2	White powder	3.46	85.73	8.50	2.88	87.45	7.77	2.62	86.73	6.84	3.34	87.18	8.13	2.98	86.61	9.51
3	White powder	3.53	85.72	8.51	2.93	87.44	7.55	2.66	86.75	8.30	3.43	87.16	8.62	3.11	86.63	8.40
4	White powder	3.48	85.71	8.68	3.01	87.46	8.84	2.63	86.75	8.54	3.52	87.14	7.70	3.09	86.57	8.89
5	White powder	3.60	85.78	8.50	3.11	87.44	9.21	2.71	86.78	7.80	3.42	87.13	8.79	3.12	86.61	8.82
6	White powder	3.55	85.75	9.13	3.02	87.44	8.08	2.72	86.76	8.03	3.44	87.15	8.16	3.16	86.63	9.51

HPLC = high-performance liquid chromatography; Reb D = rebaudioside D; Reb E = rebaudioside E

B.2 Information to Enable Identification of Rebaudioside E

Information to enable the identification of rebaudioside E, including the chemical structure, the chemical name, the molecular weight and formula, and the common name, are presented below.

B.2.1 Identity of Substance

Blue California's rebaudioside E is produced by enzymatic bioconversion of purified stevia leaf extract and the final product is a high purity preparation containing no less than 85% rebaudioside E and no less than 95% steviol glycosides. Rebaudioside E is a minor naturally occurring steviol glycoside that is present in the leaves of *S. rebaudiana* Bertoni. Rebaudioside E is an ent-kaurane diterpene glycoside with a steviol backbone conjugated to 4 glucose units, an ether at position C-13, and an ester at position C-19 (see Figure B.2.1-1).

Chemical name: 13-[(2-O-β-D-glucopyranosyl- β-D-glucopyranosyl)oxy] ent-kaur- 16-en-19-oic acid-(2-O- β-D-glucopyranosyl-β-D-glucopyranosyl) ester

Common name: Rebaudioside E

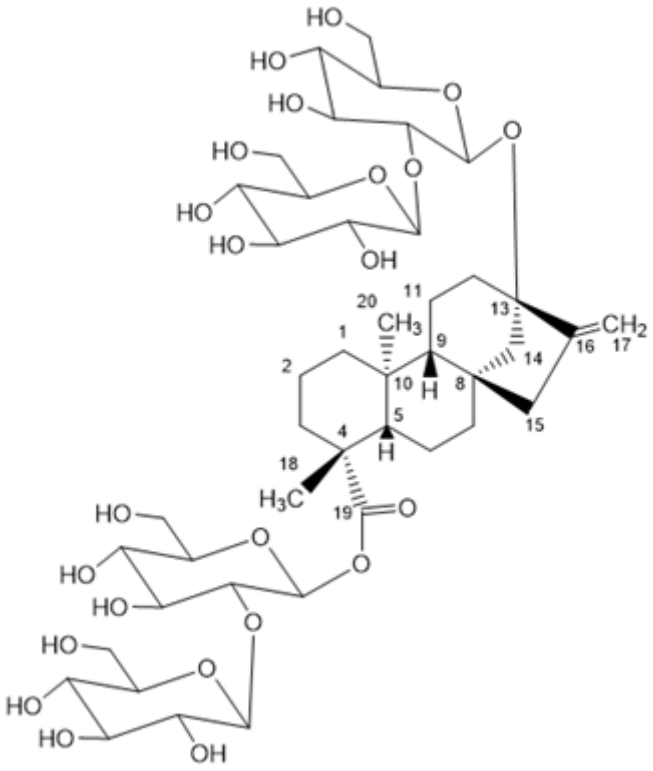
Synonyms: Reb E

Chemical formula: $C_{44}H_{70}O_{23}$

Molecular weight: 967.02 Daltons

CAS Number: 63279-14-1

Figure B.2.1-1 Chemical Structure of Rebaudioside E



B.3 Information on the Chemical and Physical Properties of Rebaudioside E

Blue California's rebaudioside E is a white to off-white powder that is slightly soluble in water with a slight characteristic odour, consistent with rebaudioside E extracted from the leaves of *S. rebaudiana* Bertoni. Steviol glycosides are a group of compounds that share a similar molecular structure, where different sugar moieties are attached to the aglycone steviol (an *ent*-kaurene-type diterpene). Steviol glycosides include any compound containing a steviol backbone conjugated to any number or combination of the principal sugar moieties, including glucose, rhamnose, xylose, fructose, deoxyglucose, galactose, and arabinose (JECFA, 2010). Based on the similar chemical structure, all steviol glycosides, including rebaudioside E, share a common metabolic fate following consumption (Purkayastha *et al.,* 2016). Specifically, steviol glycosides are hydrolysed to steviol in the large intestine, which is subsequently absorbed and conjugated with glucuronic acid to form steviol glucuronide. The glucuronide metabolite is then excreted primarily *via* the urine in humans [Kraemer and Maurer, 1994; Koyama *et al*., 2003a,b; Geuns and Pietta, 2004 (unpublished); Simonetti *et al*., 2004; Geuns *et al*., 2006, 2007; Wheeler *et al*., 2008; Roberts *et al.,* 2016].

B.4 Information on the Impurity Profile

Blue California's rebaudioside E produced *via* enzymatic bioconversion of purified stevia leaf extract consists of ≥85% rebaudioside E and ≥95% total steviol glycosides. As described in Section B.6.1, Blue California has established product specifications for rebaudioside E that 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 FAO JECFA Monograph 20 for "Steviol Glycosides from *Stevia rebaudiana* Bertoni" (JECFA, 2010). In addition to the chemical and microbiological specifications, since the starting steviol glycoside material (≥95%) is extracted from the leaves of *S. rebaudiana* Bertoni, pesticide residue analyses were conducted on 5 representative batches of the final rebaudioside D product (Lot No. 20131005, 160202-1601, 160921-1602, 160921-1603, 20170665-04). The results of the analyses provided in Appendix E demonstrate the absence of any residual pesticides in the product. The final rebaudioside E product has also been tested for residual protein to ensure that the processing enzymes have been effectively removed from the finished product. Analysis of 3 batches of final rebaudioside E product (Lot No. 160921-1603, 160202-1601, 20170665-04) using the bicinchoninic acid (BCA) assay with a limit of detection of 5 ppm confirms the absence of protein residues in the final product (results provided in Appendix F).

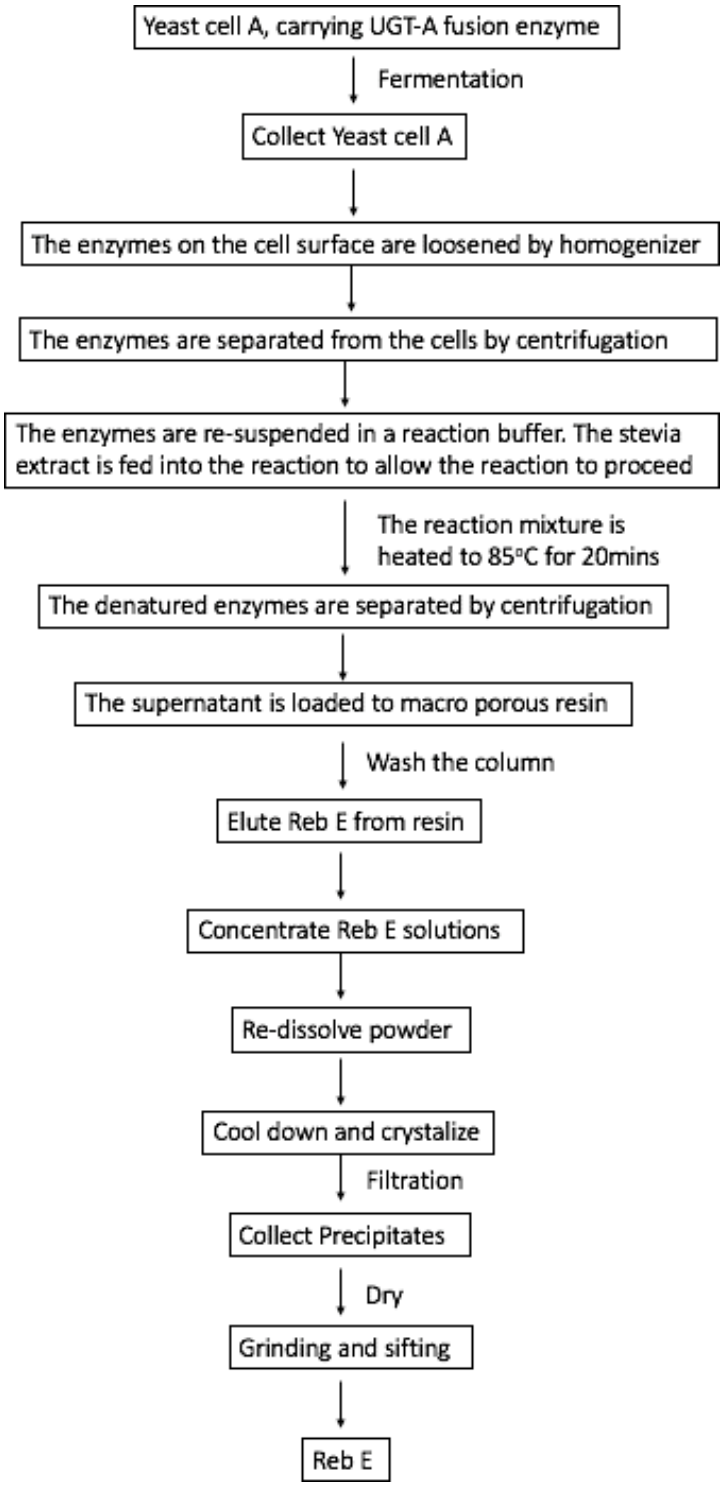
B.5 Manufacturing Process

B.5.1 Overview

The production process used to manufacture high purity rebaudioside E (≥85% rebaudioside E; ≥95% steviol glycosides) involves the use of enzymes (*e.g.,* UDP-glucosyltransferase, sucrose synthase) that facilitate the transfer of glucose to purified stevia leaf extract *via* glycosidic bonds. The enzymes are produced by a safe strain of *P. pastoris*. Blue California's rebaudioside E is manufactured in compliance with current Good Manufacturing Practices (cGMP). The manufacturing process can be broadly divided into 2 stages. In the first stage, a strain of *P. pastoris* undergoes fermentation to generate the UDP-glucosyltransferase and sucrose synthase enzymes required for the bioconversion reaction (*i.e.,* UGT-A fusion enzyme). Following the fermentation step, the enzymes are isolated from the source microorganism. In the second stage, the enzymes are mixed with stevia extract (≥95% steviol glycosides, extracted from the leaves of *S. rebaudiana* Bertoni) to generate rebaudioside E. The resulting rebaudioside E undergoes a series of purification and isolation steps to generate the final high-purity rebaudioside E (≥85% rebaudioside E; ≥95% steviol glycosides) product. A schematic overview and detailed description of the production process is presented in Figure B.5.1-1 and Section B.5.3, respectively.

It should be noted that Blue California's rebaudioside E is currently manufactured outside of Australia/New Zealand. Since the preparation will not be manufactured in Australia or New Zealand, the fermentation substrates, production organisms, and all processing aids used in the manufacturing process will not enter the territory.

Figure B.5.1-1 Schematic Overview of Rebaudioside E Production Process



B.5.2 Identity of Raw Materials and Processing Aids

All materials and processing aids utilised in the manufacture of Blue California's rebaudioside E are food-grade and comply with relevant Food Chemical Codex (FCC) or other internationally-recognised standards. A list of all raw materials, processing aids, and filtration aids used in Stage 1 of the manufacturing process to generate the enzymes is provided in Appendix A.

Stage 2 of the production process (production of rebaudioside E) requires the use of various processing and filtration aids that are already recognised for use in the manufacture of steviol glycoside preparations, in addition to the raw materials purified stevia leaf extract (≥95% steviol glycosides) and sucrose (Table B.5.2-1). A certificate of analysis for a typical batch of purified stevia leaf extract, the starting raw material, is provided in Appendix G. The UDP-glucosyltransferase and sucrose synthase enzymes (*i.e.,* UGT-A fusion enzyme) that are used as processing aids to convert the purified stevia leaf extract to rebaudioside E are already permitted for use in Australia and New Zealand in the manufacture of rebaudioside M from purified stevia leaf extract. Specifically, the UGT-A fusion enzyme containing UDP-glucosyltransferase and sucrose synthase components that is sourced from a *P. pastoris* strain expressing UGT-A, is listed in Schedule 18 as an approved processing aid for the conversion of purified stevia leaf extract to produce rebaudioside M. The UGT-A fusion enzyme that is used in this application to produce rebaudioside E is identical to the UGT-A fusion enzyme that is used to produce rebaudioside M, which was recently reviewed by FSANZ (A1157).

Table B.5.2-1 Raw Materials and Processing Aids Used in Stage 2 of the Manufacturing Process

Material	Function
Raw Material Substrates	
Stevia leaf extract (≥95% steviol glycosides)	Starting raw material
Sucrose	Substrate
UDP-glucose	Substrate
Processing Aids	
Potassium monophosphate	Buffer solution
Potassium biphosphate	Buffer solution
UDP-glucosyltransferase and sucrose synthase	Catalysts/enzymes
Water	Solvent
Ethanol	Solvent
Activated charcoal	Decolourant
Filtration Aids	
Nylon membrane cloth	Filtration aid
Macroporous resin column	Filtration aid
Filter paper	Filtration aid

UDP = uridine diphosphate

B.5.3 Details of the Manufacturing Process

B.5.3.1 Stage 1 – Enzyme Production

The first stage of the manufacturing process involves preparation of the enzymes that are utilised as processing aids in Stage 2. The enzymes are generated by a strain of *P. pastoris* that expresses the UDP-glucosyltransferase and sucrose synthase enzymes necessary to convert purified stevia leaf extract to rebaudioside E. The strain is designated Yeast A and carries the uridine 5'-diphosphoglucuronosyl transferase (UGT)-A fusion enzyme (*i.e.,* glucosyltransferase fused with sucrose synthase). This same UGT-A fusion enzyme containing UDP-glucosyltransferase and sucrose synthase components that is

sourced from a *P. pastoris* strain expressing UGT-A, is listed in Schedule 18 as a permitted processing aid for the conversion of purified stevia leaf extract to produce rebaudioside M.

The glycerol stock of Yeast A is removed from the -70°C freezer, thawed to room temperature, and grown in 50 mL yeast culture seed media. After 12 hours, the growing seed culture 1 is transferred to 2 L yeast culture seed media as seed culture 2. When the cells read OD_{600} = 10, they are transferred to 500 L fermenters. This seed culture 3 is then transferred to a 60 tonne production fermenter. The yeast cells are cultured, according to Blue California's published patents, for 48 hours. After confirming their catalytic activity in a small shaking flask, Yeast A is harvested by centrifugation and re-suspended in a reaction buffer. Yeast A is passed through a homogeniser operated at minimum pressure to release the enzymes present on the cell surface without lysing the cells. The enzymes are separated from the yeast cells *via* centrifugation, and the supernatant containing the UGT-A fusion enzyme is collected and used in the bioconversion.

B.5.3.2 Stage 2 – Rebaudioside E Production

A) Bioconversion

For the catalytic reaction needed to convert purified stevia leaf extract to rebaudioside E, the UGT-A fusion enzyme is mixed together in a 60 tonne reaction tank with slow agitation. Purified stevia leaf extract (≥95% steviol glycosides) is fed into the tank to allow the reaction to proceed. The reaction mixture containing rebaudioside E is collected in a storage tank and is heated to 85°C for 20 minutes to denature the enzymes. The mixture is filtered to remove the denatured enzymes.

B) Extraction and Purification

The remaining steps employed to purify rebaudioside E are consistent with the purification procedures described for steviol glycosides in the most recent JECFA Chemical and Technical Assessment (FAO, 2016). The supernatant is loaded onto large columns containing a macroporous resin. The supernatant flows through the column by gravity and is bound to the resin. The column is rinsed with a series of buffer solutions and rebaudioside E is eluted with food-grade ethanol numerous times. The eluent is collected and condensed in a wipe-film evaporator. The condensate is chilled to allow rebaudioside E to crystallise and precipitate from the solution. The wet crystals are collected, washed, and dissolved in ethanol. The re-dissolved rebaudioside E is treated with activated charcoal to remove remaining impurities, re-crystallised, dried, and processed to the final high-purity rebaudioside E product (≥85% rebaudioside E; ≥95% steviol glycosides).

B.6 Specification for Identity and Purity of Rebaudioside E

B.6.1 Product Specifications for Rebaudioside E

Blue California has established food-grade specifications for rebaudioside E produced *via* enzymatic bioconversion of purified stevia leaf extract. As shown in Table B.6.1-1, the product specifications 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" (JECFA, 2010). All methods of analysis are internationally-recognised methods.

Table B.6.1-1 Product Specifications for Rebaudioside E

Specification Parameters	Blue California (Rebaudioside E)	FSANZ (Steviol glycosides from *S. rebaudiana* Bertoni, S3-35)	JECFA (Steviol glycosides)	Method of Analysis
Physical parameters				
Appearance	Powder	Powder	Powder	Visual
Colour	White	White to light yellow	White to light yellow	Visual
Solubility	Soluble in water	Freely soluble in a mixture of ethanol and water (50:50)	Freely soluble in a mixture of ethanol and water (50:50)	
Purity	≥85% (rebaudioside E) ≥95% (steviol glycosides)	≥95% total steviol glycosides	≥95% total steviol glycosides	HPLC
Chemical parameters				
Residual ethanol	<1,000 ppm	≤5,000 mg/kg	≤5,000 ppm	USP 34
Residual methanol	<200 ppm	≤200 mg/kg	≤200 ppm	USP 34
Loss on drying	≤5%	≤6%	≤6%	USP 34
pH (1% solution)	4.5 to 7	4.5 to 7.0	4.5 to 7.0	USP 34
Total ash	≤1%	≤1%	≤1%	USP 34
Arsenic	<0.5 ppm	≤1 mg/kg	≤1 ppm	ICP-MS (AOAC 993.14)
Lead	<0.5 ppm	≤1 mg/kg	≤1 ppm	ICP-MS (AOAC 993.14)
Mercury	<0.5 ppm	Not specified	Not specified	ICP-MS (AOAC 993.14)
Cadmium	<0.5 ppm	Not specified	Not specified	ICP-MS (AOAC 993.14)
Microbiological parameters				
Total plate count	<1,000 CFU/g	≤1,000 CFU/g	≤1,000 CFU/g	AOAC 990.12
Total coliforms	<10 CFU/g	Not specified	Not specified	AOAC 991.14
Yeast and mould	<100 CFU/g	≤200 CFU/g	≤200 CFU/g	AOAC 997.02
Salmonella spp.	Negative in 25 g	Negative in 25 g	Negative in 25 g	AOAC-RI 100201
Escherichia coli	Negative in 1 g	Negative in 1 g	Negative in 1 g	AOAC 991.14

AOAC = Association of Analytical Communities; CFU = colony forming units; HPLC = high performance liquid chromatography; ICP-MS = Inductively coupled plasma mass spectrometry; JECFA = Joint FAO/WHO Expert Committee on Food Additives; ppm = parts-per-million; USP = United States Pharmacopeia.

B.6.2 Product Analysis

B.6.2.1 Batch Analyses

Five non-consecutive batches of Blue California's rebaudioside E produced *via* enzymatic bioconversion of purified stevia leaf extract were analysed and the results in Table B.6.2.1-1 demonstrate compliance with the defined product specifications. The certificates of analyses are provided in Appendix G.

Table B.6.2.1-1 Analytical Results for 5 Non-Consecutive Batches of Rebaudioside E

Parameter	Specification	Manufacturing Lot No.				
		20131005	160202-1601	160921-1602	160921-1603	20170665-04
Physical parameters						
Appearance	Powder	Pass	Pass	Pass	Pass	Pass
Colour	White	Pass	Pass	Pass	Pass	Pass
Solubility	Soluble in water	Pass	Pass	Pass	Pass	Pass
Purity	≥95% (steviol glycosides) ≥85% (rebaudioside E)	98.5% 90%	97% 90.5%	98.3% 91.2%	99.5% 86.7%	99.3% 85.4%

Table B.6.2.1-1 Analytical Results for 5 Non-Consecutive Batches of Rebaudioside E

Parameter	Specification	Manufacturing Lot No.				
		20131005	160202-1601	160921-1602	160921-1603	20170665-04
Chemical parameters						
Residual ethanol	<1,000 ppm	<200 ppm	<200 ppm	<200 ppm	<200 ppm	<200 ppm
Residual methanol	<200 ppm	<50 ppm	<100 ppm	<100 ppm	<100 ppm	<100 ppm
Loss on drying	≤6%	1.2%	1.48%	1.12%	1.29%	1.33%
pH (1% solution)	4.5 to 7	5.2	5.5	5.2	4.95	5.6
Total ash	≤1%	0.12%	0.15%	0.15%	0.25%	0.1%
Arsenic	<0.5 ppm	<0.5 ppm	<0.5 ppm	<0.5 ppm	<0.5 ppm	<0.5 ppm
Lead	<0.5 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm	<0.05 ppm
Mercury	<0.5 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm
Cadmium	<0.5 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm
Microbiological parameters						
Total plate count	<1,000 CFU/g	<1,000 CFU/g	<1,000 CFU/g	<1,000 CFU/g	<1,000 CFU/g	<1,000 CFU/g
Total coliforms	<10 CFU/g	<3 CFU/g	<3 CFU/g	<3 CFU/g	<3 CFU/g	<3 CFU/g
Yeast and mould	<100 CFU/g	<50 CFU/g	<50 CFU/g	<50 CFU/g	<50 CFU/g	<50 CFU/g
Salmonella spp.	Negative	ND	ND	ND	ND	ND
Escherichia coli	Negative	ND	ND	ND	ND	ND

CFU = colony forming units; ND = not detected; ppm = parts-per-million.

B.7 Information for Food Labelling

Rebaudioside E is classified as a steviol glycoside under Schedule 3 and as such, it would follow similar food labelling as for current steviol glycoside preparations. Steviol glycosides are considered intense sweeteners and flavour enhancers when added to various food products. Steviol glycosides have been assigned the INS number of 960. Rebaudioside E will be labelled under the functional class, sweetener, either as sweetener (960) or sweetener (steviol glycosides).

B.8 Analytical Method for Detection

The analytical methods used to confirm that Blue California's rebaudioside E meets the established chemical and microbial specifications (Section B.6.1) are internationally recognised (*e.g.*, Association of Analytical Communities [AOAC], U.S. Pharmacopeia [USP], JECFA). The rebaudioside E content in the final product is quantified according to the JECFA HPLC method for steviol glycosides described in FAO JECFA Monograph 10 for "Steviol Glycosides from *Stevia rebaudiana* Bertoni" (JECFA, 2010). Details of the HPLC method and chromatographic data are available in Appendix H.

B.9 Potential Additional Purposes of the Food Additive when Added to Food

As an intense sweetener, Blue California's rebaudioside E can be added to foods to replace the sweetness provided by sugars without significantly contributing to available energy. As such, rebaudioside E can be used by consumers to control caloric intake. Consumers following a weight-loss program looking to restrict their refined sugar intake or individuals with diabetes avoiding sugar consumption may also use rebaudioside E for these purposes.

C. INFORMATION RELATED TO THE SAFETY OF THE FOOD ADDITIVE

In accordance with Section 3.3.1 – Food Additives of the Food Standards Australia New Zealand *Application Handbook* (FSANZ, 2016) the safety information outlined must be provided to extend the use of a currently permitted food additive.

1. Information on the toxicokinetics and metabolism of the food additive and, if necessary, its degradation products and/or major metabolites; and,

2. Information on the toxicity of the food additive and, if necessary, its degradation products and major metabolites.

These points need only include reports of studies conducted since the last safety evaluation by FSANZ, and are addressed in the Section that follows.

Section 3.3.1 – Food Additives of the Food Standards Australia New Zealand *Application Handbook* (FSANZ, 2016) states that if available, safety assessment reports prepared by international agencies of other national government agencies should be provided. A summary of the safety assessment reports prepared by international agencies that have been published since the last safety evaluation by FSANZ are outlined in the following section.

C.1 Introduction

The safety conclusions for steviol glycosides in general, including rebaudioside E, are based on the fact that all steviol glycosides share a common metabolic fate following ingestion. Steviol glycosides 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 E, due to the shared metabolic fate.

Blue California recently submitted an application to amend the steviol glycosides specifications to include a new manufacturing process (*i.e.,* rebaudioside D produced by enzymatic bioconversion using UDP-glucosyltransferase and sucrose synthase enzymes; Application No. A1172). As part of this application, Blue California summarized safety studies on steviol glycosides that were published since the safety of steviol glycosides was last reviewed by FSANZ in 2016 when the definition of steviol glycosides was expanded to encompass all glycosides present in the *S. rebaudiana* Bertoni leaf. Therefore, for this application for a specification amendment, only safety studies conducted with steviol glycosides that were published in 2018 through 2019 were reviewed and summarised in the sections that follow. To identify scientific publications relevant to the safety of steviol glycosides and rebaudioside E, a comprehensive and detailed search of the published scientific literature was conducted up to February 2019. The search was limited to articles with full texts within peer-reviewed scientific journals and the following databases were accessed: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®.

C.2 Information on the Toxicokinetics & Metabolism of Steviol Glycosides

No new information pertaining to the toxicokinetics and metabolism of steviol glycosides published since 2018 were identified in the literature. It is well established that steviol glycosides all share a common metabolic fate in which the compound is hydrolysed to steviol by microbes residing in the colon and that steviol is then absorbed and metabolised to steviol glucuronide, which is excreted primarily *via* the urine in humans. The scientific conclusions regarding the safety of steviol glycosides are primarily based on the fact that all steviol glycosides are subject to microbial metabolism in a similar manner, ultimately generating the common primary metabolite steviol.

C.3 Information on the Toxicity of Steviol Glycosides

C.3.1 Toxicological Studies

C.3.1.1 Repeat-Dose Toxicity

The repeat-dose toxicity of steviol glycosides have been previously addressed in the safety evaluations by the scientific bodies and regulatory agencies described in Section C.4. No new data were identified in relation to this endpoint.

C.3.1.2 Genotoxicity

The genotoxicity of steviol glycosides has been previously addressed in the safety evaluations by the scientific bodies and regulatory agencies described in Section C.4. No new data were identified in relation to this endpoint.

C.3.1.3 Long-term Toxicity and Carcinogenicity

The chronic toxicity and carcinogenicity of steviol glycosides have been previously addressed in the safety evaluations by the scientific bodies and regulatory agencies described in Section C.4. No new data were identified in relation to this endpoint.

C.3.1.4 Reproductive and Developmental Toxicity

The reproductive and developmental toxicity of steviol glycosides have been previously addressed in the safety evaluations by the scientific bodies and regulatory agencies described in Section C.4. Two new reproductive and developmental toxicity studies published in 2018 were identified in the literature.

Gharheri *et al.* (2018) investigated the effects of *S. rebaudiana* extract (purity not reported) on reproduction function in diabetes-induced healthy adult male albino rats (Wistar). Diabetes mellitus was induced in rats *via* intraperitoneal injection of 50 mg streptozotocin/kg. The rats that reached fasting glucose levels greater than 250 mg/dL after 72 hours were selected for the study. Animals (7/group) were administered stevia extract at doses of 5, 50, or 100 mg/kg body weight per day by gavage for 28 days. A diabetic and non-diabetic control group received 2 mL distilled water only. Sexual behaviours of the rats were recorded for 30 minutes every 2 weeks for 1 month, including mount latency, intromission latency, mount frequency, intromission frequency, ejaculation latency, the mount latency post ejaculation, and ejaculation frequency. Following the study period, animals were killed, serum testosterone was measured, and histological examination was carried out on the right testis and epididymis. In diabetic rats, a significant increase in the frequency of intromission was observed in the low-dose group, compared to diabetic control rats. In addition, diabetic rats of the low-dose group showed a significant increase in the frequency of ejaculation, compared to the diabetic control and high-dose animals. However, a significant decrease in the latency of ejaculation was observed in the low-dose group when compared to the high-dose animals, although, the effect was not significant between the treated animals and the controls. Significant differences in other sexual behaviour parameters measured were not observed in the animals. Furthermore, a significant reduction in the number of Leydig cells in high-dose animals was noted, compared to the non-diabetic control group; however, this effect was not significantly different compared to the diabetic control rats. Organ weights and serum testosterone levels showed no significant differences among the study animals. Based on the results of the study, the authors concluded that there is no risk to reproductive parameters with the consumption of stevia and that intake of stevia may be effective in the promotion of blood glucose reduction and the prevention of the destruction of Leydig cells.

Jiang *et al.* (2018) investigated the effects of daily consumption of rebaudioside A (purity not reported) on the ovarian cycle and steroidogenesis in weanling rats. Female weanling Sprague-Dawley rats (body weight 42.3 ± 4.1 g; n=6/group) received oral doses of 0.5 or 2.5 mM rebaudioside A for 48 consecutive days. The control rats received normal water, and all animals were provided with rat chow and water *ad libitu*m. Food and water intake, and body weight were measured every third day in the morning. The day of vaginal opening was recorded (from tightly closed to open), and vaginal smears were taken daily to monitor the estrous cycle. Following the study period, rats were euthanized and blood samples and ovaries on diestrus-2 were collected. Serum progesterone levels were detected by a radioimmunoassay and the ovaries were examined through H&E staining, Western blotting, and immunohistochemistry. A significant decrease in body weight was observed in high-dose rebaudioside A-treated animals from Day 18 until Day 30, after which body weights returned to similar values in the control group. Water intake during the first 3 weeks of the study was significantly increased in high-dose rebaudioside A-treated animals, compared to the controls. During the last 3 weeks of the study, water intake was significantly higher in the high-dose rebaudioside A-treated animals compared to the low-dose rebaudioside A-treated animals. The serum progesterone levels of rats treated with rebaudioside A was significantly decreased compared to the control. Examination of the Western blot showed a higher expression of taste receptor type 2 subunit 38 (T2R38) in low- and high-dose rebaudioside A-treated groups, while

lower expression of other proteins (T1R3, Gα, StAR, CYP11A1, 3β-HSD, CYP17A1, 17β-HSD, and CYP19A1) in the ovaries was observed, compared to the controls. In addition, immunohistochemistry investigation of the ovaries also showed a lower expression of T1R3 and Gα proteins in rebaudioside A-treated groups. Based on the results of the study, the authors concluded that rebaudioside A has the potential to disrupt steroidogenesis in female weanling rats. However, there is no evidence of such effects reported in other subchronic and/or long-term studies on any steviol glycoside tested.

C.3.1.5 Other Studies

Ahmad and Ahmad (2018) examined the antidiabetic effects of aqueous extract of *S. rebaudiana* leaves (purity not reported) in rats with streptozotocin-induced diabetes. Sixty (60) adult male albino rats (body weight 152.53 g; n=10/group) were provided a basal diet *ad libitum* for 2 weeks. The diabetic rats were orally administered *S. rebaudiana* Bertoni aqueous extract dissolved in distilled water at doses of 200, 300, 400, and 500 ppm/kg body weight/day for 8 weeks. The non-diabetic and diabetic control rats were provided with distilled water. Food and water consumption were measured daily, and body weight gain was measured weekly. Following the study period, rats were fasted overnight and terminated. Blood samples were collected, and the serum of the rats were analysed for the following biochemical parameters: blood glucose, glycosylated haemoglobin (HbA1c), insulin, and liver glycogen levels. Random blood glucose and fasting blood glucose levels were significantly decreased in diabetic rats receiving stevia extract when compared to the controls after 8 weeks, while fasting blood glucose levels significantly increased in diabetic control rats compared to non-diabetic control rats. HbA1c levels were also significantly decreased after 8 weeks in diabetic rats receiving stevia extract compared to the controls, whereas HbA1c levels significantly increased in diabetic control rats compared to non-diabetic controls. Insulin and liver glycogen levels significantly increased in diabetic rats receiving stevia extract following the 8-week study period compared to the controls. When compared to the non-diabetic controls, a significant decrease in liver glycogen levels was observed in diabetic control rats. The authors concluded that stevia extract can ameliorate diabetic effects in rats with streptozotocin-induced diabetes.

Barrios-Correa *et al*. (2018) examined the brain of mice for changes in the JAK2/STAT3 signalling pathway and changes in appetite and body composition as a result of chronic intake of commercial sweeteners. Seventy-two (72) adult male and female BALB/c mice (9/sex/group) were provided with one of the following diets: sucrose (10% dilution of sucrose in 100 mL of purified water), sucralose (one 1 g packet of commercial sucralose sweetener Splenda®, equivalent to 0.012 g of sucralose, in 100 mL of purified water), or steviol glycosides (one 1 g packet of commercial steviol glycoside sweetener Svetia®, equivalent to 0.025 g of steviol glycosides in 100 mL purified water; purity not reported) for 6 weeks. The control mice were given purified water and all the animals were provided with food and water *ad libitum*. Following the 6-week study period, mice were terminated, and the brains were removed. Throughout the study period, food and water intake were measured daily, body weights were measured at the beginning of the study and then once every week until termination of the study, and energy intake was determined at the end of the study. Body composition and expression of total and phosphorylated JAK2, STAT3, and Akt, in addition to, SOCS3 and ObRb in the brain tissue were examined. Male mice provided with steviol glycosides showed significantly decreased energy intake, adiposity, as well as downregulation of feeding behaviour demonstrating a decrease in weight gain, when compared to the controls. In addition, increased expression of pJAK2 and pSTAT3 in the brain were observed in male mice supplemented with steviol glycosides when compared to the controls. In comparison, expression of JAK2 and pJAK2 was upregulated in female mice supplemented with steviol glycosides, compared to the controls. The authors concluded that chronic intake of steviol glycosides changes brain activity with respect to signalling pathways that control appetite and energy balance.

Ahmad *et al.* (2018a) examined the antihyperlipidemic effects of aqueous extract of *S. rebaudiana* leaves (purity not reported) in rats with cholesterol-induced hyperlipidaemia. Sixty (60) adult male albino rats (body weight 153.88 g; n=10/group) were orally administered *S. rebaudiana* Bertoni aqueous

extract dissolved in distilled water at doses of 0, 200, 300, 400, and 500 ppm/kg body weight/day for 8 weeks. An additional group of non-hyperlipidaemic control rats were provided with a basal diet. Food and water consumption were measured daily, and body weight gain was measured weekly. At the end of the study period, rats were fasted overnight and terminated. Blood samples were collected and the serum lipid profile was analysed for total cholesterol, triglycerides, HDL, LDL, very low density lipoproteins (VLDL), and LDL/HDL ratios. After the 8-week study period, significant decreases in total cholesterol, triglycerides, LDL levels, VLDL levels, and LDL/HDL ratios were observed at all doses of the stevia extract compared to the control. HDL levels appeared to improve among the hyperlipidaemic rats when compared to the non-hyperlipidaemic controls after 8 weeks. The authors concluded that the stevia extract can ameliorate hyperlipidaemic effects in rats with cholesterol-induced hyperlipidaemia.

El-Mesallamy *et al.*, (2018) assessed the effects of stevioside on skeletal muscle metabolic dysfunctions of diabetic rats. Male Sprague-Dawley rats (10/group) were induced with type 1 diabetes with 50 mg streptozotocin/kg body weight given intraperitoneally. Fasting blood glucose measurements were sampled one week after administration to confirm the induction of diabetes in the animals. Diabetic rats were subsequently treated with 2 mg/kg body weight per day pure stevioside for 4 weeks with control rats receiving 0.5 mL saline, both *via* gavage. A separate control group of non-diabetic rats provided with 0.5 mL saline was also included in the study. Blood samples were obtained at the end of the study and analysed for changes in blood glucose, HBA1c, fasting serum insulin, and HOMA-insulin resistance. Significantly elevated levels of fasting blood glucose, HOMA-IR, and Hb1Ac and significantly decreased body weight and insulin levels were reported in diabetic rats relative to non-diabetic control group. Changes in fasting blood glucose, insulin, and HBA1c were significantly ameliorated in diabetes-induced rats provided stevioside relative to untreated diabetic rats. Analysis of the soleus muscle for indicators of oxidative stress showed significantly decreased malondialdehyde concentration and significantly increased glutathione peroxidase, superoxide dismutase, and catalase activity in diabetic rats compared to non-diabetic rats. These effects were mitigated with the administration of stevioside in diabetic rats relative to rats of the diabetic control group. Untreated diabetic rats were also reported to have reduced AMP-activated protein kinase activity in the tissues of the soleus muscle and GLUT4 gene mRNA expression. These effects were also reported to be significantly moderated in diabetic rats with the treatment of stevioside relative to diabetic control rats. As such, the authors concluded that skeletal muscle metabolic dysfunctions in diabetic rats were improved with stevioside treatment.

The effects of aqueous extracts of stevia on cirrhosis-induced rats were studied by Ramos-Tovar *et al.* (2018). Male Wistar rats (8/group) were treated 3 times per week with thioacetamide intraperitoneally to induce cirrhosis with 0 (control) or 100 mg/kg body weight/day stevia orally. The study also included additional groups of non-cirrhotic rats provided with an oral dose of 100 mg/kg body weight/day stevia or 1 mL drinking water/day. The composition of the aqueous extract of stevia, isolated from the leaves of *S. rebaudiana* Bertoni variety Morita II, was analysed and confirmed to contain 15.5% stevioside and 3.97% rebaudioside A (wt/wt). Body weights were measured and recorded weekly, and blood and liver samples were collected following sacrifice at study termination. Following analyses of liver tissues, thioacetamide was observed to induce liver macronodular fibrosis, steatosis, hyperchromatic nuclear hepatocytes, liver parenchyma disruptions, and atypical and pleomorphic nuclei, which were mitigated with the co-treatment of the aqueous extract of stevia. Treatment with stevia was also reported to prevent the significant increases in total and direct bilirubin concentrations induced by thioacetamide. Although the liver weights were not significantly different in any groups, thioacetamide treatment resulted in decreased body weight and a corresponding increase in ratio of liver to body weight, which was reduced with the co-treatment of stevia. When compared to the control group, thioacetamide treatment significantly increased lipid peroxidation, 4-hydroxynonenal protein levels, and glutathione disulphide, and significantly decreased reduced glutathione, oxidized glutathione, nuclear factor-E2-related factor 2 proteins, the ratio of glutathione to glutathione disulphide, and the sum of glutathione and glutathione disulphide. In addition, significantly increased levels of NF-κB (p65), Il-6, IL-1β (proteins and mRNA), TNF-α (proteins and mRNA), serum ALT, and gamma-glutamyl transpeptidase were also reported in rats treated with thioacetamide relative to the control group. When thioacetamide was co-

administered with stevia, these effects were reduced and similar to untreated controls, with the exception of lipid peroxidation, 4-hydroxynonenal protein levels, oxidized glutathione, the sum of glutathione and glutathione disulphide, and nuclear factor-E2-related factor 2 proteins changes which were only mitigated. Rats treated with thioacetamide also had significantly increased area of fibrosis and collagen, collagen 1 *alpha* protein, and transforming growth factor-*beta* 1 levels. These effects were reversed to levels comparable with the control group when animals were also treated with stevia, with the exception of the changes in collagen and transforming growth factor-*beta* 1 levels. These were significantly reduced relative to the thioacetamide group, but remained significantly elevated relative to control groups. The levels of alpha-smooth muscle actin mRNA and protein, pro- and active metalloproteinase 13, metalloproteinase 9 and 2 activity, and connective tissue growth factor were also significantly increased with thioacetamide treatment relative to controls. These changes were significantly reversed when co-treated with stevia, with the exception of the alpha-smooth muscle actin protein, pro metalloproteinase 13, and connective tissue growth factor levels which were moderated with stevia, but remained significantly elevated relative to controls. The administration of stevia alone did not result in any significant differences relative to controls. It was concluded by the authors that stevia may have preventative effects on thioacetamide-induced cirrhosis in rats.

Zhao *et al.* (2018) studied the effects of 2 aqueous residue extracts of stevia on mice with impaired glucose regulation. Male ICR mice (10/group) were placed on a high fat/high fructose diet alone (control) or administered 50 or 200 mg/kg body weight of stevia extract 1 or 2 *via* gavage daily for 12 weeks. An additional control group of mice placed on a regular diet was included in the study. Aqueous stevia extracts were isolated from stevia leaves and subsequently filtrated to produce the 2 residue extracts distinguished by the composition of phenolic compounds (steviol glycoside content and purity not reported). Induction of impaired glucose tolerance was confirmed in mice fed the high fat/high fructose diet in an oral glucose tolerance test conducted following a 12-hour fast on weeks 3, 6, and 9 of the study. Results of mice given stevia were reported to be comparable with control mice fed the normal diet, with the most prominent effect reported in the high dose group given stevia extract 1. Following analysis of serum lipid levels on week 5 and 10, significantly increased total cholesterol, triglycerides, LDL-C and HDL-C levels were reported in mice fed the high fat/high fructose diet. These effects were significantly moderated in mice that were also given high dose stevia extract 1 and were comparable to normal diet controls. Antioxidant levels were assessed at study termination and mice fed the high fat/high fructose diet were reported to have significantly reduced levels of superoxide dismutase, malondialdehyde, and total antioxidant activity. These effects were reported to be significantly moderated in mice co-treated with high dose stevia extract 1 to levels similar to normal diet controls. Histological examinations of liver tissues in mice fed the high fat/high fructose diet were also reported to have increased lipid deposits relative to controls fed the normal diet. This effect was reported to be significantly reduced in mice also receiving high dose stevia extract 1. In conclusion, the authors suggested that the effects of certain residue extracts of stevia may be beneficial to mice with impaired glucose regulation.

Han *et al.* (2019) investigated the inclusion of stevioside in the diet of goats and its effects on feed intake and digestibility. Stevioside was provided in the diet of male Xiangdong Black goats (3/group) (rice straws) at 0, 400, or 800 mg/kg forage (dry matter) for 20 days. Feed concentrate was provided with the feed twice daily at 0.5% body weight. Stevioside was isolated from the leaves of *S. rebaudiana* at 97% purity and dissolved in water. Animals were allocated into treatment groups using a replicated 3 x 3 Latin square design. On Days 12 to 17, faecal samples were collected and analysed for nutrient digestibility and chemical composition and total digestibility was calculated. On Day 18, observations were conducted on the feeding behaviour including eating, ruminating, and resting over a 24-hour period. Blood samples were collected on Day 19 and assessed for serum metabolites, glucose, total protein, albumin, globulin, triglyceride, and total cholesterol. On Day 19 and 20, rumen fluid samples were collected and analysed for pH, concentrations of volatile fatty acid, and ammoniacal nitrogen (NH_3-N) concentration. The dry intake of forage and total diet were reported to significantly increase in a linear relationship in animals given a diet containing stevioside, which was suggested to increase the

palatability of the forage. Animals given stevioside in the diet were also reported to have a significant quadratic decrease in total volatile fatty acids and significant quadratic increase in rumen pH. Levels of isobutyrate and isovalerate in animals given stevioside between 0 and 400 mg/kg stevioside were increased and decreased between 400 and 800 mg/kg stevioside in a significant quadratic relationship. Animals treated with stevioside were also reported to have an increase in a statistically significant linear and quadratic relationship in neutral and acid detergent fibre digestibility, with increased digestibility at 0 to 400 mg/kg stevioside, and decreased digestibility from 400 to 800 mg/kg stevioside. Serum parameters were not significantly affected with the treatment of stevioside. In conclusion, feed containing stevioside increased dry matter intake and digestibility of neutral and acid detergent fibre in goats.

C.3.2 Human Studies

Human safety studies have been previously addressed in the safety evaluations by the scientific bodies and regulatory agencies described in Section C.4. Two new human evaluations published in 2018 were identified in the literature.

Ahmad *et al*. (2018b) investigated the potential effects of stevia leaf powder (prepared from dried stevia leaves; steviol glycoside content not reported) on postprandial glycemia, appetite, palatability, gastrointestinal discomfort, and anthropometric parameters in a randomized single-blinded, crossover placebo-controlled study in healthy humans. Healthy males and females (10/group; mean age 24.1 ± 1.33 years; BMI 22.09 ± 3.88 kg/m^2) were fasted overnight and provided with either a placebo (140 g cookie made from 100% wheat flour) or cookies containing stevia leaf powder (3% w/w; approximately equivalent to 4.2 g stevia) once in the morning. A 1- to 2-week washout period was carried out before and after each treatment period. The subjects were to avoid any vigorous physical activity prior to each study visit, while also maintaining the same dietary patterns in the evening prior to each visit. At baseline and following each treatment, fasting blood glucose concentration, appetite, hunger levels, and gastrointestinal discomfort were measured. The following parameters were also measured: blood pressure, weight, height, and BMI. The palatability of test foods was also recorded using a 9-point hedonic scale. Following the consumption of stevia, a decrease in appetite was noted by the authors, compared to the control cookies. However, this observed effect was only significant at 30 minutes following intake. In addition, the stevia cookies had a lower rating for texture based on the palatability testing, when compared to the control cookies. No other significant differences were observed in relation to palatability parameters, and the stevia-containing cookies did exceed the score required to be considered acceptable. The results also demonstrated no significant effects on any of the anthropometric parameters, blood glucose response, or gastrointestinal discomfort. The study authors concluded that consumption of stevia leaf powder in cookies decreased hunger, when compared to the control cookies.

Rizwan *et al.* (2018) investigated the beneficial effect of *S. rebaudiana* (purity not reported) in a prospective, interventional, randomized, single-blind, placebo-controlled preliminary trial in stage I to stage III chronic kidney disease (CKD) patients. Ninety-seven (97) male and female patients (Group 1 [n=44]: 55 ± 11.75 years, BMI 26.34 ± 3.46 kg/m2; Group 2 [n=43]: 53.60 ± 11.27 years, BMI 25.79 ± 3.31 kg/m2; Group 3 [n=10]: 47.20 ± 4.87 years, BMI 25.45 ± 4.11 kg/m2) were enrolled in the study and received either a stevia capsule (250 mg; purity not reported) or matching placebo twice daily, as well as Angiotensin-II Receptor Blocker and/or Calcium Channel Blocker for 9 months. The patients were separated into 3 groups as follows: study Group 1 (STV), stevioside capsule plus conventional antihypertensive treatment and CKD treatment; study Group 2 (PLC), matching placebo and a similar treatment regimen; study Group 3 (CL), control group with healthy participants. Follow-up visits were scheduled every 3 months, and a washout period was conducted after 9 months. Data from the first 3 months of the study were only considered in this study. Blood and urine samples were collected at the first follow-up, 3 months after the initial stage of the study (baseline). Significant changes were observed in systolic and diastolic blood pressure, serum creatinine, serum uric acid, fasting blood sugar,

postprandial blood sugar, and microalbumin levels in Group 1 compared to baseline. In comparison, significant differences in systolic and diastolic blood pressure, serum uric acid, sodium, chloride, urine for albumin, and urine for protein were observed in the Group 2 compared to baseline. Moreover, significant differences at baseline were observed in diastolic blood pressure, blood urea, serum creatinine, serum total protein, calcium, and inorganic phosphate between Groups 1 and 2. In the first follow-up (3 months), significant differences were observed in diastolic blood pressure and urine for protein between Groups 1 and 2. In addition, very highly significant ($p<0.001$) results were observed for estimated glomerular filtration rate in Group 1 compared with controls. Based on the results of the study, the authors concluded that stevia has the potential to significantly improve some biochemical parameters in CKD patients after 3 months of treatment, and the constructive effect of stevia can be confirmed after 9 months of treatment.

C.4 Safety Assessment Reports Prepared by International or National Agencies

The safety of steviol glycosides has been reviewed by several scientific bodies and regulatory agencies, such as FSANZ, the U.S. FDA, JECFA, EFSA, European Commission's Scientific Committee on Food (SCF), and Health Canada. The large consumer and industry interest into the use of steviol glycosides as sweeteners has prompted extensive safety testing of these compounds and, as a result, a large safety database exists. This database includes a thorough evaluation of the metabolic fate and pharmacokinetics of various steviol glycosides in experimental animals and humans, acute toxicity studies, short-term and long-term toxicity and carcinogenicity studies, reproductive and developmental toxicity studies, *in vitro* and *in vivo* mutagenicity and genotoxicity studies, and clinical studies. Many of the early toxicology studies examined the safety of stevioside due to its predominance in *S. rebaudiana* leaves (Aze *et al*., 1991; Toyoda *et al.,* 1997). However, due to the shared metabolic fate of steviol glycosides (*i.e.,* hydrolysis into steviol), regulatory agencies and authoritative bodies have expanded their safety opinions to encompass the safety of all steviol glycosides rather than individual glycosides. The most recent opinions/reports are summarised below.

C.4.1 Joint FAO/WHO Expert Committee on Food Additives (JECFA)

The safety of steviol glycosides has been extensively reviewed by JECFA at their 51st, 63rd, 68th, 69th, and 82nd meetings in 1998, 2004, 2007, 2008, and 2016 respectively (JECFA, 1999, 2006, 2007, 2009, 2017). Initially, the Committee established a temporary ADI for steviol glycosides of 0 to 2 mg/kg body weight, expressed as steviol, based on a NOAEL of 970 mg/kg body weight/day (383 mg/kg body weight/day as steviol) from a 2-year study in rats (Toyoda *et al.*, 1997) and application of a safety factor of 200 (JECFA, 2006). In 2008, following review of additional animal and human studies evaluating the effects of steviol glycosides on blood pressure and blood glucose, the Committee concluded that the results from these studies were sufficient to remove the additional safety factor of 2, and established a full ADI of 0 to 4 mg/kg body weight (expressed as steviol) for steviol glycosides.

The JECFA Committee recently re-evaluated the safety, dietary intake, and specifications for steviol glycosides at its 82nd meeting in 2016. The safety of steviol glycosides as well as the ADI of 0 to 4 mg/kg body weight, expressed as steviol, were confirmed. Details of a new manufacturing process for rebaudioside A utilising a strain of *Y. lipolytica* that was genetically modified to overexpress the steviol glycoside biosynthetic pathway were submitted to and reviewed by the Committee. As a result, the Committee issued a new specification monograph for "Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica*" (JECFA, 2016). The Committee also reviewed data demonstrating the shared metabolism of all steviol glycosides and issued new specifications for "Steviol Glycosides from *Stevia rebaudiana* Bertoni" (JECFA, 2017a), expanding the definition of steviol glycosides to "a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties (glucose, rhamnose, xylose, fructose, arabinose, galactose and deoxyglucose) in any of

the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni". The purity of steviol glycosides from *S. rebaudiana* Bertoni must be no less than 95% total steviol glycosides on the dried basis.

C.4.2 U.S. Food and Drug Administration (FDA)

Since the beginning of 2018, 13 GRAS notices regarding purified steviol glycosides (≥95% purity), including stevia leaf extract, glucosylated steviol glycosides, steviol glycosides manufactured using genetically modified yeast, and steviol glycosides manufactured *via* enzymatic bioconversion have been submitted to the U.S. FDA. A summary of the steviol glycoside GRAS notices submitted to the U.S. FDA since the beginning of 2018 and the Agency's corresponding response, where available, is presented in Table C.4.2-1. With the exception of the most recent GRAS notifications currently pending review, the U.S. FDA has raised no objections to the GRAS status of these steviol glycoside products for use as general purpose sweeteners in foods. Of particular relevance to this submission, Blue California submitted a GRAS notice pertaining to the GRAS status of rebaudioside E that is the subject of this application, and was filed by the Agency under GRN No. 823 (U.S. FDA, 2019a). The status of GRN No. 823 is still pending. The production process of rebaudioside E is similar to the processes described in GRN No. 667 and 715 for rebaudiosides M and D, respectively, produced by enzymatic bioconversion which received "no questions" from the U.S. FDA regarding their GRAS status (U.S. FDA, 2017a,b)

Table C.4.2-1 Summary of GRAS Notices Submitted to the U.S. FDA for Steviol Glycosides in 2016, 2017, 2018, and 2019

Company	Substance	FDA Response	GRAS Notice No.
Shangdong Shengxiangyuan Biotechnology	Purified steviol glycosides	No questions	GRN 000733 (U.S. FDA, 2018a)
PureCircle Limited	Steviol glycosides consisting primarily of rebaudioside M	No questions	GRN 000744 (U.S. FDA, 2018b)
PureCircle Limited	Steviol glycosides consisting primarily of rebaudioside M	No questions	GRN 000745 (U.S. FDA, 2018c)
DSM Food Specialties	Steviol glycosides consisting primarily of rebaudioside M produced in *Yarrowia lipolytica*	No questions	GRN 000759 (U.S. FDA, 2018d)
Sichuan Ingia Biosynthetic Co., Ltd.	Rebaudioside D	No questions	GRN 000764 (U.S. FDA, 2018e)
Cargill, Inc.	Stevia leaf extract	No questions	GRN 000768 (U.S. FDA, 2018f)
Tate and Lyle	Rebaudioside M	No questions	GRN 000780 (U.S. FDA, 2018g)
GLG Life Tech Corporation	Steviol glycosides (minimum purity 95%)	No questions	GRN 000790 (U.S. FDA, 2018h)
Steviana Bioscience (Suzhou) Inc.	Purified steviol glycosides	No questions	GRN 000795 (U.S. FDA, 2018i)
Sichuan Ingia Biosynthetic Co., Ltd.	Rebaudioside M	No questions	GRN 000799 (U.S. FDA, 2018j)
Amyris, Inc.	Rebaudioside M	Pending	GRN 000812 (U.S. FDA, 2019b)
Haigen-BGG Natural Ingredients Limited	Glucosylated steviol glycosides	Pending	GRN 000821 (U.S. FDA, 2019c)
Blue California	Rebaudioside E	Pending	GRN 000823 (U.S. FDA, 2019a)

FDA = Food and Drug Administration; GRAS = Generally Recognized as Safe; U.S. = United States.

C.4.3 Health Canada

In 2017, Health Canada expanded the definition further to include all the steviol glycosides in the *S. rebaudiana* Bertoni plant (Health Canada, 2017b). Detailed safety assessments were conducted by Health Canada in both cases and the agency concluded that the expanded definitions of steviol glycosides raised no safety concerns. Expansion of the definition confirms that the safety data generated from 1 specific steviol glycoside can be used to support safety of another steviol glycoside.

C.4.4 European Food Safety Authority (EFSA)

In a recent evaluation in response to a proposed amendment of the specifications of steviol glycosides, EFSA did not agree to expand the definition of steviol glycosides to include all individual steviol glycosides, due to uncertainties on the rate and extent of the metabolism of the different steviol glycosides to steviol (EFSA, 2018a). Likewise, in a recent evaluation of glucosylated steviol glycosides, EFSA concluded that the data provided was not sufficient to assess the safety of glucosylated steviol glycosides due to the limited evidence on the complete hydrolysis of glucosylated steviol glycosides, metabolic fate data for steviol glycosides cannot be used in a read-across approach (EFSA, 2018b).

D. INFORMATION RELATED TO THE DIETARY EXPOSURE TO THE FOOD ADDITIVE

In accordance with Section 3.3.1 – Food Additives of the Food Standards Australia New Zealand *Application Handbook* (FSANZ, 2016) the following dietary exposure information must be provided:

1. A list of the foods or food groups proposed to contain the food additive.

2. The maximum proposed level and/or concentration range of the food additive for each food group or food.

3. For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption (Not applicable).

Each point is addressed in turn in the Section that follows.

D.1 Proposed Food Uses and Use-Levels of Rebaudioside E

The currently approved food uses and use-levels for steviol glycosides in Australia and New Zealand are presented in Table D.1-1 below (FSANZ, 2017). Blue California intends to market rebaudioside E for use as an intense sweetener under the same conditions of use as those presently authorised for steviol glycosides under Schedule 15.

Table D.1-1 Summary of Currently Permitted Food Uses and Use-Levels for Steviol Glycosides in Australia and New Zealand

Category No	Food Description	Steviol Glycoside Concentration (mg/kg) as Steviol Equivalents
1.1.2	Liquid milk products and flavoured milk	115
1.2.2	Fermented milk products and rennetted milk products	175
3	Ice cream and edible ices	200
4.3.2	Fruits and vegetables in vinegar, oil, brine, or alcohol	160
4.3.4.1	Low joule chutneys, low joule jams, and low joule spreads	450
4.3.6	Fruit and vegetable preparations including pulp	210
5.1	Chocolate and cocoa products	550
5.2	Sugar confectionary	1100
6.3	Processed cereal and meal products	250
7.1.1	Fancy breads	160
7.2	Biscuits, cakes, and pastries	160
11.4	Tabletop sweeteners	GMP
13.3	Formula meal replacements and formulated supplementary foods	175
13.4	Formulated supplementary sports foods	175
14.1.2.1	Fruit and vegetable juices	50
14.1.2.2.2	Low joule fruit and vegetable juice products	125
14.1.2.2.3	Soybean beverage (plain)	100 (plain)
	Soybean beverage (flavoured)	200 (flavoured)
14.1.3	Water based flavoured drinks	200
14.1.4	Formulated beverages	200
14.1.5	Coffee, coffee substitutes, tea, herbal infusions, and similar products	100
20.2.0.1	Custard mix, custard powder, and blancmange powder	80
20.2.0.2	Jelly	260
20.2.0.3	Dairy and fat based desserts, dips, and snacks	150 (only dairy and fat based dessert products)
20.2.0.4	Sauces and toppings (including mayonnaises and salad dressings)	320

GMP = good manufacturing practice

D.2 Exposure Data

As previously noted, rebaudioside E is intended for use as an intense sweetener under the same conditions of use as those presently authorised for steviol glycosides. Therefore, intakes of rebaudioside E will be the same as for steviol glycosides currently on the Australian/New Zealand marketplace as it is intended to be a direct replacement for other steviol glycosides. As such, a separate intake assessment for rebaudioside E was not performed for the purpose of this application. Furthermore, 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.

D.3 Use of the Food Additive in Other Countries

Blue California's rebaudioside E produced *via* enzymatic bioconversion of purified stevia leaf extract for use as a table top sweetener and a general purpose non-nutritive sweetener in foods has been concluded to be GRAS by an independent panel of experts under the GRAS procedure in the U.S. These GRAS conclusions on rebaudioside E produced by enzymatic bioconversion of purified stevia leaf extract, the subject of this application, were filed under GRN 823 by the U.S. FDA (U.S. FDA, 2019a). The status is currently "pending".

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