Application for the Approval of Those Steviol Glycosides Present in *Stevia rebaudiana* under Australia and New Zealand Food Standard Code Standard 1.3.1– Food Additives

PureCircle Limited
915 Harger Road, Suite 250
Oak Brook, Illinois, 60523
USA

16 May 2016

Application for the Approval of Those Steviol Glycosides Present in *Stevia rebaudiana* under Australia and New Zealand Food Standard Code Standard 1.3.1– Food Additives

Table of Contents

Page

A. GENERAL REQUIREMENTS 3
A.1 Format of the Application 4
A.2 Applicant Details 4
A.3 Purpose of the Application 5
A.4 Justification of the Application 5
A.5 Information to support the application 6
A.6 Assessment Procedure 7
A.7 Confidential Commercial Information (CCI) 7
A.8 Other Confidential Information 7
A.9 Exclusive Capturable Commercial Benefit (ECCB) 7
A.10 International and Other National Standards 8
A.11 Statutory Declaration 10
A.12 Checklist 10

B. TECHNICAL INFORMATION ON THE FOOD ADDITIVE 11
B.1 Nature and Technological Purpose of Steviol Glycosides 12
B.1.1 Taste Attributes 12
B.1.2 Stability 13
B.2 Information to Enable Identification of Steviol Glycosides 20
B.2.1 Identity of Substance 20
B.2.2 Composition 23
B.3 Information on the Chemical and Physical Properties of SG Mixtures 24
B.4 Information on the Impurity Profile 24
B.5 Manufacturing Process 24
B.6 Specification for Identity and Purity 24
B.6.1 Current Specifications for Steviol Glycosides 24
B.6.2 Product Analysis 27
B.7 Information for Food Labelling 30
B.8 Analytical Method for Detection 30
B.9 Potential Additional Purposes of the Food Additive when Added to Food 31

C. INFORMATION RELATED TO THE SAFETY OF THE FOOD ADDITIVE 32
C.1 Introduction 33
C.2 Metabolic Fate 33
C.3 Common Metabolic Fate of Steviol Glycosides 36
C.4 Safety of Steviol Glycosides 41

D. INFORMATION RELATED TO THE DIETARY EXPOSURE TO THE FOOD ADDITIVE 44
D.1 Current Permitted Food Uses and Use Levels of Steviol Glycosides 45
D.2 Exposure Data 46

REFERENCES 47

List of Appendices

APPENDIX A Statutory Declaration

APPENDIX B Checklists for Submission

APPENDIX C Sensory Analysis of Major and Minor Steviol Glycosides

APPENDIX D Study Reports for the Stability of Steviol Glycoside Mixtures

APPENDIX E HPLC Methodology for Analysis of Steviol Glycoside Mixtures

APPENDIX F Certificates of Analysis for Steviol Glycoside Mixtures

APPENDIX G Pesticide Analysis Reports for Steviol Glycoside Mixtures

List of Figures and Tables

Figure B.2.1-1 Backbone Structure Steviol Glycosides 21
Figure C.3-1 Structure of Fructosylated Rebaudioside A 40

Table B.1.2-1 Storage Stability of RA50 (Lot A0912266) when Stored at 40°C and 75% RH (glass vials), as percent (%) dry basis 14
Table B.1.2-2 Storage Stability of A95 (Lot PT21052015) when Stored at 40°C and 75% RH (aluminium bags), as percent (%) dry basis 14
Table B.1.2-3 Stability of A95 (Lot PT21052015) and RA50 (Lot A0912266) at 5°C in Solution at Varying pH 16
Table B.1.2-4 Stability of A95 (Lot PT21052015) and RA50 (Lot A0912266) at 25°C in Solution at Varying pH 17
Table B.1.2-5 Stability of A95 (Lot PT21052015) and RA50 (Lot A0912266) at 37°C in Solution at Varying pH 18
Table B.1.2-6 Stability of A95 (Lot PT21052015) and RA50 (Lot A0912266) at 56°C in Solution at Varying pH 19
Table B.2.1-1 Molecular Formula, and R-Groups in Identified Steviol Glycosides (see Figure B.2.1-1) 21
Table B.6.1-1 Physical and Chemical Specifications for SG Mixtures 26
Table B.6.1-2 Microbiological Specifications for SG Mixtures 27
Table B.6.2-1 Summary of the Chemical Product Analysis for Different Lots of SG Mixtures 27
Table B.6.2-2 Steviol Glycoside Distribution for 3 Lots of SG Mixture RA50 29
Table B.6.2-3 Steviol Glycoside Distribution for 5 Lots of SG Mixture A95 29
Table B.6.2-4 Summary of the Microbiological Product Analysis for Different Lots of SG Mixtures 30
Table C.3-1 Metabolism of Steviol Glycosides Containing Glucose, Xylose, and Rhamnose Moieties to Steviol by Human Faecal Homogenates 37
Table C.3-2 Metabolism of Fructosylated Rebaudioside A to Steviol by Human Faecal Homogenates 40
Table D.1-1 Summary of Currently Permitted Uses and Use Levels for Steviol Glycosides in Australia/New Zealand 45
Table D.1-2 Conversion Factors of Steviol Glycosides In Order to Determine Steviol Equivalents 46

Application for the Approval of Those Steviol Glycosides Present in *Stevia rebaudiana* under Australia and New Zealand Food Standard Code Standard 1.3.1– Food Additives

A. GENERAL REQUIREMENTS

In accordance with Section 3.1.1 – General Requirements of the Food Standards Australia New Zealand (FSANZ) *Application Handbook* (FSANZ, 2016a) 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
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 Application Handbook (FSANZ, 2016a) 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

D. Information on dietary exposure to the food additive.

The application is presented in this format. At the start of each section (A-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 within the *Application Handbook* (FSANZ, 2016a).

A.2 Applicant Details

PureCircle Limited (hereafter PureCircle) is the world's leading producer of high purity steviol glycoside ingredients for the global food and beverage industry. PureCircle obtains the stevia ingredients following extraction of the *Stevia rebaudiana* (*S. rebaudiana*) plant. The contact details for the person responsible for this application are listed below.

[redacted]
VP Head of Global Scientific & Regulatory Affairs
PureCircle Limited
915 Harger Road, Suite 250
Oak Brook, Illinois, 60523
USA
Telephone (Office):
Telephone (Mobile):
Fax:
Email:

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

[redacted]
Intertek Scientific & Regulatory Consultancy
2233 Argentia Road, Suite 201
Mississauga, Ontario, Canada L5N 2X7
Telephone (Office): [redacted]
Fax:
Email:

A.3 Purpose of the Application

PureCircle is submitting this application concerning steviol glycosides to FSANZ seeking the approval to expand the definition of steviol glycosides for use as a sweetener to include all steviol glycosides present in *S. rebaudiana* (*i.e.*, steviol conjugated with glucose, rhamnose, xylose, fructose, deoxyglucose and/or other sugar moieties in any orientation, quantity, or combination). The application which has currently identified more than 30 different glycosides will cover all those current and potential steviol glycoside mixtures (hereinafter referred to as "SG mixtures") produced by the PureCircle. Currently as listed in Schedule 3 – Purity and Identity of the Food Standards Code, steviol glycosides are a mixture comprising of not less than 95% of the 10 named steviol glycosides, which include rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, rebaudioside M, stevioside, dulcoside A, rubusoside, and steviolbioside. All other parameters relating to the identity and purity of steviol glycosides are consistent with those standards established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Commission, and the Food Chemical Codex (FCC).

A.4 Justification of the Application

Technological Function for the Food Additive

Although steviol glycoside preparations for use as high-intensity sweeteners are already approved for use in Australia and New Zealand, expanding the definition of steviol glycosides to include all steviol glycosides present in *S. rebaudiana* (*i.e.*, steviol conjugated with glucose, rhamnose, xylose, fructose, deoxyglucose and/or other sugar moieties in any orientation, quantity, or combination) will provide certain additional functional and technological advantages that are presently not attainable with the currently permitted 10 steviol glycosides. Based on sensory panel studies (see Section B.1.1), the inclusion of the SG mixtures overall improved the flavour and taste characteristics of the sample foods and improved the overall liking, as reported by the study participants, in comparison to sample foods prepared with a combination of some of the original major steviol glycosides. With the continued strive to limit unwanted taste characteristics that are often associated with the use of high-intensity sweeteners in foods and beverages, the specific flavour profiles

imparted by high-intensity sweeteners are critical determinants for their practical use as sugar replacement in food.

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

Steviol glycosides are already approved for many food uses at specified use levels within Australia and New Zealand and since PureCircle intends to market SG mixtures in the same approved food-uses and use-levels, there is no perceived benefit or added cost to government.

Expanding the definition of steviol glycosides to include all minor steviol glycosides identified in *S. rebaudiana* is of clear interest to the food industry in Australia and New Zealand since SG mixtures provide improved sensory characteristics which will allow for the replacement of greater amounts of sugar in food and beverages at the presently permitted use-levels for steviol glycosides.

It is expected that SG mixtures will present an attractive alternative as a sweetener for food manufacturers in Australia and New Zealand. It is anticipated that SG mixtures may be imported into Australia and New Zealand which manufacturers would then incorporate into their products and in addition, global companies may also import their finished products. SG mixtures would benefit individuals who want to maintain a reduced-calorie diet or individuals with specific medical conditions that require reduced sugar intakes including diabetics, as steviol glycosides do not interfere with glucose homeostasis (EFSA, 2010). This application to expand the definition of steviol glycosides to include all steviol glycosides identified in *S. rebaudiana* in Australia and New Zealand is part of a global regulatory strategy for PureCircle with the equivalent submissions prepared and submitted in the United States (U.S.) (Generally Recognized as Safe; GRAS) and Canada and is also on the upcoming agenda at the WHO/FAO JECFA meeting.

A.5 Information to support the application

In addition to the technical information specific to SG mixtures, as presented in Section B, information to support the safety of SG mixtures has been compiled utilising the numerous reviews and opinions by scientific bodies and regulatory authorities. Additionally, a detailed summary of the metabolic fate of steviol glycosides is presented in Section C. In order to corroborate previous conclusions regarding the shared metabolic pathway for steviol glycosides, *in vitro* studies comparing the microbial metabolism and hydrolysis rates of various steviol glycosides (rebaudioside A, B, C, D, E, F, M, steviolbioside, dulcoside A) are also discussed in Section C. Based on the findings of the available information, it was deemed appropriate to extend the safety conclusions for steviol glycosides to all glycosylated derivatives of the aglycone steviol (purified).

A.6 Assessment Procedure

PureCircle considers the most appropriate assessment procedure for the application herein is related to an amendment to *Standard 1.3.1 – Food Additives* of the Australia New Zealand Food Standards Code in order to expand the definition of steviol glycosides to include all those glycosides identified in *S. rebaudiana* (*i.e.*, steviol conjugated with glucose, rhamnose, xylose, fructose, deoxyglucose and/or other sugar moieties in any orientation, quantity, or combination). This revision is expected to fall under the General Procedure (Subdivision D of the FSANZ Act), Cost Category Level 2. PureCircle also requests that the application undergo the expedited procedure.

A.7 Confidential Commercial Information (CCI)

PureCircle requests the information contained within Section B.5.1 be considered confidential commercial information. This section provides a detailed description of the manufacturing process for SG mixtures including proprietary information and is therefore of significant commercial value to the company.

A.8 Other Confidential Information

PureCircle requests that the study report by Kwok (2015) cited in Section C.3 be considered as confidential. The study report represents unpublished research that is intended to be published in a peer-reviewed journal in the future.

A.9 Exclusive Capturable Commercial Benefit (ECCB)

Steviol glycoside preparations are currently produced by other manufacturing companies, aside from PureCircle. However, SG mixtures are only produced by PureCircle using a specific manufacturing process. On this basis, PureCircle is seeking permission for the exclusive use of SG mixtures; therefore, PureCircle anticipates that this application would confer Exclusive Capturable Commercial benefit (ECCB) in accordance with Section 8 of the Food Standards Australia New Zealand (FSANZ) Act which states:

An **exclusive, capturable commercial benefit** is conferred upon a person who applies for the development of a food regulatory measure or the variation of a food regulatory measure under Section 22 if:

> (a) the applicant can be identified as a person or body that may derive a financial gain from the coming into effect of the draft standard or draft variation of the standard that would be prepared in relation to the application; and
>
> (b) any other unrelated persons or bodies, including unrelated commercial entities, would require the agreement of the applicant in order to benefit financially from the approval of the application.

As such, PureCircle is expected to pay the full costs of processing this application.

A.10 International and Other National Standards

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

The safety of steviol glycosides was reviewed by the JECFA at 4 separate meetings (51st, 63rd, 68th, and 69 h) in 1998, 2004, 2007, and 2008 (JECFA, 1999, 2006b, 2007c, 2009). In 2004, the Committee established a temporary acceptable daily intake (ADI) of 2 mg/kg body weight/day (expressed as steviol) for steviol glycosides based on a study in which pharmacological effects were observed in patients. In 2008, the Committee received additional data on steviol glycosides and upon review of the new data, the Committee concluded that the results from the new human studies were sufficient to remove the additional safety factor of 2 and to establish a full ADI of 4 mg/kg body weight (expressed as steviol) for steviol glycosides. The specifications for steviol glycosides were revised further, requiring not less than 95% of the 7 named steviol glycosides. In 2010, JECFA revised the specifications for steviol glycosides to include 2 additional steviol glycosides, rebaudioside D and rebaudioside F, within the purity criteria (JECFA, 2010). For further details regarding the conclusions of the JECFA meetings, see Section C.4.

In March 2011, at the 43rd Session of the Codex Committee on Food Additives (CCFA) recommendations for steviol glycosides provisions in the General Standards of Food Additives (GSFA) were considered (CCFA, 2011).

United States

A notification to the U.S. Food and Drug Administration (FDA) for purified steviol glycosides with rebaudioside M as the principal component (GRN No. 000473) was recently submitted and the FDA has raised no objections regarding the petitioners' determinations of Generally Recognized as Safe (GRAS) status of the steviol glycoside products for use as general-purpose sweeteners in foods (U.S. FDA, 2013a).

In the U.S., approximately 38 GRAS notices (GRN 252, 253, 275, 278, 282, 287, 303, 304, 318, 323, 329, 337, 348, 349, 354, 365, 367, 369, 375, 380, 388, 389, 393, 395, 418, 448, 452, 456, 461, 467, 473, 493, 512, 516, 536, 548, 555, 607, 619, 626, 632) for major individual steviol glycosides (stevioside and rebaudiosides A, C, D, and X/M), mixtures of steviol glycosides (*e.g.,* rebaudioside A and stevioside as the principal components), or glucosylated steviol glycosides have been submitted to the FDA for review since 2008. With the exception of the most recent GRAS notifications (GRN 607, 619, 626, and 632), which have not yet been reviewed, the FDA has raised no objections regarding the petitioners' determinations of GRAS status of the steviol glycoside products for use as general purpose sweeteners in foods (U.S. FDA, 2008a,b, 2009a-d, 2010a-e, 2011a-i, 2012a-e, 2013a-f, 2014a-c, 2015a-d, 2016a-c). These GRAS notifications all specify a total steviol glycoside content of no less than 95%, and indicate that each steviol glycoside (prepared individually or as a dominant component of a mixture) is safe for use in foods and beverages. Some of these notifications describe steviol glycoside preparations (>95%) that do not specifically meet the JECFA specifications of not less than 95% of the 9 named steviol glycosides

(stevioside, rebaudioside A, B, C, D, F, dulcoside A, rubusoside, and steviolbioside), but rather include steviol glycosides, such as rebaudioside X/M, that have not been defined in JECFA's purity specification (*e.g.,* GRN 473, 512) (U.S. FDA, 2013a, 2014b)

European Union

In 1985, the European Commission's Scientific Committee on Food (SCF) evaluated stevioside as a sweetener and concluded that its use was "not toxicologically acceptable" due to limited data on metabolism, mutagenicity, long-term, and reproductive and developmental toxicity (SCF, 1985). In a subsequent evaluation, the SCF examined newly available data on metabolism, genotoxicity, and long-term toxicity, but maintained that these data were inadequate to sufficiently assess the safety of stevioside (SCF, 1999). Specifically, the SCF continued to raise concerns related to the potential reproductive effects of steviol glycosides and recommended that a study in a rat strain other than the F344 rat be conducted [rat strain used in the 2 carcinogenicity studies on stevioside (Yamada *et al*., 1985; Toyoda *et al*., 1997), since it is not possible to evaluate any potential effects on the testicular system in this strain of rats as it normally seems to develop testicular changes. The SCF (1999) also questioned the relevance of numerous other studies because the composition of the test material was not clearly defined. The potential mutagenic effects of steviol also continued to be a concern (SCF, 1999). Based on the SCF's review of stevioside, the European Commission rejected *Stevia* and stevioside for use as a sweetener (Geuns, 2003). However, in an independent review of the safety data previously reviewed by JECFA at its 69th meeting (JECFA, 2009), EFSA corroborated JECFA's conclusion regarding the safety and concurred with the ADI previously established by JECFA of 4 mg/kg body weight/day for steviol glycosides, expressed as steviol equivalents (EFSA, 2010). Moreover, EFSA recently confirmed JECFA's previous determination that safety studies conducted with an individual steviol glycoside can extend to other steviol glycosides due to the shared metabolic fate (EFSA, 2015).

Canada

Consistent with other international scientific reviews on the safety of steviol glycosides, Health Canada established an ADI of 4 mg steviol equivalents/kg body weight in July 2012 and recommended that steviol glycosides be approved for use as a sweetening agent (Health Canada, 2012a). Following a brief consultation period, Health Canada approved the use of steviol glycosides as a sweetening agent in a variety of food and beverage categories at levels of up to 0.35%, calculated as steviol equivalents (Health Canada, 2012b). Moreover, the recent expansion of the definition of steviol glycosides in Canada to include rebaudioside M further corroborates the common metabolic fate of steviol glycosides to steviol and confirms that safety data generated from one steviol glycoside can represent all steviol glycosides, in general, regardless of the number and location of sugar moieties (Health Canada, 2016).

Asia

Steviol glycosides are approved for use as a food additive (sweetening agent) in Japan, South Korea, China, Malaysia, Indonesia, Singapore, and Taiwan. In Japan, the Ministry of Health and Welfare approved 3 types of stevia extracts: α-glucosyltransferase-treated stevia, powdered stevia, and stevia extract (Japan Food Chemical Research Foundation, 2014). In addition, purified stevioside (as a crude extract, 50% pure extract, and ≥90% pure extract) and *Stevia rebaudiana* leaf extracts are accepted for general use as sweeteners in a variety of foods and beverages including pickling gum, pickles, dried seafood, meat, fish, soy sauce, bean pastes, sugarless chewing gums, juices, cola, table-top sweeteners, and ice cream in Japan (Marie, 1991; Das *et al.*, 1992; Ferlow, 2005). In India, the Food Safety and Standards Authority of India (FSSAI) has approved the use of steviol glycosides in a number of food and beverage categories as per the official gazette notification (FSSAI, 2012; MOHFW, 2015).

Central/South America

Stevioside, *S. rebaudiana* leaves, and highly refined extracts are permitted for use as low-calorie sweeteners in Brazil, Argentina, Paraguay, Uruguay, Mexico, Peru, and Colombia.

Nigeria

In May of 2014, the National Agency for Food and Drug Administration and Control permitted rebaudioside M for use in foods and beverages. The use-levels of rebaudioside M must be in accordance with maximum levels established for steviol glycosides under Codex Alimentarius Commission's General Standards for Food Additives (GSFA).

Other Jurisdictions

Other countries permitting the use of steviol glycosides include Israel, Russia, Switzerland, Turkey, and Ukraine.

A.11 Statutory Declaration

A signed Statutory Declaration for Australia is provided as Appendix A.

A.12 Checklist

A completed checklist relating to the information required for submission with this application is provided in Appendix B.

B. TECHNICAL INFORMATION ON THE FOOD ADDITIVE

In accordance with Section 3.3.1 – Food Additives of the FSANZ *Application Handbook* (FSANZ, 2016a) 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.
9. Potential additional purposes of the food additive when added to food

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

B.1 Nature and Technological Purpose of Steviol Glycosides

In Australia and New Zealand, food additives must comply with a monograph published from a specified list of sources, such as JECFA (FSANZ, 2016b). The specifications for steviol glycosides as established by JECFA (2010) stipulate that steviol glycoside products contain not less than 95% of the combined 9 named steviol glycosides, which include stevioside, rebaudioside A, B, C, D, and F, dulcoside A, rubusoside, and steviolbioside. Additionally, the definition of steviol glycosides in Australia and New Zealand was expanded recently to include rebaudioside M. However, while steviol glycoside preparations are already available for use in food as sweeteners throughout Australia and New Zealand and many other parts of the world, the steviol glycoside products presently available have technological limitations regarding sweetness quality.

The number of steviol glycosides that have been identified in the *S. rebaudiana* plant has now increased to 40 different glycosides, including those already established as part of the FSANZ definition of steviol glycosides (major steviol glycosides). This number is expected to grow over time. As presented in Section B.2.1, below, these glycosides share the same steviol backbone and are conjugated with glucose, rhamnose, xylose, fructose, deoxyglucose and/or other sugar moieties in any orientation, quantity, or combination. SG mixtures, similar to other already permitted steviol glycoside preparations for use in food and beverages in Australia and New Zealand, would be used as high-intensity sweeteners for the replacement of sucrose in reduced-calorie or no-sugar-added products. Expanding the definition of steviol glycosides to include minor steviol glycosides is not expected to alter the sweetness potency of steviol glycosides as previously reviewed by FSANZ and JECFA. It is well-recognised that the usefulness of high-intensity sweeteners extends beyond its ability to provide sweetness, but also needs to take into account other taste attributes (*i.e.*, the taste quality); therefore, sensory studies were undertaken to understand the effects of the minor steviol glycosides on the taste characteristics of various foods.

B.1.1 Taste Attributes

To evaluate the impact of the inclusion of minor steviol glycosides on the sensory profile of steviol glycosides, PureCircle evaluated the sensory characteristics of various sugar-reduced foods, sweetened with major steviol glycosides (*e.g.*, rebaudioside A, stevioside, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside M) with and without the addition of minor glycosides. The foods tested were sugar-reduced chocolate milk, sugar-reduced barbeque flavoured peanuts, and acidified water. The inclusion of the minor steviol glycosides with major steviol glycosides overall improved the flavour and taste characteristics of the sample foods and improved the overall liking, as reported by the study participants, in comparison to sample foods prepared with only those major steviol glycosides. Therefore, minor steviol glycosides did not adversely affect the sensory profile of steviol glycosides currently accepted for use as a general purpose sweetener, and in fact improved the flavour and taste of the foods. Please see Appendix C for the complete study report.

B.1.2 Stability

The stability of steviol glycosides has been previously reviewed by a number of the scientific advisory bodies involved in the evaluation of steviol glycoside safety [JECFA, the European Food Safety Authority (EFSA), and FSANZ] and is also discussed in several published studies (Chang and Cook, 1983; Kroyer, 1999). Specifically, JECFA evaluated the stability of steviol glycosides under conditions mimicking their use in foods at the 68th meeting (JECFA, 2007b). The Committee noted that steviol glycosides do not undergo browning or caramelisation when heated, and are reasonably stable under elevated temperatures used in food processing. Under acidic conditions (pH 2 to 4), steviol glycosides (approximately 90 to 94% purity), are stable for at least 180 days when stored at temperatures up to 24°C. When exposed to elevated temperatures (80°C, in water, 8 hours), however, 4 and 8% decomposition was observed in solutions of steviol glycosides at pH 4.0 and 3.0, respectively, indicating that the stability of steviol glycosides is pH and temperature dependent. When the temperature was increased to 100°C, expectedly higher rates of steviol glycoside decomposition (10 and 40% at pH 4.0 and 3.0, respectively) were observed. Based on the above findings, as well as additional publicly available stability studies, JECFA concluded that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions.

Although the stability of each steviol glycoside identified within JECFA's definition was not specifically addressed during JECFA's evaluation, it is expected that the stability of SG mixtures containing differing distributions of steviol glycosides would be similar to individual steviol glycosides given the similarities in structure. To confirm this viewpoint, additional stability studies of example SG mixtures powders were conducted under normal and/or accelerated storage conditions as well as in solution at various pH levels and temperatures. These studies are summarised below and demonstrate that the stability of SG mixtures are similar to individual steviol glycosides, as previously concluded by JECFA.

Storage Stability of SG Mixtures

The storage stability of SG mixture RA50 (Lot A0912266) and A95 (Lot PT21052015) were assessed (see Appendix D for study reports). RA50 powder samples were stored in sealed amber glass bottles for up to 122 weeks at 40°C and 75% relative humidity (RH). A95 powder samples were stored in sealed amber glass vials or aluminium bags for up to 13 weeks at 1) 25°C and ambient RH (50 to 55%) and 2) 40°C and 75% RH. To assess storage stability, RA50 and A95 samples were tested by high-performance liquid chromatography (HPLC) at baseline and at various time points thereafter, based upon measured values of individual steviol glycosides as well as total steviol glycosides.

For RA50, minor changes in the individual steviol glycosides and minimal degradation of total steviol glycosides (<4%) were measured over 122 weeks at 40°C and 75% RH (Table B.1.2-1). A95 powder stored in aluminium bags at 40°C and 75% RH for 13 weeks was reported to be stable in its individual steviol glycoside content as well as total steviol glycosides (<1.5% degradation) (Table B.1.2-2). Similar results were reported for A95

stored in amber vials at 40°C and 75% RH and in both aluminium bags and amber vials stored at 25°C (maximum degradation of total steviol glycosides reported was <2.5%) (see Appendix D).

Table B.1.2-1 Storage Stability of RA50 (Lot A0912266) when Stored at 40°C and 75% RH (glass vials), as percent (%) dry basis

Week	Rub	Dul A	Stev	Reb C	Reb F	Reb A	Reb D	Sbio	Reb B	TSG
0	0.30	0.46	18.22	9.58	2.41	64.47	ND	0.30	1.06	96.80
4	0.31	0.48	18.00	9.61	2.45	64.43	ND	0.33	1.22	96.82
8	0.25	0.39	17.91	9.7	2.06	64.44	ND	0.26	0.97	95.97
12	0.25	0.4	18.03	9.75	2.02	63.28	ND	0.26	1.02	95.01
16	0.26	0.41	18.08	9.77	2.01	63.83	ND	0.27	1.10	95.72
20	0.25	0.41	18.10	9.8	2.01	64.11	ND	0.29	1.11	96.08
24	0.25	0.40	18.12	9.87	2.18	64.44	ND	0.29	1.11	96.66
28	0.26	0.38	17.97	9.7	2.17	64.17	ND	0.26	1.11	96.01
36	0.19	0.35	17.55	9.51	2.53	63.61	ND	0.30	1.14	95.17
44	0.19	0.35	17.55	9.51	2.53	63.61	ND	0.30	1.14	95.17
52	0.20	0.36	17.69	9.60	2.39	63.83	ND	0.39	1.18	95.63
64	0.23	0.37	17.81	9.58	2.11	64.28	ND	0.30	1.18	95.86
72	0.27	0.39	17.75	9.63	2.04	63.84	ND	0.34	1.31	95.58
80	0.25	0.40	17.86	9.65	2.06	63.34	ND	0.36	1.29	95.22
96	0.25	0.35	17.76	9.66	1.93	63.27	ND	0.35	1.24	94.90
106	0.25	0.36	17.56	9.42	1.94	62.49	ND	0.38	1.44	93.84
122	0.26	0.40	17.35	9.38	1.98	61.54	ND	0.37	1.64	92.93

Source: "Storage stability of Reb A 50 powder" report, see Appendix D.
Dul = dulcoside; ND = not detected; Reb = rebaudioside; RH = relative humidity; Rub = rubusoside; Sbio = steviolbioside; Stev = stevioside; TSG = total steviol glycosides

Table B.1.2-2 Storage Stability of A95 (Lot PT21052015) when Stored at 40°C and 75% RH (aluminium bags), as percent (%) dry basis

Week	Reb E	Reb O	Reb D	Reb N	Reb M	Reb A	Stev	Reb F	Reb C	Dul A	Rub	Reb B	Sbio	TSG
0	0.89	1.39	66.48	3.00	25.38	1.37	0.04	0.01	0.02	ND	ND	0.17	ND	98.75
1	0.83	1.35	64.28	2.87	25.95	1.35	0.03	0.01	0.01	ND	ND	0.18	ND	96.86
4	0.83	1.32	64.25	2.79	26.72	1.35	0.03	0.01	0.01	ND	ND	0.22	ND	97.53
8	0.85	1.35	65.98	2.93	22.94	1.35	0.04	0.01	0.02	ND	ND	0.24	ND	95.71
13	0.84	1.39	66.29	2.99	24.08	1.38	0.03	ND	ND	ND	ND	0.30	ND	97.30

Source: "Storage stability of A95 powder" report, see Appendix D.
Dul = dulcoside; ND = not detected; Reb = rebaudioside; RH = relative humidity; Rub = rubusoside; Sbio = steviolbioside; Stev = stevioside; TSG = total steviol glycosides

pH Stability of SG Mixtures

The general stability of SG mixtures RA50 (Lot A0912266) and A95 (Lot PT21052015) were assessed over a pH range of 2.0 to 8.0 for a total of 17 or 48 weeks at 4 different temperatures, 5, 25, 37, and 56°C (see Appendix D for study reports). Samples were prepared at concentrations of approximately 1,000 mg/L for RA50 and 50,000 mg/L for A95

in 500 mL of buffer solution and stored in amber glass vials. Buffer was prepared by mixing different ratios of 0.1 M phosphate buffer, 0.1 M phosphorous acid, or 0.1 M sodium hydrogen phosphate buffer to obtain the target pH. Total steviol glycosides present in the stability samples were measured by HPLC at baseline as well as various time points over the study period, determined by the sum of the measured concentrations of the following specific steviol glycosides: dulcoside A, rebaudiosides A, B, C, F, rubusoside, steviolbioside, and stevioside for RA50; dulcoside A, rebaudiosides A, B, C, D, E, F, M, N, O, rubusoside, steviolbioside, and stevioside for A95. Tables B.1.2-3 to B.1.2-6 summarise the results for solutions of the SG mixtures, RA50 and A95, at the different pH values (2.0 to 8.0) and temperatures (5, 25, 37, and 56°C) for 17 and 48 weeks, respectively.

The extent and rate of degradation of both SG mixtures A95 and RA50, based on measured total steviol glycosides, were shown to be dependent on pH, temperature, and time. A95, at all pH levels tested (2.0 to 8.0), was most stable when stored at 5°C and the least stable at 56°C. Over the 17-week study period, samples tested at pH 3.0 to 8.0 at 5, 25, and 37°C remained stable within at least 7% of the starting material percentage value. A significant loss in stability was noted when samples were stored at 56°C at the majority of pH levels, with the pH 4.0 samples remaining the most stable based on a maximal loss of 9% of starting material reported during the 17 weeks. Similarly, RA50 at all pH levels tested was most stable when stored at 5°C and the least stable at 56°C. Over the 48-week study period, samples tested at pH 3.0 to 6.0 at 5, 25, and 37°C remained stable within at least 7% of the starting material percentage value. Samples at a pH of 7.0 showed stability at 5 and 25°C, whereas approximately 11% degradation was observed at 37°C. At a higher pH of 8.0, all samples at 5, 25, and 37°C showed very little degradation over 48 weeks (maximum 5%). None of the samples were stable for 48 weeks at 56°C. Overall, at pH values ranging from 3 to 8, no significant degradation (within approximately 12% of baseline) of RA50 was observed over 48 weeks at 5, 25, and 37°C.

Similar to individual steviol glycosides, the stability of the SG mixtures followed the same degradation pathway and was pH-, temperature-, and time-dependent. Therefore, the conclusions regarding the stability of steviol glycosides made by JECFA and other scientific bodies (that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions) can be extended to include the SG mixtures that are the subject of this safety assessment.

Table B.1.2-3 Stability of A95 (Lot PT21052015) and RA50 (Lot A0912266) at 5°C in Solution at Varying pH							
Week	**pH 2.0**	**pH 3.0**	**pH 4.0**	**pH 5.0**	**pH 6.0**	**pH 7.0**	**pH 8.0**
A95	**Total steviol glycosides, mg/mL (percent of baseline, %)**						
0 (baseline)	859.68[a] (100.0)	861.32 (100.0)	869.66 (100.0)	874.61 (100.0)	856.27 (100.0)	862.88 (100.0)	867.45 (100.0)
1	872.25 (101.5)	871.65 (101.2)	880.50 (101.2)	877.88 (100.4)	875.30 (102.2)	875.55 (101.5)	879.31 (101.4)
2	859.80 (100.0)	873.47 (101.4)	880.81 (101.3)	868.44 (99.29)	871.32 (101.8)	875.02 (101.4)	870.11 (100.3)
4	863.33 (100.4)	871.08 (101.1)	877.61 (100.9)	875.86 (100.1)	871.05 (101.7)	877.16 (101.7)	877.64 (101.2)
8	735.33 (85.54)	852.57 (98.98)	861.67 (99.08)	851.21 (97.32)	849.91 (99.26)	862.23 (99.92)	855.72 (98.65)
13	751.00 (87.36)	874.57 (101.5)	880.90 (101.3)	881.32 (100.8)	868.70 (101.5)	888.32 (102.9)	887.98 (102.4)
17	787.35 (91.59)	889.27 (103.2)	883.69 (103.2)	875.98 (100.2)	903.55 (105.5)	880.12 (102.0)	883.35 (101.8)
RA50	**Percentage of total steviol glycosides, % (percent of baseline, %)**						
0 (baseline)	95.02[b] (100.0)	95.21 (100.0)	95.37 (100.0)	95.57 (100.0)	96.12 (100.0)	95.81 (100.0)	95.54 (100.0)
2	96.28 (101.3)	95.89 (100.7)	95.41 (100.0)	96.10 (100.6)	96.11 (99.99)	96.28 (100.5)	95.75 (100.2)
4	96.09 (101.1)	95.78 (100.6)	94.47 (99.06)	96.37 (100.8)	96.46 (100.4)	96.79 (101.0)	95.72 (100.2)
6	95.50 (100.5)	94.18 (98.92)	94.58 (99.17)	95.88 (100.3)	96.29 (100.2)	96.63 (100.9)	95.20 (99.64)
8	96.19 (101.2)	95.94 (100.8)	95.85 (100.5)	96.01 (100.5)	96.12 (100.0)	96.06 (100.3)	95.81 (100.3)
12	95.54 (100.5)	95.26 (100.1)	94.96 (99.57)	94.70 (99.09)	94.89 (98.72)	95.41 (99.58)	95.14 (99.58)
14	94.97 (99.95)	94.27 (99.01)	94.02 (98.58)	94.10 (98.46)	94.75 (98.57)	94.83 (98.98)	94.37 (98.78)
16	94.97 (99.95)	94.35 (99.10)	94.65 (99.25)	94.41 (98.79)	94.96 (98.79)	94.10 (98.22)	94.20 (98.60)
20	94.44 (99.39)	96.13 (101.0)	95.63 (100.3)	94.87 (99.27)	95.75 (99.62)	96.48 (100.7)	94.16 (98.56)
24	95.59 (100.6)	94.95 (99.73)	94.93 (99.54)	94.80 (99.19)	94.92 (98.75)	95.57 (99.75)	94.92 (99.35)
28	93.82 (98.74)	93.74 (98.46)	93.92 (98.48)	94.10 (98.46)	94.72 (98.54)	93.89 (98.00)	93.52 (97.89)
32	93.36 (98.25)	92.92 (97.59)	92.58 (97.07)	92.78 (97.08)	92.77 (96.51)	93.06 (97.13)	92.51 (96.83)
36	94.22 (99.16)	93.24 (97.93)	93.17 (97.69)	93.53 (97.87)	93.97 (97.76)	94.12 (98.24)	93.93 (98.31)
40	94.99 (99.97)	94.57 (99.33)	93.47 (98.01)	95.27 (99.69)	95.83 (99.70)	95.02 (99.18)	94.41 (98.82)
48	95.77 (100.8)	95.85 (100.7)	95.32 (99.95)	96.30 (100.8)	95.73 (99.59)	95.31 (99.48)	94.97 (99.40)

Source: "pH stability of Reb A 50" and "pH stability of A95" reports, see Appendix D.
[a] A95 values represent a concentration of total steviol glycosides based on dulcoside A, rebaudiosides A, B, C, D, E, F, M, N, O, rubusoside, steviolbioside, and stevioside.
[b] RA50 values represent a percentage of total steviol glycosides based on dulcoside A, rebaudiosides A, B, C, F, rubusoside, steviolbioside, and stevioside.

Table B.1.2-4 Stability of A95 (Lot PT21052015) and RA50 (Lot A0912266) at 25°C in Solution at Varying pH							
Week	**pH 2.0**	**pH 3.0**	**pH 4.0**	**pH 5.0**	**pH 6.0**	**pH 7.0**	**pH 8.0**
A95	**Total steviol glycosides, mg/mL (percent of baseline, %)**						
0 (baseline)	859.68[a] (100.0)	861.32 (100.0)	869.66 (100.0)	874.61 (100.0)	856.27 (100.0)	862.88 (100.0)	867.45 (100.0)
1	828.10 (96.33)	865.52 (100.5)	871.28 (100.2)	871.49 (99.64)	863.32 (100.8)	877.90 (101.7)	871.77 (100.5)
2	802.56 (93.36)	858.55 (99.68)	879.90 (101.2)	875.98 (100.2)	874.24 (102.1)	872.40 (101.1)	882.36 (101.7)
4	738.87 (85.95)	864.92 (100.4)	876.93 (100.8)	869.46 (99.41)	864.87 (101.0)	874.71 (101.4)	873.74 (100.7)
8	555.23 (64.59)	849.37 (98.61)	865.04 (99.47)	852.03 (97.42)	842.03 (98.34)	854.68 (99.05)	852.61 (98.29)
13	548.53 (63.81)	869.53 (101.0)	886.87 (102.0)	882.44 (100.9)	861.44 (100.6)	847.99 (98.27)	872.01 (100.5)
17	463.40 (53.90)	872.26 (101.3)	902.60 (103.8)	891.69 (102.0)	887.03 (103.6)	832.34 (96.46)	873.90 (100.7)
RA50	**Percentage of total steviol glycosides, % (percent of baseline, %)**						
0 (baseline)	95.02[b] (100.0)	95.21 (100.0)	95.37 (100.0)	95.57 (100.0)	96.12 (100.0)	95.81 (100.0)	95.54 (100.0)
2	96.41 (101.5)	95.92 (100.7)	95.72 (100.4)	95.63 (100.1)	96.42 (100.3)	95.81 (100.0)	95.59 (100.1)
4	95.85 (100.9)	95.40 (100.2)	95.23 (99.85)	95.20 (99.61)	96.05 (99.93)	95.91 (100.1)	94.42 (98.83)
6	96.19 (101.2)	97.31 (102.2)	96.74 (101.4)	97.07 (101.6)	97.72 (101.7)	97.06 (101.3)	97.09 (101.6)
8	95.15 (100.1)	95.61 (100.4)	96.67 (101.4)	96.48 (101.0)	95.56 (99.42)	96.78 (101.0)	96.26 (100.8)
12	90.95 (95.72)	94.33 (99.08)	94.51 (99.10)	94.08 (98.44)	94.40 (98.21)	94.01 (98.12)	95.68 (100.2)
14	92.60 (97.45)	95.76 (100.6)	94.74 (99.34)	94.62 (99.01)	94.56 (98.38)	94.69 (98.83)	94.02 (98.41)
16	90.81 (95.57)	95.63 (100.4)	95.07 (99.69)	95.51 (99.94)	94.78 (98.61)	94.31 (98.43)	94.54 (98.95)
20	88.55 (93.19)	96.61 (101.5)	94.88 (99.49)	94.49 (98.87)	94.49 (98.30)	96.08 (100.3)	99.09 (103.7)
24	84.13 (88.54)	96.00 (100.8)	94.67 (99.27)	94.56 (98.94)	94.56 (98.39)	95.55 (99.73)	94.69 (99.11)
28	80.96 (85.20)	94.44 (99.19)	94.23 (98.80)	92.41 (96.69)	92.41 (96.14)	93.47 (97.56)	93.90 (98.28)
32	76.72 (80.74)	93.30 (97.99)	92.45 (96.94)	92.43 (96.71)	92.12 (95.84)	92.01 (96.03)	92.18 (96.48)
36	72.05 (75.83)	93.86 (98.58)	93.16 (97.68)	92.28 (96.56)	92.18 (95.90)	93.46 (97.55)	93.35 (97.71)
40	66.59 (70.08)	94.67 (99.43)	94.93 (99.54)	94.88 (99.28)	94.96 (98.79)	94.45 (98.58)	93.54 (97.91)
48	58.20 (61.25)	94.93 (99.71)	94.80 (99.40)	94.91 (99.31)	92.93 (96.68)	94.33 (98.46)	94.62 (99.04)

Source: "pH stability of Reb A 50" and "pH stability of A95" reports, see Appendix D.
[a] A95 values represent a concentration of total steviol glycosides based on dulcoside A, rebaudiosides A, B, C, D, E, F, M, N, O, rubusoside, steviolbioside, and stevioside.
[b] RA50 values represent a percentage of total steviol glycosides based on dulcoside A, rebaudiosides A, B, C, F, rubusoside, steviolbioside, and stevioside.

Table B.1.2-5 Stability of A95 (Lot PT21052015) and RA50 (Lot A0912266) at 37°C in Solution at Varying pH							
Week	**pH 2.0**	**pH 3.0**	**pH 4.0**	**pH 5.0**	**pH 6.0**	**pH 7.0**	**pH 8.0**
A95	**Total steviol glycosides, mg/mL (percent of baseline, %)**						
0 (baseline)	859.68[a] (100.0)	861.32 (100.0)	869.66 (100.0)	874.61 (100.0)	856.27 (100.0)	862.88 (100.0)	867.45 (100.0)
1	623.94 (72.58)	859.80 (99.82)	875.30 (100.6)	872.13 (99.72)	875.19 (102.2)	882.15 (102.2)	881.07 (101.6)
2	462.78 (53.83)	863.97 (100.3)	877.76 (100.9)	859.77 (98.30)	863.46 (100.8)	875.79 (101.5)	869.52 (100.2)
4	284.12 (33.05)	851.43 (98.85)	871.24 (100.2)	865.44 (98.95)	867.09 (101.3)	872.54 (101.1)	870.47 (100.3)
8	110.25 (12.82)	807.55 (93.76)	851.01 (97.86)	836.91 (95.69)	847.75 (99.00)	852.62 (98.81)	853.15 (98.35)
13	58.60 (6.82)	812.54 (94.34)	868.78 (99.90)	860.38 (98.37)	869.02 (101.5)	874.35 (101.3)	877.79 (101.2)
17	44.99 (5.23)	801.24 (93.02)	869.80 (100.0)	852.18 (97.44)	875.04 (102.2)	864.32 (100.2)	879.09 (101.3)
RA50	**Percentage of total steviol glycosides, % (percent of baseline, %)**						
0 (baseline)	95.02[b] (100.0)	95.21 (100.0)	95.37 (100.0)	95.57 (100.0)	96.12 (100.0)	95.81 (100.0)	95.54 (100.0)
2	95.09 (100.1)	95.69 (100.5)	95.73 (100.4)	95.69 (100.1)	96.24 (100.1)	95.63 (99.81)	94.91 (99.34)
4	88.53 (93.17)	96.35 (101.2)	96.34 (101.0)	95.98 (100.4)	95.87 (99.74)	96.72 (100.9)	94.54 (98.95)
8	79.15 (83.30)	95.90 (100.7)	95.86 (100.5)	96.16 (100.6)	95.87 (99.74)	95.99 (100.2)	95.76 (100.2)
12	72.31 (76.10)	95.20 (99.99)	94.95 (99.56)	94.57 (98.95)	94.51 (98.33)	94.56 (98.70)	94.12 (98.51)
14	66.22 (69.69)	96.19 (101.0)	96.39 (101.1)	95.91 (100.4)	94.26 (98.06)	95.59 (99.77)	96.00 (100.5)
16	59.27 (62.38)	95.54 (100.3)	95.33 (99.96)	95.08 (99.49)	94.22 (98.02)	94.70 (98.84)	94.98 (99.41)
20	48.70 (51.25)	95.26 (100.1)	95.32 (99.95)	93.19 (97.51)	93.26 (97.02)	93.11 (97.18)	92.85 (97.18)
24	42.28 (44.50)	94.74 (99.51)	95.05 (99.66)	94.17 (98.54)	92.98 (96.73)	92.68 (96.73)	93.15 (97.50)
28	36.45 (38.36)	93.38 (98.08)	95.00 (99.61)	93.25 (97.57)	91.39 (95.08)	90.82 (94.79)	92.08 (96.38)
32	28.29 (29.77)	91.39 (95.99)	92.86 (97.37)	93.18 (97.50)	89.35 (92.96)	89.73 (93.65)	91.46 (95.73)
36	23.59 (24.83)	90.16 (94.70)	92.04 (96.51)	91.59 (95.84)	89.61 (93.23)	87.94 (91.79)	90.36 (94.58)
40	16.90 (17.79)	89.51 (94.01)	93.27 (97.80)	90.90 (95.11)	86.70 (90.20)	84.90 (88.61)	90.69 (94.92)
48	12.15 (12.79)	88.45 (92.90)	94.81 (99.41)	92.05 (96.32)	89.46 (93.07)	85.36 (89.09)	90.76 (95.00)

Source: “pH stability of Reb A 50” and “pH stability of A95” reports, see Appendix D.
[a] A95 values represent a concentration of total steviol glycosides based on dulcoside A, rebaudiosides A, B, C, D, E, F, M, N, O, rubusoside, steviolbioside, and stevioside.
[b] RA50 values represent a percentage of total steviol glycosides based on dulcoside A, rebaudiosides A, B, C, F, rubusoside, steviolbioside, and stevioside.

Table B.1.2-6 Stability of A95 (Lot PT21052015) and RA50 (Lot A0912266) at 56°C in Solution at Varying pH							
Week	**pH 2.0**	**pH 3.0**	**pH 4.0**	**pH 5.0**	**pH 6.0**	**pH 7.0**	**pH 8.0**
A95	**Total steviol glycosides, mg/mL (percent of baseline, %)**						
0 (baseline)	859.68[a] (100.0)	861.32 (100.0)	869.66 (100.0)	874.61 (100.0)	856.27 (100.0)	862.88 (100.0)	867.45 (100.0)
1	241.75 (28.12)	850.64 (98.76)	877.68 (100.9)	881.68 (100.8)	873.82 (102.0)	877.55 (101.7)	873.80 (100.7)
2	68.68 (7.99)	809.88 (94.03)	874.56 (100.6)	871.89 (99.69)	861.12 (100.6)	848.41 (98.32)	839.36 (96.76)
4	25.17 (2.93)	724.92 (84.16)	850.00 (97.74)	855.10 (97.77)	834.73 (97.48)	768.72 (89.09)	814.09 (93.85)
8	7.95 (0.92)	552.33 (64.13)	817.44 (94.00)	822.10 (94.00)	691.29 (80.73)	664.74 (77.04)	758.27 (87.41)
13	6.64 (0.77)	438.45 (50.90)	795.56 (91.48)	803.29 (91.85)	518.95 (60.61)	579.60 (67.17)	668.54 (77.07)
17	18.58 (2.16)	163.83 (19.02)	804.52 (92.51)	729.90 (83.45)	444.48 (51.91)	535.36 (62.04)	626.21 (72.19)
RA50	**Percentage of total steviol glycosides, % (percent of baseline, %)**						
0 (baseline)	95.02[b] (100.0)	95.21 (100.0)	95.37 (100.0)	95.57 (100.0)	96.12 (100.0)	95.81 (100.0)	95.54 (100.0)
2	35.10 (36.94)	93.15 (97.84)	94.24 (98.82)	94.43 (98.81)	91.99 (95.70)	91.66 (95.67)	93.31 (97.67)
4	16.70 (17.58)	87.00 (91.38)	94.88 (99.49)	93.40 (97.73)	91.08 (94.76)	84.56 (88.26)	89.57 (93.75)
6	7.52 (7.91)	82.40 (86.55)	93.08 (97.60)	92.66 (96.96)	87.85 (91.40)	76.55 (79.90)	86.12 (90.14)
8	13.72 (14.44)	81.84 (85.96)	93.99 (98.55)	93.47 (97.80)	88.38 (91.95)	74.65 (77.91)	84.14 (88.07)
12	4.02 (4.23)	73.34 (77.03)	90.14 (94.52)	89.26 (93.40)	79.87 (83.09)	70.09 (73.16)	78.76 (82.44)
14	0.54 (0.57)	67.52 (70.92)	90.06 (94.43)	88.59 (92.70)	82.23 (85.55)	66.61 (69.52)	77.67 (81.30)
16	1.53 (1.61)	61.27 (64.35)	87.67 (91.93)	85.79 (89.77)	78.43 (81.60)	61.91 (64.62)	75.53 (79.06)
20	0.49 (0.52)	49.36 (51.84)	84.04 (88.12)	82.69 (86.52)	72.32 (75.24)	54.63 (57.02)	65.79 (68.86)
24	0.44 (0.46)	36.60 (38.44)	79.25 (83.10)	77.66 (81.26)	68.83 (71.61)	50.46 (52.67)	61.62 (64.50)
28	0.37 (0.39)	32.11 (33.73)	78.72 (82.54)	76.32 (79.86)	67.53 (70.26)	48.29 (50.40)	62.27 (65.18)
32	0.81 (0.85)	24.06 (25.27)	75.95 (79.64)	71.51 (74.82)	61.16 (63.63)	43.79 (45.71)	53.87 (56.38)
36	0.26 (0.27)	20.74 (21.78)	67.29 (70.56)	68.76 (71.95)	57.57 (59.89)	39.37 (41.09)	50.91 (53.29)
40	1.32 (1.39)	27.58 (28.97)	61.55 (64.54)	62.74 (65.65)	36.88 (38.37)	54.75 (57.14)	49.96 (52.29)
48	0.13 (0.14)	8.37 (8.79)	55.83 (58.54)	60.34 (63.14)	49.80 (51.81)	34.88 (36.41)	41.72 (43.67)

Source: "pH stability of Reb A 50" and "pH stability of A95" reports, see Appendix D.
[a] A95 values represent a concentration of total steviol glycosides based on dulcoside A, rebaudiosides A, B, C, D, E, F, M, N, O, rubusoside, steviolbioside, and stevioside.
[b] RA50 values represent a percentage of total steviol glycosides based on dulcoside A, rebaudiosides A, B, C, F, rubusoside, steviolbioside, and stevioside.

Conclusion Pertaining to Stability

Similar to publicly available literature demonstrating the stability of rebaudioside A and stevioside, SG mixtures have been demonstrated to be stable under bulk storage accelerated conditions for up to 122 weeks and in solutions of varying temperatures and pH conditions. Therefore, stability data conducted with rebaudioside A and stevioside in food matrices can be extrapolated to steviol glycosides in general. Furthermore, at the 68th meeting of JECFA, the Committee concluded, based on the studies conducted by (Chang and Cook, 1983; Kroyer, 1999; Clos *et al*., 2008; Prakash *et al.,* 2008), that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions.

B.2 Information to Enable Identification of Steviol Glycosides

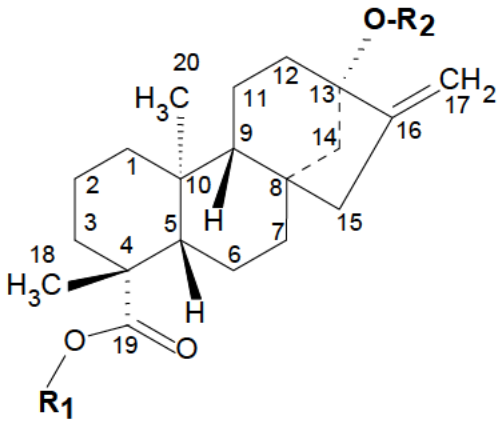
Information to enable the identification of steviol glycosides, including the chemical structure, the chemical name, the molecular weight and formula, and the common name, are presented below.

B.2.1 Identity of Substance

PureCircle's SG mixtures will contain not less than 95% of those steviol glycosides present in *S. rebaudiana*, in any combination or quantity. In addition to the 10 steviol glycosides already permitted for use in steviol glycoside products in Australia and New Zealand (stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, rebaudioside M, dulcoside A, rubusoside, and steviolbioside), PureCircle's SG mixtures may contain additional minor steviol glycosides containing the those sugar groups glucose, rhamnose, and xylose contained within the current approved glycosides, as well as more diverse sugar moieties, such as fructose and deoxyglucose.

All steviol glycosides extracted from the stevia leaf are glycosylated derivatives of the aglycone steviol and as such, all share the same backbone structure (Figure B.2.1-1) and differ only with respect to the type and number of glycoside units at positions R_1 and R_2. The chemical structures of a number of different steviol glycosides have been identified from the leaves of the *S. rebaudiana* plant, including those minor components, all containing the sugar moieties glucose, xylose, rhamnose, fructose and/or deoxyglucose (Ceunen and Geuns, 2013). The common names and trivial formulas, as well as the chemical (R_1 and R_2 groups) formulas for all identified steviol glycosides to date, are summarised in Table B.2.1-1.

Figure B.2.1-1 Backbone Structure Steviol Glycosides



Note: steviol - R_1 and R_2 = H.

Table B.2.1-1 Molecular Formula, and R-Groups in Identified Steviol Glycosides (see Figure B.2.1-1)

#	Common Name	Trivial Formula	R_1	R_2	Reference
1) Steviol + Glucose (SvGn)					
1.1	Steviolmonoside	SvG1	H	Glcβ1-	Ohta *et al.* (2010)
1.2	Steviol-19-O-β-D-glucoside	SvG1	Glcβ1-	H	Gardana *et al.* (2010)
1.3	Rubusoside	SvG2	Glcβ1-	Glcβ1-	Ohta *et al.* (2010)
1.4	Steviolbioside	SvG2	H	Glcβ(1-2)Glcβ1-	Kohda *et al.* (1976)
1.5	Stevioside	SvG3	Glcβ1-	Glcβ(1-2)Glcβ1-	Bridel and Lavieille (1931)
1.6	Stevioside A	SvG3	Glcβ(1-2)Glcβ1-	Glcβ1-	Wu *et al.* (2012)
1.7	Rebaudioside *B*	SvG3	H	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Kohda *et al.* (1976)
1.8	Rebaudioside *G*	SvG3	Glcβ1-	Glcβ(1-3)Glcβ1-	Ohta *et al.* (2010)
1.9	Stevioside B	SvG3	Glcβ(1-3)Glcβ1-	Glcβ1-	Chaturvedula and Zamora (2014)
1.10	Rebaudioside *E*	SvG4	Glcβ(1-2)Glcβ1-	Glcβ(1-2)Glcβ1-	Sakamoto *et al.* (1977a)
1.11	Rebaudioside *A*	SvG4	Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Kohda *et al.* (1976)
1.12	Rebaudioside A2	SvG4	Glcβ1-	Glcβ(1-6)Glcβ(1-2)Glcβ1-	Chaturvedula and Prakash (2011a)
1.13	Rebaudioside *D*	SvG5	Glcβ(1-2)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Sakamoto *et al.* (1977a)
1.14	Rebaudioside *I*	SvG5	Glcβ(1-3)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Ohta *et al.* (2010)
1.15	Rebaudioside *L*	SvG5	Glcβ1-	Glcβ(1-6)Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Ohta *et al.* (2010)

Table B.2.1-1 Molecular Formula, and R-Groups in Identified Steviol Glycosides (see Figure B.2.1-1)

#	Common Name	Trivial Formula	R_1	R_2	Reference
1.16	Rebaudioside *Q2*	SvG5	Glcα(1-2)Glcα(1-4)Glcβ1-	Glcβ(1-2)Glcβ1-	Chaturvedula and Prakash (2011b)
1.17	Rebaudioside *Q*	SvG5	Glcβ1-	Glcα(1-4)Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	-
1.18	Rebaudioside *I2*	SvG5	Glcβ1-	Glcα(1-3)Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Chaturvedula *et al.* (2011a)
1.19	Rebaudioside *Q3*	SvG5	Glcβ1-	Glcα(1-4)Glcβ(1-3)[Glcβ(1-2)]Glcβ1-	Chaturvedula *et al.* (2011a)
1.20	Rebaudioside *I3*	SvG5	Glcβ(1-2)[Glcβ(1-6)]Glcβ1-	Glcβ(1-2)Glcβ1-	Chaturvedula *et al.* (2011a)
1.21	Rebaudioside *M*	SvG6	Glcβ(1-2)[Glcβ (1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Ohta *et al.* (2010)
2) Steviol + Rhamnose + Glucose (SvR1Gn)					
2.1	Dulcoside *A*	SvR1G2	Glcβ1-	Rhaα(1-2)Glcβ1-	Kobayashi *et al.* (1977)
2.2	Dulcoside *B*	SvR1G2	H	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Ohta *et al.* (2010)
2.3	Rebaudioside *C*	SvR1G3	Glcβ1-	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Sakamoto *et al.* (1977b)
2.4	Rebaudioside C *(isomer)*	SvR1G3	Rhaα(1-2)Glcβ1-	Glcβ(1-3)Glcβ1-	-
2.5	Rebaudioside S	SVR1G3	Rhaα(1-2)Glcβ1-	Glcα(1-2)Glcβ1-	Ihrahim *et al.* (2016)
2.6	Rebaudioside *H*	SvR1G4	Glcβ1-	Glcβ(1-3)Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Ohta *et al.* (2010)
2.7	Rebaudioside *K*	SvR1G4	Glcβ(1-2)Glcβ1-	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Ohta *et al.* (2010)
2.8	Rebaudioside *J*	SvR1G4	Rhaα(1-2)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Ohta *et al.* (2010)
2.9	Rebaudioside *N*	SvR1G5	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Ohta *et al.* (2010)
2.10	Rebaudioside *O*	SvR1G6	Glcβ(1-3)Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Ohta *et al.* (2010)
3) Steviol + Xylose + Glucose (SvX1Gn)					
3.1	Stevioside F	SvX1G2	Glcβ1-	Xylβ(1-2)Glcβ1-	Chaturvedula and Prakash (2011c)
3.2	Rebaudioside *F*	SvX1G3	Glcβ1-	Xylβ(1-2)[Glcβ(1-3)]Glcβ1-	Starratt *et al.* (2002)
3.3	Rebaudioside *F2*	SvX1G3	Glcβ1-	Glcβ(1-2)[Xylβ(1-3)]Glcβ1-	Chaturvedula and Prakash (2011c)
3.4	Rebaudioside *F3*	SvX1G3	Xylβ(1-6)Glcβ1-	Glcβ(1-2)Glcβ1-	Chaturvedula *et al.* (2011b)
3.5	Rebaudioside R	SvX1G3	Glcβ1-	Gluβ(1-2)[Glcβ(1-3)]]Xylβ1-	Ihrahim *et al.* (2016)

Table B.2.1-1 Molecular Formula, and R-Groups in Identified Steviol Glycosides (see Figure B.2.1-1)					
#	**Common Name**	**Trivial Formula**	**R_1**	**R_2**	**Reference**
4) Steviol + Fructose + Glucose (SvF1Gn)					
4.1	Rebaudioside A3	SvF1G3	Glcβ1-	Glcβ(1-2)[Fruβ(1-3)]Glcβ1-	Chaturvedula *et al.* (2011c)
5) Steviol + deoxyGlucose + Glucose (SvdG1Gn)					
5.1	Stevioside D	SvdG1G2	Glcβ1-	6-deoxyGlcβ(1-2)Glcβ1-	Chaturvedula and Prakash (2011d)
5.2	Stevioside E	SvdG1G3	Glcβ1-	6-deoxyGlcβ(1-2)[Glcβ(1-3)]Glcβ1-	Chaturvedula and Prakash (2011d)
5.3	Stevioside E2	SvdG1G3	6-deoxyGlcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Chaturvedula *et al.* (2011d)

deoxyGlc, deoxyglucose; Fru, fructose; Glc, glucose; Rha, rhamnose; Xyl, xylose

B.2.2 Composition

PureCircle's SG mixtures will comprise not less than 95% (on dried basis) of steviol glycosides listed in Table B.2.1-1, in any combination of glycoside as well as quantity. It has been recognised that the composition of *S. rebaudiana* extracts depends upon the composition of the harvested leaves, which, in turn, is influenced by soil, climate, and the manufacturing process (Wallin, 2008). In addition to those well characterised steviol glycoside molecules listed in Table B.2.1-1, a large number of other steviol glycosides, which remain to be fully characterised, have also been detected in stevia leaf extracts. It is possible that some of these steviol glycosides are generated during the extraction process from the stevia leaf (Kohda *et al*., 1976; Kinghorn *et al.*, 1999; Kennelly, 2002), for example, rebaudioside A and stevioside may undergo hydrolysis under certain extraction conditions to form steviol glycosides with fewer sugar moieties (Kobayashi *et al.*, 1977). The alkaline hydrolysis of rebaudioside A and stevioside to form rebaudioside B and steviolbioside are in fact well documented (Ahmed *et al.*, 1980; Chaturvedula and Prakash, 2011e), and although rebaudioside B exists only as a minor glycoside in the *S. rebaudiana* plant, a commercial process has been developed to produce this steviol glycoside by alkaline hydrolysis (Bridges *et al.*, 2012; Furlano *et al.*, 2012; Markosyan, 2013). Steviol glycosides can also undergo hydrolysis during product storage, for instance, stevioside added to an acidic carbonated beverage at 0.1% was reported to hydrolyse to steviolbioside after 2 months of storage at 37°C (Chang and Cook, 1983; Wölwer-Rieck *et al.*, 2010). Since hydrolysis of steviol glycosides may occur during the extraction process, different pH and temperature conditions can be employed during the extraction and purification of stevia leaf extracts to generate SG mixtures with different distributions of individual steviol glycosides.

B.3 Information on the Chemical and Physical Properties of SG Mixtures

SG mixtures are white to off-white powders that have a clean taste with no abnormal or off odour. Steviol glycoside extracts are purified from the hot water extraction of *S. rebaudiana* leaves. These hot water extract solutions of steviol glycosides can be up to 350 times sweeter than sucrose and are consistent with the sweetness profile of steviol glycosides in general (JECFA, 2007a).

B.4 Information on the Impurity Profile

Impurities occurring in steviol glycosides consist primarily of compounds extracted from the *Stevia* leaves (*e.g.*, pigments, saccharides) (JECFA, 2005). The existing purity specifications for steviol glycosides, as adopted by JECFA at the 73rd meeting, indicate limits for total ash content and loss on drying of 1 and 6%, respectively (JECFA, 2010). Residues of the solvents used in the manufacturing process of steviol glycosides may also carry over to the final preparation. The current specifications for steviol glycosides limit levels of methanol at 200 mg/kg and ethanol at 5000 mg/kg. Lead and arsenic are not permitted at concentrations above 1 mg/kg. As SG mixtures comply with the existing specifications of impurities, as demonstrated in Section B.6, below, no further discussion regarding the impurity profile of SG mixtures is presented.

B.5 Manufacturing Process

While there are some differences in the production of steviol glycosides among different manufacturers, overall the process involves hot-water extraction of stevia leaves followed by isolation and step-wise purification using ion-exchange resins and alcohol solvents (methanol and/or ethanol). The production process is consistent with the methodologies for the manufacture of steviol glycosides as described in the respective Chemical and Technical Assessment (CTA) published by JECFA (2007a). All SG mixtures are manufactured in a facility certified under Food Safety System Certification (FSSC) 22000:2010 and all raw materials used in the production process to obtain SG mixtures are already recognised for use in the manufacture of steviol glycoside preparations.

B.6 Specification for Identity and Purity

B.6.1 Current Specifications for Steviol Glycosides

Physical and Chemical Specifications

FSANZ recently updated the specifications for identity and purity of steviol glycosides under Schedule 3 – Identity and Purity of the Foods Standards Code to include separate specifications for rebaudioside M and steviol glycoside mixtures including rebaudioside M. In addition to complying with the current specification established for steviol glycosides by JECFA, the European Commission, or the FCC, the specifications for rebaudioside M and steviol glycoside mixtures including rebaudioside M established by FSANZ further define the

assay parameter for these additives. With respect to rebaudioside M, the final material must not contain less than 95% rebaudioside M on a dried basis. Steviol glycoside mixtures including rebaudioside M must contain rebaudioside M and one or more of the 9 prescribed steviol glycosides (*i.e.*, rebaudiosides A, B, C, D, or F, stevioside, dulcoside A, rubusoside, and steviolbioside), such that the final preparation contains at least 95% of the mixture on a dried basis.

Based on the specifications for steviol glycoside mixtures including rebaudioside M outlined in Schedule 3 – Identity and Purity, and those established by JECFA for steviol glycosides following their 73rd meeting (JECFA, 2010), as well as the specifications for rebaudioside A published in FCC (2016a) and the FCC specification for steviol glycosides (FCC, 2016b), PureCircle established specification for SG mixtures, as outlined in Table B.6.1-1. Although SG mixtures contain a number of other steviol glycosides (*e.g.,* rebaudiosides N, O, E, stevioside A, rebaudioside C isomers) that are not included within the specifications for steviol glycosides mixtures including rebaudioside M listed within Schedule 3, these other steviol glycosides are present in steviol glycoside preparations at less than 5% of the total steviol glycoside content and have similar structures to the 10 specified glycosides. The steviol glycoside constituents are glycosylated derivatives of the aglycone steviol and, as presented in Section B.2, all share the same backbone structure differing only with respect to the type and number of glycoside units at positions R_1 and R_2 (see Figure B.2.1-1 and Table B.2.1-1). Since the majority of constituents (>95%) in PureCircle's SG mixtures are in fact steviol glycosides, the purity specification for SG mixtures (not less than 95% total steviol glycosides) is consistent with the purity specifications and thus may include some or all of the 10 specified steviol glycosides along with other steviol glycosides present in *S. rebaudiana*.

Table B.6.1-1 Physical and Chemical Specifications for SG Mixtures			
Specification Parameter	**Specification**		**Method of Analysis**
	SG Mixtures	**Current JECFA/FCC Specifications for steviol glycosides (JECFA, 2010; FCC, 2016b)**	
Appearance	White to off-white powder	White to light yellow powder	Sensory Evaluation
Total Steviol Glycosides, % (anhydrous basis)	> 95.0	> 95% of the total of the 9 named steviol glycosides[a]	HPLC[b]
Loss on Drying, %	≤ 6.0	< 6% (105°, 2h)	FAO/JECFA (2006) (Vol 4)[c] (p. 61)
pH (1% solution)	4.5 to 7.0	4.5 to 7.0	FAO/JECFA (2006) (Vol 4) (pp. 36-38)
Residual Ethanol, %	< 0.50	< 0.5	USP (2012)[d], Method <467>
Residual Methanol, %	< 0.02	< 0.02	USP (2012), Method <467>
Total Ash, %	< 1.0	< 1	AOAC 945.46
Lead (as Pb), ppm	< 1.0	< 1	AOAC (2005)[e], Method 993.14
Arsenic (as As), ppm	< 1.0	< 1	AOAC (2005), Method 993.14
Cadmium (as Cd), ppm	< 1.0	Not specified	AOAC (2005), Method 993.14
Mercury (as Hg), ppm	< 1.0	Not specified	AOAC (2005), Method 993.14

FCC = Food Chemicals Codex; HPLC = high performance liquid chromatography; NS = not specified

[a] Nine glycosides are stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside, and steviolbioside.

[b] HPLC method is based upon the JECFA method (JECFA, 2010) but has been optimised to detect other glycosides, in addition to the nine glycosides specified by JECFA (see Appendix E).

[c] FAO/JECFA (2006). *Combined Compendium of Food Additive Specifications [Online Edition]. General Specifications for Enzymes Analytical Methods, Volume 4: Analytical Methods, Test Procedures and Laboratory Solutions Used by and Referenced in the Food Specifications*. 1st to 65th JECFA Meetings, 1956–2005. (FAO JECFA Monographs 1). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf [Last updated (Web version): August 2011].

[d] USP (2012). United States Pharmacopeia, 35th edition & National Formulary, 30th edition [Online]. Rockville (MD): U.S. Pharmacopeia (USP) Convention Inc. Available at: http://www.uspnf.com/ [Subscription Only].

[e] AOAC (2005). *Official Methods of Analysis of the Association of Official Analytical Chemists: Vols. 1&2, 18th edition* (Current through Revision 1, 2006). Arlington (VA): Association of Official Analytical Chemists (AOAC).

Microbiological Specifications

SG mixtures are produced from a natural source (*i.e.,* leaves of the *S. rebaudiana* Bertoni plant). As such, microbiological specification parameters have been established to ensure safe use in food, similar to those for other food ingredients derived from natural sources. Standard microbial tests appropriate for food ingredients are employed. The tests and limits are presented in Table B.6.1-2.

Table B.6.1-2 Microbiological Specifications for SG Mixtures		
Specification Parameter	**Specification**	**Method of Analysis**
Total Plate Count, CFU/g	< 1,000	AOAC (2005)[a], Method 966.23
Yeast & Mould, CFU/g	< 200	Standards Australia (1997)[b], Method 1766.2.2
Total Coliforms, MPN/g	Not detected	ISO 4831 (BSi, 1991)[c]
Escherichia coli count, MPN/g	Not detected	ISO 7251 (BSi, 1993)[d]
Salmonella sp.	Absent in 25 g	ISO 6579 (BSi, 2012)[e]

CFU = colony forming units; MPN = most probable number

[a] AOAC (2005). *Official Methods of Analysis of the Association of Official Analytical Chemists: Vols. 1&2, 18th edition* (Current through Revision 1, 2006). Arlington (VA): Association of Official Analytical Chemists (AOAC).

[b] Standards Australia (1997). *Food microbiology. Method 2.2: Examination for specific organisms—Colony count of yeasts and moulds*. (Australian/New Zealand Standard AS 1766.2.2). Sydney, Australia: Standards Association of Australia/SAI Global.

[c] BSi (1991). *Methods for Microbiological examination of food and animal feeding stuffs — Part 3: Enumeration of coliforms — Most probable number technique*. (British Standard (BS) / International Organization for Standardization (ISO), BS 5763-3:1991 ISO 4831:1991). London, Engl.: British Standards Institution (BSi).

[d] BSi (1993). *Methods for Microbiological examination of food and animal feeding stuffs — Part 8: Enumeration of presumptive Escherichia coli. Most probable number technique*. (British Standard (BS) / International Organization for Standardization (ISO), BS 5763-8:1994 ISO 7251:1993). London, Engl.: British Standards Institution (BSi).

[e] BSi (2012). Microbiology of Food and Animal Feed. Horizontal Method for the Detection, Enumeration and Serotyping of Salmonella. Enumeration by a miniaturized most probable number technique. (PD CEN ISO/TS 6579-2:2012). London, Engl.: British Standards Institution (BSi). Information available at: http://shop.bsigroup.com/en/ProductDetail/?pid=000000000030255346.

B.6.2 Product Analysis

Compliance with Physical and Chemical Specifications

Analysis of 3 to 5 non-consecutive lots of SG mixtures (RA50 and A95 as two example preparations) demonstrates that the manufacturing process, as described in Section B.5, produces a consistent product that conforms to the defined specification parameters. Table B.6.2-1 summarises the results of the batch analyses for the production lots of these SG mixtures. The Certificates of Analysis can be found in Appendix F.

Table B.6.2-1 Summary of the Chemical Product Analysis for Different Lots of SG Mixtures

RA50				
Specification Parameter	**Limit**	**Manufacturing Lot**		
		C1-002-0115-0007	**C1-002-0115-0011**	**C1-002-0115-0026**
Appearance	White to off-white powder	Conforms	Conforms	Conforms
Total Steviol Glycosides, % (anhydrous basis)	≥ 95.0	96.39	96.33	96.32
Loss on Drying, %	≤ 6.0	4.59	4.78	4.89
pH (1% solution)	4.5 to 7.0	5.76	6.08	5.87
Residual Ethanol, %	< 0.50	0.13	0.17	0.12
Residual Methanol, %	< 0.02	ND	ND	ND
Total Ash, %	< 1.0	0.05	0.04	0.02

Table B.6.2-1 Summary of the Chemical Product Analysis for Different Lots of SG Mixtures

RA50				
Specification Parameter	**Limit**	**Manufacturing Lot**		
		C1-002-0115-0007	**C1-002-0115-0011**	**C1-002-0115-0026**
Lead (as Pb), ppm	< 1.0	0.007	0.008	< 0.005
Arsenic (as As), ppm	< 1.0	0.015	0.011	< 0.005
Cadmium (as Cd), ppm	< 1.0	< 0.005	< 0.005	< 0.005
Mercury (as Hg), ppm	< 1.0	< 0.005	< 0.005	< 0.005

A95						
Specification Parameter	**Limit**	**Manufacturing Lot**				
		PT010915	**PT040915**	**PT180815**	**PT240815**	**PT280815**
Appearance	White to off-white powder	Conforms	Conforms	Conforms	Conforms	Conforms
Total Steviol Glycosides, % (anhydrous basis)	> 95.0	96.90	95.76	95.44	95.03	96.66
Loss on Drying, %	≤ 6.0	1.01	1.00	0.99	1.26	1.27
pH (1% solution)	4.5 to 7.0	5.81	5.77	5.75	5.87	5.79
Residual Ethanol, %	< 0.50	0.079	0.071	0.091	0.072	0.104
Residual Methanol, %	< 0.02	ND	ND	ND	ND	ND
Total Ash, %	< 1.0	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Lead (as Pb), ppm	< 1.0	0.036	0.041	0.049	0.039	0.039
Arsenic (as As), ppm	< 1.0	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Cadmium (as Cd), ppm	< 1.0	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Mercury (as Hg), ppm	< 1.0	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005

Source: Certificates of Analyses, see Appendix F.
ND = not detected; ppm = parts per million

As described in Section B.5, the distribution of steviol glycosides in the final SG mixture is dependent upon adsorption to and desorption from the divinyl benzene adsorption resin. Because different sections of the column system adsorb different proportions of steviol glycosides and these different sections are desorbed separately, SG mixtures with different ratios of steviol glycosides may be produced. Therefore, the steviol glycoside distribution is specific to each individual SG mixture and could contain any of the identified steviol glycosides, such as those listed in Table B.2.1-1. As an example, the steviol glycoside distributions, measured by HPLC analysis, are provided for RA50 and A95 in Tables B.6.2-2 and B.6.2-3 (see Appendix E) and demonstrate that although the distribution of steviol glycosides is different between the 2 preparations, the total steviol glycosides measured is consistently >95%.

Table B.6.2-2 Steviol Glycoside Distribution for 3 Lots of SG Mixture RA50				
Steviol Glycoside (%)	**Manufacturing Lot**			**Average**
	C1-002-0115-0007	**C1-002-0115-0011**	**C1-002-0115-0026**	
Rebaudioside A	57.88	59.18	58.71	58.59
Stevioside	25.77	24.80	25.24	25.27
Rebaudioside C	7.03	7.07	7.04	7.05
Rebaudioside F	1.41	1.43	1.48	1.44
Rebaudioside D	0.58	0.57	0.57	0.57
Rebaudioside B	0.53	0.30	0.23	0.35
Rubusoside	0.44	0.41	0.43	0.43
Dulcoside A	0.40	0.40	0.41	0.40
Rebaudioside M	0.24	0.25	0.20	0.23
Rebaudioside N	0.21	0.21	0.20	0.21
Rebaudioside O	0.15	0.17	0.17	0.16
Rebaudioside E	0.13	0.12	0.10	0.12
Steviolbioside	0.03	0.02	0.02	0.02
Stevioside A	1.25	1.13	1.20	1.19
Rebaudioside C *(isomer)*	0.34	0.27	0.32	0.31
Total Steviol Glycosides (%)	**96.39**	**96.33**	**96.32**	**96.35**

Source: HPLC analysis of SG mixtures, see Appendix E.

Table B.6.2-3 Steviol Glycoside Distribution for 5 Lots of SG Mixture A95						
Steviol Glycoside (%)	**Manufacturing Lot**					**Average**
	PT010915	**PT040915**	**PT180815**	**PT240815**	**PT280815**	
Rebaudioside A	1.34	1.67	2.04	2.12	1.40	1.74
Stevioside	0.08	0.17	0.05	0.08	0.03	0.08
Rebaudioside C	ND	0.07	ND	ND	ND	0.01
Rebaudioside F	ND	ND	ND	ND	ND	ND
Rebaudioside D	69.30	62.15	59.89	65.27	63.09	63.94
Rebaudioside B	0.20	0.37	0.19	0.21	0.17	0.23
Rubusoside	ND	ND	ND	ND	ND	ND
Dulcoside A	ND	ND	ND	ND	ND	ND
Rebaudioside M	21.57	27.30	27.55	22.34	26.98	25.15
Rebaudioside N	2.67	2.63	3.25	2.80	2.83	2.84
Rebaudioside O	1.10	0.90	1.71	1.56	1.32	1.32
Rebaudioside E	0.64	0.59	0.76	0.65	0.82	0.69
Steviolbioside	ND	ND	ND	ND	ND	ND
Total Steviol Glycosides (%)	**96.90**	**95.76**	**95.44**	**95.03**	**96.66**	**95.90**

Source: HPLC analysis of SG mixtures, see Appendix E.
ND = not detected

Compliance with Microbiological Specifications

Analysis of 3 to 5 non-consecutive lots of SG mixtures, RA50 and A95, demonstrates that the microbiological specifications outlined in Section B.6.1 are consistently met. A summary

of the microbiological analyses for RA50 and A95 are presented in Table B.6.2-4 (see Appendix F for Certificates of Analysis).

Table B.6.2-4 Summary of the Microbiological Product Analysis for Different Lots of SG Mixtures

RA50				
Specification Parameter	**Limit**	**Manufacturing Lot**		
		C1-002-0115-0007	**C1-002-0115-0011**	**C1-002-0115-0026**
Total Plate Count, (CFU/g)	< 1,000	< 250	< 250	ND
Yeast & Mould, (CFU/g)	< 200	ND	ND	ND
Total Coliforms, (MPN/g)	ND	ND	ND	ND
Escherichia coli count, (MPN/g)	ND	ND	ND	ND
Salmonella sp. (in 25 g)	Absent	Absent	Absent	Absent

A95						
Specification Parameter	**Limit**	**Manufacturing Lot**				
		PT010915	**PT040915**	**PT180815**	**PT240815**	**PT280815**
Total Plate Count, (CFU/g)	< 1,000	ND	ND	ND	ND	ND
Yeast & Mould, (CFU/g)	< 200	ND	ND	ND	ND	ND
Total Coliforms, (MPN/g)	ND	ND	ND	ND	ND	ND
Escherichia coli count, (MPN/g)	ND	ND	ND	ND	ND	ND
Salmonella sp. (in 25 g)	Absent	Absent	Absent	Absent	Absent	Absent

CFU = colony forming units; MPN = most probable number; ND = not detected

Other Chemical Analysis

Pesticide analyses are conducted on all final SG Mixtures. The same non-consecutive lots of A95 tested above and Lot # C1-002-0115-0011 of RA50 were subject to a multi-residue pesticide screen that covered a range of commonly applied pesticides. No pesticide residues were detected in the finished products (see Appendix G for the analytical reports).

B.7 Information for Food Labelling

Steviol glycosides are considered to be intense sweeteners and flavour enhancers when added to various food products. Steviol glycosides have been assigned the INS number of 960. Steviol glycosides will be labelled under its functional class, sweetener, either as sweetener (960) or sweetener (steviol glycosides).

B.8 Analytical Method for Detection

The analytical methods used to confirm that SG mixtures meet the established chemical and microbial specifications (Section B.6) are internationally recognised [*e.g.*, Association of Official Analytical Chemists (AOAC), U.S. Pharmacopeia (USP), FCC, JECFA]. In addition, PureCircle has developed a method to detect the presence of steviol glycosides in food matrices using HPLC, based on the assay method described by JECFA (2010) to detect

9 steviol glycosides. PureCircle's HPLC method has been optimised to account for the differences in molecular weights among the individual steviol glycosides, thereby improving the sensitivity of the assay and confirming that the HPLC method described by JECFA is sufficient to detect a variety of steviol glycosides, regardless of the glycosidic moieties conjugated to the steviol backbone. Please see Appendix E for complete details.

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

In addition to its use as a high-intensity sweetener, SG mixtures when added to food have the potential to act as a sweetness enhancer, as demonstrated in the sensory analyses presented in Section B.1.1.

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, 2016a) the safety information outlined must be provided for new food additives.

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

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

These points are addressed in the Section that follows.

Section 3.3.1 – Food Additives of the Food Standards Australia New Zealand *Application Handbook* (FSANZ, 2016a) 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 are outlined in the following section.

C.1 Introduction

Over the last few decades, the safety of steviol glycosides has been considered by several scientific bodies and regulatory agencies, including the FDA, JECFA, SCF, EFSA, FSANZ, and Health Canada. Interest in the use of steviol glycosides as sweeteners has encouraged extensive testing of the compounds and as such a large safety database exists. This database includes a thorough examination of the comparative metabolism and pharmacokinetics of steviol glycosides in experimental animals and humans, acute toxicity studies, short- and long-term toxicity and carcinogenicity studies, reproductive and developmental toxicology studies, *in vitro* and *in vivo* mutagenicity/genotoxicity studies, and human studies. Although many earlier studies examining the safety of steviol glycosides were conducted with stevioside due to the predominance of stevioside in *S. rebaudiana* leaves (Aze *et al.*, 1991; Toyoda *et al.*, 1997), the database pertaining to the safety of steviol glycosides was expanded following the completion of additional short-term toxicity, reproductive toxicity, *in vitro* and *in vivo* mutagenicity/genotoxicity studies, and human studies on rebaudioside A (Curry and Roberts, 2008; Curry *et al*., 2008; Nikiforov and Eapen, 2008; Williams and Burdock, 2009). Although the majority of toxicity studies have been conducted with either purified stevioside or rebaudioside A, the extensive database on the common metabolic fate of steviol glycosides has permitted the scientific bodies and regulatory agencies to extend their safety opinion to all steviol glycosides, rather than just individual glycosides.

As the safety of steviol glycosides has been reviewed by numerous scientific bodies and regulatory authorities, presented below is a detailed summary of the conclusions and the data deemed pivotal in determining the safety of steviol glycosides. Additionally, a detailed summary of the metabolic fate of steviol glycosides is provided. In order to corroborate previous conclusions regarding the shared metabolic pathway for steviol glycosides, studies comparing the microbial metabolism and hydrolysis rates of various steviol glycosides (rebaudioside A, B, C, D, E, F, M, steviolbioside, dulcoside A) *in vitro* are also discussed below. Based on the findings of these studies, it was deemed appropriate to extend the safety conclusions for steviol glycosides to all glycosylated derivatives of the aglycone steviol (purified).

C.2 Metabolic Fate

In vitro and *ex vivo* studies have demonstrated that steviol glycosides are not hydrolysed by digestive enzymes of the upper gastrointestinal tract and are not absorbed through the upper portion of the gastrointestinal tract (Hutapea *et al*., 1997; Geuns *et al*., 2003, 2007; Koyama *et al*., 2003a). Therefore, steviol glycosides enter the colon intact, where they are subject to microbial degradation by members of the *Bacteroidaceae* family, resulting in the release of the aglycone steviol (Gardana *et al*., 2003; Renwick and Tarka, 2008). Several *in vitro* studies mimicking the anaerobic conditions of the colon, reviewed by Renwick and Tarka (2008), have confirmed the ability of gut microflora from mice, rats, hamsters, and humans to hydrolyse steviol glycosides completely to steviol (Wingard *et al*., 1980; Hutapea *et al*., 1997;

Gardana *et al*., 2003; Koyama *et al*., 2003b). Specifically, Koyama *et al*. (2003b) investigated the degradation of a stevia mixture containing rebaudioside A, stevioside, rebaudioside C, and dulcoside A (percent composition not reported) in the presence of human faecal homogenates under anaerobic conditions. Similar to studies conducted with individual steviol glycosides, the stevia mixture was degraded completely to steviol within 24 hours of incubation (Koyama *et al*., 2003b). Rebaudioside E incubated *in vitro* with crude pectinase, an enzyme associated with the resident pectinolyic bacteria of the human intestine, was found to hydrolyse to steviol (Jensen and Canale-Parola, 1985). More recently, rebaudioside D was incubated with rat caecal contents for 90 minutes and hydrolysis to stevioside and steviol was found to be comparable to that of rebaudioside A (Nikiforov *et al.*, 2013).

Steviol glycosides are hydrolysed sequentially, removing one sugar moiety at a time, with differences in the degradation rates depending on the structural complexities of the steviol glycoside (Wingard *et al*., 1980; Koyama *et al*., 2003b). Stevioside is degraded to steviolbioside, steviolmonoside, and finally steviol, with glucose released with each sequential hydrolysis, whereas rebaudioside A is first converted to either stevioside (major pathway) or rebaudioside B (minor pathway) prior to being ultimately degraded to steviol (Nakayama *et al*., 1986; Gardana *et al*., 2003; Koyama *et al*., 2003b). The hydrolysis of rebaudioside A to steviol appears to be slower than that of stevioside to steviol partly due to the presence of one additional glucose moiety, indicating that microbes hydrolyse steviol glycosides sequentially by removing one glucose molecule at a time. Additionally, the metabolism of differing steviol glycosides appears to be stereoselective such that that degradation of rebaudioside C, which has an α(1>2) rhamnose on the 13-position, is faster than rebaudioside A, which has a β(1>2) glucose on the same position (Koyama *et al.*, 2003b).

Steviol is absorbed systemically into the portal vein and distributed to a number of organs and tissues, including the liver for additional metabolism, spleen, adrenal glands, fat, and blood (Nakayama *et al*., 1986; Sung, 2002; Koyama *et al*., 2003b; Wang *et al*., 2004; Roberts and Renwick, 2008). Peak concentrations of steviol are detected in the plasma of Sprague-Dawley rats within 15 to 30 minutes of oral steviol administration, whereas maximum levels of steviol were attained approximately 8 hours following oral administration of steviol glycosides to rats, including a mixture of rebaudioside A (28.8%), rebaudioside C (25.2%), stevioside (17.0%), and dulcoside A (10.2%) (Nakayama *et al*., 1986; Koyama *et al*., 2003a; Roberts and Renwick, 2008). Generally, the delay between the time of administration of steviol glycosides and the appearance of steviol in the plasma is attributed to the fact that glycosides are first cleaved to steviol in the colon before absorption (Koyama *et al*., 2003a).

Following absorption from the colon, steviol primarily undergoes conjugation with glucuronic acid to steviol glucuronide in the liver. In rats, free steviol (82 to 86% of chromatographed radioactivity), steviol glucuronide (10 to 12% of chromatographed radioactivity), and 2 unidentified metabolites (5 to 6% of chromatographed radioactivity) were identified in the plasma 8 hours after oral administration with either rebaudioside A or stevioside (Roberts

and Renwick, 2008). Similarly, steviol glucuronide was detected in the plasma following ingestion of stevioside or rebaudioside A in humans, with maximal concentrations detected 8 and 12 hours after administration, respectively (Geuns and Pietta, 2004 [unpublished]; Simonetti *et al*., 2004; Geuns *et al*., 2007; Wheeler *et al*., 2008). The differences in the time to reach maximum steviol glucuronide plasma concentrations between stevioside and rebaudioside A are due to the simpler structure and faster bacterial degradation of stevioside (Wheeler *et al.,* 2008). Moreover, significant inter-individual variability in maximum plasma steviol glucuronide levels, and in the time required to reach peak plasma levels, was noted in study participants following stevioside ingestion (Geuns *et al*., 2007). Such variations can likely be attributed to differences in the time required to release steviol from the glycoside in the gut as a result of inter-individual variability in the microflora composition or in gastric emptying.

In rats, free and conjugated steviol, as well as any unhydrolysed fraction of the administered glycosides, are excreted primarily in the faeces *via* the bile (generally within 48 hours), with smaller amounts appearing in the urine (less than 3%) (Wingard *et al*., 1980; Nakayama *et al*., 1986; Sung, 2002; Roberts and Renwick, 2008). Two (2) steviol conjugates were identified by Nakayama *et al*. (1986) in the bile of Wistar rats, one which was hydrolysed by a weak acid and another which was hydrolysed by a weak acid and β-glucuronidase; therefore, steviol is available to be released again from its conjugated form by the action of the microflora and may enter enterohepatic circulation following elimination in the bile. In contrast, in humans elimination of steviol glycosides, primarily as steviol glucuronide with very small amounts of the unchanged glycoside or steviol, occurs *via* the urine (Kraemer and Maurer, 1994; Geuns and Pietta, 2004 [unpublished]; Simonetti *et al*., 2004; Geuns *et al*., 2006, 2007; Wheeler *et al*., 2008). Relative to amounts recovered in urine, larger amounts of steviol (unabsorbed steviol released from steviol glycosides in the colon or from small amounts of steviol glucuronide secreted back into the gut *via* the bile) were also eliminated in the faeces (Geuns and Pietta, 2004 [unpublished]; Simonetti *et al*., 2004; Geuns *et al*., 2007; Wheeler *et al*., 2008).

The inter-species difference in the route of elimination of systemically absorbed steviol as steviol glucuronide (*via* the bile in rats and in the urine in humans) occurs as a result of the lower molecular weight threshold for biliary excretion in rats (325 Da) as compared to humans (500 to 600 Da; molecular weight of steviol glucuronide is 495 Da) (Renwick, 2007). Notably, in bile-duct ligated rats, excretion of steviol glucuronide occurred primarily in the urine (Wingard *et al*., 1980). While the primary routes of elimination of steviol glucuronide differ between rats and humans, the metabolism and pharmacokinetics of steviol glycosides are quite similar, which confirms the rat as an acceptable model for risk assessment in humans. The difference in the route of elimination is considered to be of no toxicological significance due to the fact that the water soluble phase II metabolites are rapidly cleared in both species. Therefore, toxicology data generated in rats are applicable to the assessment of the safety of steviol glycosides in humans given the similarities in metabolic fate.

Circulating steviol glycosides have not been detected in the plasma of humans, nor in the majority of animal studies conducted, indicating that the parent compound is not absorbed

into systemic circulation. The parent glycoside stevioside, as well as the hydrolysis product steviol, were not detected in the plasma following 3 days of stevioside consumption (3 to 4.5 mg/kg/day steviol equivalents) in healthy human subjects (Geuns *et al.*, 2007), whereas measurable concentrations of steviol glucuronide have been detected in the plasma (Geuns *et al*., 2007; Wheeler *et al*., 2008). Similarly, parent compound was not detected in the plasma of rats dosed orally with rebaudioside A or stevioside (5 mg/kg body weight, single dose) (Roberts and Renwick, 2008) or pigs dosed orally with stevioside (0.167% in the diet for 2 days) (Geuns *et al*., 2003). In contrast to these reports, a recent study in rats by Nikiforov *et al.* (2013) reported the detection of very low plasma levels of parent compound (≤1.5 µg/mL) following administration of rebaudioside A or D at 2,000 mg/kg body weight/day in the diet for 1 day and 21 days. Free steviol (≤12 µg/mL) and glucuronide-conjugated steviol (≤40 µg/mL) were the primary metabolites detected in the plasma. Although these low levels of parent compound were reported, this is considered to have no safety consequence as all the toxicological studies conducted with rebaudioside A and D showed no adverse toxicological findings, and no parent steviol glycoside has been detected in human plasma or urine.

C.3 Common Metabolic Fate of Steviol Glycosides

In order to confirm the similarities in the microbial metabolism among all steviol glycosides as previously observed by Wingard *et al*. (1980), Hutapea *et al*. (1997), Gardana *et al*. (2003), and Koyama *et al*. (2003b), 6 *in vitro* metabolism studies were conducted to compare the metabolism of rebaudioside A with individual steviol glycosides containing glucose, rhamnose, xylose, and fructose moieties, including rebaudioside B, C, D, E, F, M, steviolbioside, dulcoside A, and fructosylated rebaudioside A (Purkayastha *et al.,* 2014*,* 2015, 2016; Kwok, 2015). The purity of all steviol glycosides tested was >95%. Human faecal homogenates were prepared based on pooling of faecal samples from 6 healthy male and 6 healthy female volunteers. Male and female faecal homogenate samples were incubated with individual steviol glycosides (0.2 or 2.0 mg/mL, depending on solubility) at 37°C for up to 48 hours under anaerobic conditions. Liquid chromatography-mass spectrometry (LC/MS) was employed to measure the rate of formation and amount of steviol generated from the microbial hydrolysis of the individual steviol glycosides. The details of the 5 metabolic comparison studies, as reported in Purkayastha *et al.* (2016), are provided in Table C.3-1 along with a summary at each time point of the percent of steviol glycoside metabolised to steviol.

Table C.3-1 Metabolism of Steviol Glycosides Containing Glucose, Xylose, and Rhamnose Moieties to Steviol by Human Faecal Homogenates

Steviol Glycoside	Incubation Time (h)	Male Faecal Homogenates		Female Faecal Homogenates	
		Percent Metabolised[a]		Percent Metabolised	
Experiment 1					
Rebaudioside A (2.0 mg/mL) (SvG4)	4	3.5[b] ± 8.1		2.6[b] ± 4.7	
	8	14.5 ± 5.0		11.1 ± 2.2	
	24	50.5 ± 4.0		52.4 ± 3.4	
	48	84.3 ± 4.4		78.9 ± 4.2	
Rebaudioside B (2.0 mg/mL) (SvG3)	4	4.2 ± 11.6		3.3 ± 7.5	
	8	13.8 ± 5.9		8.6 ± 7.0	
	24	56.4 ± 5.7		40.6 ± 3.6	
	48	87.7 ± 9.7		62.0 ± 11.9	
Rebaudioside D (2.0 mg/mL) (SvG5)	4	3.7 ± 5.8		3.3 ± 8.6	
	8	16.6 ± 2.5		13.5 ± 2.7	
	24	85.6 ± 5.6		66.0 ± 2.1	
	48	113.8 ± 4.0		110.9 ± 6.3	
Experiment 2					
Rebaudioside A (2.0 mg/mL) (SvG4)	4	1.40[b] ± 4.2		2.05[b] ± 3.2	
	8	4.70 ± 3.9		9.35 ± 4.7	
	24	40.1 ± 4.1		47.0 ± 2.0	
	48	53.3 ± 16.6		59.5 ± 3.3	
Rebaudioside C (2.0 mg/mL) (SvR1G3)	4	0.4 ± 6.2		0.5 ± 4.5	
	8	1.4 ± 4.1		1.5 ± 5.3	
	24	26.5 ± 6.9		33.7 ± 4.7	
	48	85.3 ± 3.3		97.1 ± 5.9	
Experiment 3					
Rebaudioside A (0.2 mg/mL) (SvG4)	8	64.1[b] ± 2.1		97.3[b] ± 3.4	
	16	98.5 ± 1.9		103.7 ± 1.6	
	24	97.5 ± 2.6		100.8 ± 2.2	
Rebaudioside M (0.2 mg/mL) (SvG6)	8	64.6 ± 1.0		86.7 ± 2.2	
	16	111.6 ± 2.2		108.4 ± 2.7	
	24	111.8 ± 3.0		107.4 ± 2.2	
Experiment 4					
		Asian[c]	*Caucasian*	*Asian*	*Caucasian*
Rebaudioside A (2.0 mg/mL) (SvG4)	4	1.3[d] ± 2.8	1.1 ± 2.8	2.1 ± 0.8	1.6 ± 2.6
	8	4.5 ± 3.7	3.1 ± 2.3	10.7 ± 2.3	5.9 ± 4.0
	16	26.5 ± 2.2	16.0 ± 2.5	61.9 ± 4.7	32.7 ± 0.9
	24	60.1 ± 1.6	34.8 ± 0.3	78.9 ± 3.0	64.7 ± 2.4
Rebaudioside E (2.0 mg/mL) (SvG4)	4	1.8 ± 0.9	2.2 ± 2.0	2.0 ± 1.0	2.3 ± 1.9
	8	4.6 ± 6.7	5.9 ± 1.4	11.2 ± 2.2	7.9 ± 1.5
	16	28.0 ± 1.7	26.1 ± 4.3	56.2 ± 1.2	37.5 ± 0.7
	24	58.4 ± 0.5	54.2 ± 5.0	67.8 ± 1.9	59.4 ± 6.4
Steviolbioside (2.0 mg/mL) (SvG2)	4	5.4 ± 1.1	6.2 ± 1.5	7.9 ± 0.5	7.2 ± 4.3
	8	13.8 ± 2.6	14.6 ± 0.4	25.3 ± 1.9	20.0 ± 4.4
	16	35.3 ± 2.5	31.4 ± 0.4	60.5 ± 3.2	62.4 ± 3.6
	24	54.0 ± 3.1	50.4 ± 1.5	77.9 ± 4.2	66.8 ± 0.6

Table C.3-1 Metabolism of Steviol Glycosides Containing Glucose, Xylose, and Rhamnose Moieties to Steviol by Human Faecal Homogenates			
Steviol Glycoside	**Incubation Time (h)**	**Male Faecal Homogenates**	**Female Faecal Homogenates**
		Percent Metabolised[a]	**Percent Metabolised**
Experiment 5			
Rebaudioside A (0.2 mg/mL) (SvG4)	4	4.0 ± 1.3[d]	6.5 ± 1.6
	8	7.6 ± 3.1	22.9 ± 10.6
	16	34.4 ± 13.0	80.3 ± 14.9
	24	74.8 ± 6.0	101.5 ± 4.6
	48	114.0 ± 3.9	114.4 ± 1.9
Rebaudioside F (0.2 mg/mL) (SvX1G3)	4	0.7 ± 0.1	0.9 ± 0.2
	8	1.6 ± 0.7	3.1 ± 0.4
	16	4.7 ± 1.3	12.2 ± 4.1
	24	15.9 ± 9.4	41.2 ± 6.0
	48	63.6 ± 22.3	82.0 ± 7.8
Dulcoside A (0.2 mg/mL) (SvR1G2)	4	2.2 ± 0.7	2.3 ± 1.2
	8	5.5 ± 2.0	6.4 ± 4.0
	16	15.0 ± 7.4	19.7 ± 4.1
	24	42.8 ± 19.8	60.3 ± 11.4
	48	96.7 ± 3.7	89.3 ± 16.2
Rebaudioside M (0.2 mg/mL) (SvG6)	4	3.2 ± 1.3	5.6 ± 1.8
	8	7.2 ± 2.6	24.9 ± 14.1
	16	32.3 ± 10.2	67.6 ± 11.6
	24	75.5 ± 8.5	96.6 ± 5.6
	48	102.2 ± 3.2	105.0 ± 2.0
Rebaudioside A (2.0 mg/mL) (SvG4)	4	0.5 ± 0.1	0.6 ± 0.2
	8	1.2 ± 0.4	2.4 ± 0.9
	16	4.5 ± 1.4	10.3 ± 4.9
	24	12.2 ± 4.7	32.5 ± 7.0
	48	66.2 ± 11.4	87.5 ± 10.9
Rebaudioside F (2.0 mg/mL) (SvX1G3)	4	0.1 ± 0.0	0.1 ± 0.0
	8	0.3 ± 0.1	0.4 ± 0.0
	16	2.0 ± 1.6	1.9 ± 0.2
	24	2.9 ± 0.9	6.6 ± 1.0
	48	15.0 ± 6.6	31.1 ± 6.5
Dulcoside A (2.0 mg/mL) (SvR1G2)	4	0.3 ± 0.1	0.4 ± 0.1
	8	1.1 ± 0.5	1.3 ± 0.8
	16	5.1 ± 1.6	5.4 ± 1.6
	24	43.2 ± 9.5	29.5 ± 16.7
	48	104.5 ± 7.9	75.4 ± 37.7

Trivial formula: Sv = steviol; G = glucose; R = rhamnose; X = xylose.
All results are expressed as mean ± standard deviation.
[a] The percent metabolised to steviol was calculated based on the theoretical maximum concentration of steviol that could be formed from nominal complete metabolism (*i.e.*, percent remaining = 100% - [(mean steviol concentration reported/theoretical maximum steviol concentration generated) x 100%].
[b] Results for experiments 1, 2, and 3 are expressed as the average of results obtained with 2 different faecal homogenate pools.
[c] Faecal homogenates were obtained from Asian and Caucasian volunteers.
[d] Results for experiments 4 & 5 are expressed as the average of 3 replicates from one faecal homogenate pool.

The results from these experiments demonstrate a remarkable similarity with respect to the rate of hydrolysis of the individual steviol glycosides to steviol, particularly during the first 24 hours of incubation. The type of faecal homogenate used (male *vs.* female, Asian *vs.* Caucasian) did not have a significant impact upon the hydrolysis rate or amount of steviol formed. The results of experiment 1 demonstrate that the rates of metabolism for 3 steviol glycosides containing glucose moieties, rebaudiosides A, B, and D were very similar (2.0 mg/mL test concentration), particularly at the earlier time points (4- and 8-hour). Rebaudioside D showed slightly higher percent hydrolysis at the 24- and 48-hour time points compared to both rebaudiosides A and B. In experiment 2, although rebaudioside C (rhamnose conjugate) was found to have a much slower initial rate of hydrolysis compared to rebaudioside A (both tested at 2.0 mg/mL), after the 48-hour incubation period a higher percentage of rebaudioside C (68.6%, males; 97.1%, females) had in fact been metabolised to steviol compared to rebaudioside A (53.3%, males; 59.5%, females). In experiment 3, since rebaudioside M (glucose conjugate) was not completely soluble at the 2.0 mg/mL concentration, metabolism was assessed at a lower sample concentration of 0.2 mg/mL over a shorter timeframe (24 hours). Metabolism of both rebaudioside A and M was extensive at this concentration, with about 64 to 97% hydrolysed by 8 hours, and it was established that both compounds were completely converted to steviol by 16 hours (~100%). Experiment 4 compared the metabolism of rebaudioside A, E, and steviolbioside, 3 glucose containing glycosides, at a sample concentration of 2.0 mg/mL over a 24-hour incubation period. Steviolbioside was found to have a slightly higher metabolism rate over the first 8 hours compared to rebaudiosides A and E, but by 24 hours the percent metabolised to steviol for all 3 compounds was remarkably similar. In the last experiment, the hydrolysis of rebaudiosides A, F (xylose conjugate), M, and dulcoside A (rhamnose conjugate) were compared at the lower concentration of 0.2 mg/mL over a 48-hour incubation time. The rates of hydrolysis for rebaudiosides A, M were quite similar from 4 to 48 hours, and although dulcoside A hydrolysis was initially slower, hydrolysis to steviol for all 3 compounds was essentially complete by 48 hours (~90 to 100%). The initial rate of hydrolysis for rebaudioside F, between 4 to 16 hours, was similar to that for dulcoside A, but by 48 hours rebaudioside F was not completely metabolised to steviol (~64 to 82%). At the higher concentration of 2.0 mg/mL, rebaudioside A and dulcoside A displayed similar rates of hydrolysis over the complete timeframe (4 to 48 hours) whereas rebaudioside F had an overall slower rate of metabolism, with a maximum of 31.1% hydrolysis at 48 hours. The results of this last experiment directly demonstrate that independent of the type and number of sugar moieties attached to the steviol backbone (rebaudioside A, SvG4; rebaudioside M, SvG6; dulcoside A, SvR1G2; rebaudioside F, SvX1G2), the glucose (SvGn), rhamnose (SvR1Gn), and xylose (SvX1Gn) containing steviol glycoside groups have a similar metabolic fate (*i.e.,* hydrolysis to steviol).

A separate unpublished *in vitro* pilot study was conducted comparing the metabolism of rebaudioside A to a steviol glycoside containing fructose (Kwok, 2015). Since extraction methods were not able to yield sufficient quantities of steviol glycosides containing fructose moieties from the stevia leaf (*i.e.,* Rebaudioside A3, see Table B.2.1-1) for *in vitro* incubations, a representative fructosylated steviol glycoside, fructosylated rebaudioside A

(Figure C.3-1), was prepared by conjugating the terminal glucose of the rebaudioside A R1 side chain with fructose. The final reaction product for use in the *in vitro* incubations contained a mixture of fructosylated rebaudioside A (RAF) and rebaudioside A (93% and 7% w/w, respectively). As described above, human faecal homogenates were prepared based on pooling of faecal samples from 6 healthy male and 6 healthy female volunteers. Male and female faecal homogenates were incubated with rebaudioside A or RAF (0.2 mg/mL) at 37°C for 0, 24, and 48 hours under anaerobic conditions. LC/MS was employed to measure the amount of steviol generated from the microbial hydrolysis of rebaudioside A and RAF and to determine the percentage of starting material metabolised (Table C.3-2). Both rebaudioside A and RAF were completely metabolised to steviol by 24 hours (both >100%) demonstrating that despite containing different sugar moieties, fructosylated rebaudioside A and rebaudioside A are hydrolysed to steviol at similar rates. This pilot study demonstrated for the first time that a fructosylated rebaudioside A (laboratory prepared) was hydrolysed to steviol at a similar rate to that of rebaudioside A, and based on the results of this study, it is expected that steviol glycosides containing fructose moieties present in the stevia leaf (*i.e.,* rebaudioside A3) would be metabolised in a similar manner.

Figure C.3-1 Structure of Fructosylated Rebaudioside A

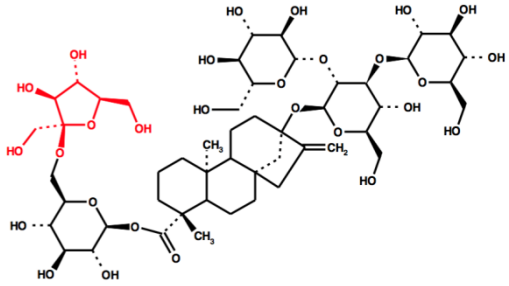


Table C.3-2 Metabolism of Fructosylated Rebaudioside A to Steviol by Human Faecal Homogenates

Steviol Glycoside	Incubation Time (h)	Male Faecal Homogenates	Female Faecal Homogenates
		Percent Metabolised[a]	Percent Metabolised
Rebaudioside A (0.2 mg/mL) (SvG4)	0	0.6[b] ± 0	0.6[b] ± 0
	24	122.3 ± 10.5	138.6 ± 6.8
	48	124.3 ± 5.9	119.8 ± 14.5
RAF (0.2 mg/mL) [7% rebaudioside A/93% fructosylated rebaudioside A (SvF1G3)]	0	0.7 ± 0	0.6 ± 0
	24	109.7 ± 13.0	128.7 ± 11.0
	48	98.3 ± 13.1	110.7 ± 11.0

Trivial formula: Sv = steviol; G = glucose; F = fructose. All results are expressed as mean ± standard deviation.
[a] The percent metabolised to steviol was calculated based on evaluating the molar equivalent of rebaudioside A/RAF calculated from the measured amount of steviol and comparing to the starting concentration of rebaudioside A/RAF.
[b] Results are expressed as the average of results obtained with 3 different faecal homogenate pools.

These experiments demonstrate that steviol glycosides are metabolised by human faecal homogenates to steviol at generally similar hydrolysis rates, indicating that the number and location of sugar units attached at the R1 and R2 positions (see Figure B.2.1-1) does not significantly impact the rate of hydrolysis. Furthermore, since none of the steviol glycosides tested demonstrated a significantly faster rate of hydrolysis than rebaudioside A, there is no concern that consumption of any of these compounds would increase the circulating plasma levels of the aglycone metabolite (steviol) relative to those achieved by rebaudioside A. While subtle differences in the rate of individual steviol glycoside hydrolysis may exist, it does not appear that these differences would be great enough to significantly impact the absorption rate of steviol (that could potentially alter the level of systemic metabolism and clearance of steviol). The results of these studies indicate that the major steviol glycosides as well as the many "minor" steviol glycosides recently identified share a common metabolic fate, and, therefore, the safety database established for steviol glycosides can be extended to all glycosylated derivatives of the aglycone steviol (purified), including the 2 SG mixtures outlined within the dossier (RA50 and A95).

C.4 Safety of Steviol Glycosides

The safety of steviol glycosides was reviewed by JECFA at 4 separate meetings (51st, 63rd, 68th, and 69th) in 1998, 2004, 2007, 2008 (JECFA, 1999, 2006, 2007c, 2009). At the first meeting in 1998, JECFA was asked to specifically review the safety of stevioside. Following review of the available information, the Committee concluded that the data on stevioside were limited and highlighted the need for specifications for commercial materials. An ADI could not be established.

Subsequently in 2004, the Committee determined that the material of commerce for which tentative specifications were developed should be known as "steviol glycosides". New data as per the requests made at the earlier meeting were provided to the Committee for review. The Committee reviewed the newly available data which demonstrated that stevioside and rebaudioside A were not genotoxic and that the positive *in vitro* results for steviol and its oxidative derivatives were not confirmed *in vivo*. Although the Committee reviewed the results of a developmental study showing adverse effects on fertility following treatment of male rats with a crude aqueous extract of *S. rebaudiana*, the Committee referred back to the studies reviewed at the preceding meeting noting that in studies conducted with higher purity material, no reproductive or developmental effects were observed, and thus, the reproductive effects noted following administration of the crude extract were unlikely to be related to steviol glycosides. Although the Committee did not raise any further questions regarding the potential toxicity of steviol glycosides at this review, the Committee noted that pharmacological effects in patients with hypertension or type 2 diabetes were observed at doses of 12.5 to 25 mg/kg body weight/day of steviol glycosides (5 to 10 mg/kg body weight/day as steviol equivalents). Consequently, further information regarding the potential effects of steviol glycosides in subjects with diabetes and in normotensive and hypotensive populations was requested. At this time, a temporary ADI of 2 mg/kg body weight/day (expressed as steviol) for steviol glycosides was allocated, based on a no-observed-

adverse-effect level (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 a safety factor of 200 (JECFA, 2006).

In 2007, the Committee received additional data pertaining to the potential pharmacological effects of steviol glycosides in humans; yet, none of these studies were conducted with a material that met the specifications for steviol glycosides. However, the Committee was made aware of an ongoing human study that was designed to specifically address the Committee's previous concerns (Maki *et al*., 2008a,b) and thus the temporary ADI was extended until 2008. The specifications were revised and the tentative designation was removed.

At the final safety evaluation of steviol glycosides in 2008, the Committee was presented with new data pertaining to the metabolic fate of steviol glycosides in rats and humans (Roberts and Renwick, 2008; Wheeler *et al*., 2008), subchronic and reproductive/ developmental toxicity of rebaudioside A specifically (Curry and Roberts, 2008; Curry *et al*., 2008; Nikiforov and Eapen, 2008), and the potential pharmacological effects of steviol glycosides in diabetic populations and individuals with normal or low-normal blood pressure (Maki *et al*., 2008a,b). The Committee concluded that the results of the human studies evaluating the effects of steviol glycosides on blood pressure and blood glucose were sufficient to remove the additional safety factor of 2 and establish a full ADI of 4 mg/kg body weight (expressed as steviol) for steviol glycosides. The specifications for steviol glycosides were revised further, requiring not less than 95% of the 7 named steviol glycosides (stevioside, rebaudioside A, B, C, dulcoside A, rubusoside, and steviolbioside).

During the Committee's 73rd meeting in 2010, JECFA revised the specifications for steviol glycosides to include 2 additional steviol glycosides, rebaudioside D and rebaudioside F, within the purity criteria[1] (JECFA, 2010). Although no specific studies have been conducted with these steviol glycosides individually, their inclusion within JECFA's purity specification further confirms that the safety of steviol glycosides is based on the general recognition that all steviol glycosides are degraded to the aglycone steviol and that the safety demonstrated for one glycoside is relevant to all glycosides in general.

Immediately prior to JECFA's 69th meeting (JECFA, 2009), FSANZ conducted their own evaluation of the safety of steviol glycosides (FSANZ, 2008). In its assessment, FSANZ considered the data previously reviewed by JECFA, as well as supplementary data consisting of published and unpublished studies. FSANZ considered the toxicological database for stevioside to cover a range of toxicological endpoints, and concluded that the supplementary data were sufficient to revise JECFA's temporary ADI to a full ADI of 4 mg/kg body weight/day by removing the additional uncertainty factor of 2. Moreover, FSANZ recently approved the inclusion of rebaudioside M to the definition of steviol glycosides on the basis of the common metabolic fate of steviol glycosides to steviol (FSANZ, 2015).

[1] Not less than 95% of the following 9 steviol glycosides, on a dried weight basis: stevioside, rebaudioside A, B, C, D, and F, dulcoside A, rubusoside, and steviolbioside.

Therefore, the approval by FSANZ confirms that safety data generated from one steviol glycoside can represent all steviol glycosides, in general, regardless of the number and location of sugar moieties.

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, 2016a) 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. The percentage of the food group in which the food additive is proposed to be used or the percentage of the market likely to use the new food additive.
4. Information relating to the use of the food additive in other countries.

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

D.1 Current Permitted Food Uses and Use Levels of Steviol Glycosides

FSANZ approved the use of steviol glycosides in specific food-uses at specified use levels as shown below in Table D.1-1 (FSANZ, 2016c). PureCircle intends to market SG mixtures for use as intense sweeteners under the same conditions as those presently approved for steviol glycoside preparations; see Table D.1-1).

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

As the current steviol glycoside concentrations are provided in terms of steviol equivalents, conversion factors for all potential steviol glycosides present in *S. rebaudiana* would normally be required to be provided and added to the conversion factor table. Presently, as indicated in *Standard 1.3.1 – Food Additives* of the Standard Code, the conversion factor is provided for each of the 10 permitted steviol glycosides (see Table D.1-2).

Table D.1-2 Conversion Factors of Steviol Glycosides In Order to Determine Steviol Equivalents	
Column 1	**Column 2**
Steviol glycoside	**Conversion factor**
Dulcoside A	0.40
Rebaudioside A	0.33
Rebaudioside B	0.40
Rebaudioside C	0.33
Rebaudioside D	0.28
Rebaudioside F	0.34
Rebaudioside M	0.25
Rubusoside	0.50
Steviol	1.00
Steviolbioside	0.50
Stevioside	0.40

Adapted from *Standard 1.3.1 – Food Additives* of the Standard Code (FSANZ, 2016d)

As the current application submitted by PureCircle is intended to expand the definition of steviol glycosides to include all those identified in the *S. rebaudiana* plant, applying multiple conversion factors to a mixture of steviol glycosides are considered impractical. Therefore, PureCircle proposes to utilise a single conversion factor of 0.33, which is representative of typical SG mixtures and all potential steviol glycosides. For example, as presented in Section B.6.2, rebaudioside A is generally the predominant glycoside present in commercial preparations, as highlighted within PureCircle's RA50 mixture. Although, the conversion factor of 0.33 is higher than that of the predominant glycosides present in PureCircle's other commercial preparation (A95), the value of 0.33 represents the median conversion factor of all major steviol glycosides present in the final preparations (0.25 to 0.4), with the minor glycosides typically representing much smaller amounts of the final preparation (approximately 5% as presented in Section B.6.1). Therefore, the conversion factor of 0.33 will need to be added to *Standard 1.3.1 – Food Additives* of the Standard Code to represent SG mixtures.

D.2 Exposure Data

Since the SG mixtures including the minor glycosides outlined above are intended for use as an intense sweetener in food-uses under the same conditions of use as those presently authorised for steviol glycosides, intakes of SG mixtures (as steviol equivalents) will be the same as for steviol glycosides, which are already available in the Australian/New Zealand marketplace. Accordingly, a separate intake assessment for SG mixtures specifically was not performed for the purpose of this food additive application. It should be further noted that the use-levels for steviol glycosides are expressed as steviol equivalents and as such are not specified for any one particular steviol glycoside, but rather 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.

REFERENCES

Ahmed MS, Dobberstein RH, Farnsworth NR (1980). Stevia rebaudiana. I. Use of *p*-bromophenacyl bromide to enhance ultraviolet detection of water-soluble organic acids (steviolbioside and rebaudioside B) in high-performance liquid chromatographic analysis. J Chromatogr A 192(2):387-393.

Aze Y, Toyoda K, Imaida K, Hayashi S, Imazawa T, Hayashi Y et al. (1991). Subchronic oral toxicity study of stevioside in F344 rats. Eisei Shikenjo Hokoku [Bull Natl Inst Hyg Sci] (109):48-54 [Japanese].

Bridel M, Lavieille R (1931). Le principe à saveur sucrée du Kaa-hé-á (Stevia rebaudiana Bert.). J Pharm Chim 14:99-113 [No English – not obtained].

Bridges JR, Carlson A, Patton PA, inventors; Decatur (IL): Tate & Lyle Ingredients Americas, assignee (2012). Stevia Blends Containing Rebaudioside B. US Patent Application US 2012/0269954 A1 [Oct. 25, 2012].

CCFA (2011). Steviol glycosides. In: *Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission, Thirty fourth Session*. Jul. 4-9, 2011, Geneva, Switz. / *Report of the Forty-Fourth Session of the Codex Committee on Food Additives.* Mar. 14-18, 2011, Xiamen, China. (REP11/FA). Rome, Italy: Codex Committee on Food Additives (CCFA), Codex Alimentarius Commission (CAC). Available at: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-43%252FREP11_FAe.pdf.

Ceunen S, Geuns JM (2013). Steviol glycosides: chemical diversity, metabolism, and function. J Nat Prod 76(6):1201-1228.

Chang SS, Cook JM (1983). Stability studies of stevioside and rebaudioside A in carbonated beverages. J Agric Food Chem 31(2):409-412.

Chaturvedula VSP, Prakash I (2011a). Utilization of RP-HPLC fingerprinting analysis for the identification of diterpene glycosides from *Stevia rebaudiana*. Int J Res Phytochem Pharmacol 1(2):88-92.

Chaturvedula VSP, Prakash I (2011b). Diterpene glycosides from *Stevia rebaudiana*. J Med Plants Res 5:4838-4842.

Chaturvedula VSP, Prakash I (2011c). Additional minor diterpene glycosides from Stevia rebaudiana. Nat Prod Commun 6(8):1059-1062 [abstract only].

Chaturvedula VSP, Prakash I (2011d). Structures of the novel diterpene glycosides from Stevia rebaudiana. Carbohydr Res 346(8):1057-1060.

Chaturvedula VSP, Prakash I (2011e). Acid and alkaline hydrolysis studies of stevioside and rebaudioside A. J Appl Pharm Sci 1(8):104-108.

Chaturvedula VSP, Zamora J (2014). Isolation and structural characterization of a new minor diterpene glycoside from Stevia rebaudiana. Nat Prod Commun 9:1677-1679 [abstract only].

Chaturvedula VSP, Upreti M, Prakash I (2011a). Structures of the novel α-glucosyl linked diterpene glycosides from *Stevia rebaudiana*. Carbohydr Res 346(13):2034-2038.

Chaturvedula VSP, Clos JF, Rhea J, Milanowski D, Mocek U, Dubois GE et al. (2011b). Minor diterpenoid glycosides from the leaves of *Stevia rebaudiana*. Phytochem Lett 4(3):209-212.

Chaturvedula VSP, Rhea J, Milanowski D, Mocek U, Prakash I (2011c). Two minor diterpene glycosides from the leaves of *Stevia rebaudiana*. Nat Prod Commun 6(2):175-178 [abstract only].

Chaturvedula VSP, Rhea J, Milanowski D, Mocek U, Prakash I (2011d). Isolation and structure elucidation of two new minor diterpene glycosides from *Stevia rebaudiana*. Organic Chem Curr Res 1:102. doi:10.4172/2161-0401.1000102.

Clos, JF, DuBois, GE, Prakash, I (2008). Photostability of rebaudioside A and stevioside in beverages. J Agric Food Chem 56(18):8507–8513.

Curry LL, Roberts A (2008). Subchronic toxicity of rebaudioside A. Food Chem Toxicol 46(7, Suppl.):S11-S20.

Curry LL, Roberts A, Brown N (2008). Rebaudioside A: two-generation reproductive toxicity study in rats. Food Chem Toxicol 46(7, Suppl.):S21-S30.

Das S, Das AK, Murphy R A, Punwani IC, Nasution MP, Kinghorn AD (1992). Evaluation of the cariogenic potential of the intense natural sweeteners stevioside and rebaudioside A. Caries Res 26(5):363-366.

EFSA (2010). EFSA Panel on Food Additives and Nutrient Sources (ANS); Scientific Opinion on safety of steviol glycosides for the proposed uses as a food additive (question no EFSA-Q-2007-071, EFSA-Q-2008-387, EFSA-Q-2008-401, adopted on 10 March 2010 by European Food Safety Authority). EFSA J 8(4):1537. [85 pp.]. doi:10.2903/j.efsa.2010.1537. Available at: http://www.efsa.europa.eu/fr/efsajournal/pub/1537.htm.

EFSA (2015). Scientific opinion on the safety of the proposed amendment of the specifications for steviol glycosides (E 960) as a food additive. (EFSA Panel on Food Additives and Nutrient Sources Added to Food/ANS) (Question no EFSA-Q-2014-00002, adopted on 17 November 2015 by European Food Safety Authority). EFSA J 13(12):4316 [29 pp.]. doi: 10.2903/j.efsa.2015.4316. Available at: http://www.efsa.europa.eu/en/efsajournal/pub/4316.

FCC (2016a). Rebaudioside A. In: *Food Chemicals Codex, 10th edition*. Rockville (MD): United States Pharmacopeial Convention (USP), pp. 1099-1103.

FCC (2016b). Steviol glycosides. In: *Food Chemicals Codex, 10th edition*. Rockville (MD): United States Pharmacopeial Convention (USP), pp. 1234-1238.

Ferlow K (2005). Stevia - The sweetest substance on Earth. NutraCos 4(2, Suppl.):10-11.

FSANZ (2008). *Final Assessment Report: Application A540: Steviol Glycosides as Intense Sweeteners*. Canberra, Australia / Wellington, NZ: Foods Standards Australia New Zealand (FSANZ). Available at: http://www.foodstandards.gov.au/code/applications/documents/FAR_A540_Steviol_glycosides.pdf.

FSANZ (2015). *Approval Report – Application A1108: Rebaudioside M as a Steviol Glycoside Intense Sweetener*. Canberra, Australia / Wellington, NZ: Foods Standards Australia New Zealand (FSANZ). Available at: http://www.foodstandards.gov.au/code/applications/Documents/A1108-AppR.pdf.

FSANZ (2016a). *Food Standards Australia New Zealand Application Handbook. 1 March 2016*. Canberra, Australia / Wellington, NZ: Foods Standards Australia New Zealand (FSANZ). Available at: http://www.foodstandards.gov.au/code/changes/pages/applicationshandbook.aspx.

FSANZ (2016b). Schedule 3: Identity and purity. In: *Australia New Zealand Food Standards Code*. Canberra, Australia / Wellington, New Zealand: Food Standards Australia New Zealand (FSANZ). Available at: https://www.legislation.gov.au/Details/F2016C00188.

FSANZ (2016c). Schedule 15: Substances that may be used as food additives. In: *Australia New Zealand Food Standards Code*. Canberra, Australia / Wellington, New Zealand: Food Standards Australia New Zealand (FSANZ). Available at: https://www.legislation.gov.au/Series/F2015L00439.

FSANZ (2016d). Standard 1.3.1 - Food Additives. In: *Australia New Zealand Food Standards Code*. Canberra, Australia / Wellington, New Zealand: Food Standards Australia New Zealand (FSANZ). Available at: https://www.legislation.gov.au/Details/F2016C00165.

FSSAI (2012). *Minutes of National Workshop on Regulatory and Enforcement Procedures – reg.* [File No. 1(48) 2011/Seminar/FSSAI]. New Delhi, India: Food Safety and Standards Authority of India (FSSAI), Ministry of Health & Family Welfare, Government of India. Available at: http://www.fssai.gov.in/Portals/0/Pdf/Workshop_Minutes(14-05-2012).pdf.

Furlano BL, Myerson AS, Ohmes AK, Rhonemus TA, Tyler CA, inventors; Wayzata (MN): Cargill, Incorporated, assignee (2012). *Crystalline Forms of Rebaudioside B*. International Patent Application WO/2012/082493 A1 [June 21, 2012].

Gardana C, Simonetti P, Canzi E, Zanchi R, Pietta P (2003). Metabolism of stevioside and rebaudioside A from Stevia rebaudiana extracts by human microflora. J Agric Food Chem 51(22):6618-6622.

Gardana C, Scaglianti M, Simonetti P (2010). Evaluation of steviol and its glycosides in *Stevia rebaudiana* leaves and commercial sweetener by ultra-high-performance liquid chromatography-mass spectrometry. J Chromatogr A 1217(9):1463-1470.

Geuns JMC (2003). Stevioside. Phytochemistry 64(5): 913-921.

Geuns JMC, Pietta, P (2004) [unpublished]. *Stevioside metabolism by human volunteers*. Report from Segrate (MI), Italy: Laboratory Functional Biology, Kuleuven, Leuven Belgium and ITB-CNR. Submitted to WHO by Belgium: Federal Ministry of Social Affairs, Public Health and the Environment. Cited In: JECFA, 2006.

Geuns JM, Augustijns P, Mols R, Buyse JG, Driessen B (2003). Metabolism of stevioside in pigs and intestinal absorption characteristics of stevioside, rebaudioside A and steviol. Food Chem Toxicol 41(11):1599-1607.

Geuns JMC, Buyse J, Vankeirsbilck A, Temme EHM, Compernolle F, Toppet S (2006). Identification of steviol glucuronide in human urine. J Agric Food Chem 54(7):2794-2798.

Geuns JMC, Buyse J, Vankeirsbilck A, Temme EHM (2007). Metabolism of stevioside by healthy subjects. Exp Biol Med 232(1):164-173.

Health Canada (2012a). *Information and Consultation Document on Health Canada's Proposal to Allow the Use of the Food Additive Steviol Glycosides as a Table-Top Sweetener and as a Sweetener in Certain Food Categories.* Ottawa (ON): Health Canada, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch. Available at: http://www.hc-sc.gc.ca/fn-an/consult/steviol/document-consultation-eng.php [Date Modified: 2012-11-30].

Health Canada (2012b). *Notice of Modification to the Lists of Permitted Food Additives to Enable the Use of Steviol Glycosides as a Table-Top Sweetener and as a Sweetener in Certain Food Categories*. Ottawa (ON): Health Canada, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch. Available at: http://www.hc-sc.gc.ca/fn-an/consult/nom-adm-0002/index-eng.php [Date Modified: 2012-11-30].

Health Canada (2016). *Notice of Modification to the List of Permitted Sweeteners to Enable the Use of Rebaudioside M as a Sweetener in Various Unstandardized Foods*. (Reference Number: NOM/ADM-0065). Ottawa (ON): Health Canada, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch. Available at: http://www.hc-sc.gc.ca/fn-an/consult/nom-adm-0065/index-eng.php [Date Modified: 2016-01-15].

Hutapea AM, Toskulkao C, Buddhasukh D, Wilairat P, Glinsukon T (1997). Digestion of stevioside, a natural sweetener, by various digestive enzymes. J Clin Biochem Nutr 23(3):177-186.

Ibrahim MA, Rodenburg DL, Alves K, Perera WH, Fronczek FR, Bowling J et al. (2016). Rebaudiosides R and S, minor diterpene glycosides from the leaves of *Stevia rebaudiana*. J Nat Prod [Epub ahead of print-Apr. 27, 2016].

Japan Food Chemical Research Foundation (2014). *List of Existing Food Additives* [Complied and published by the Ministry of Health and Welfare on April 16, 1996]. Tokyo, Japan: Japan Food Chemical Research Foundation. Available at: http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/list-exst.add [Effective from January 30, 2014, Last update: 06/30/2014].

JECFA (1999). Stevioside. In: *Safety Evaluation of Certain Food Additives*. 51st Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 9-18, 1998. (WHO Food Additives Series, no 42). Geneva, Switz.: World Health Organization (WHO) / International Programme on Chemical Safety (IPCS), pp. 119-143. Available at: http://www.inchem.org/documents/jecfa/jecmono/v042je07.htm.

JECFA (2005). Steviol glycosides. In: Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 8-17, 2004, Geneva. Geneva: World Health Organization (WHO); 34-39, 138. (WHO Technical Report Series, no. 928; http://whqlibdoc.who.int/trs/WHO_TRS_928.pdf)

JECFA (2006). Steviol glycosides. In: *Safety Evaluation of Certain Food Additives*. Sixty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives, June 8-17, 2004, Geneva, Switz. (WHO Food Additives Series, no 54). Geneva, Switz.: World Health Organization (WHO) / International Programme on Chemical Safety (IPCS), pp. 117-144, 638. Available at: http://whqlibdoc.who.int/publications/2006/9241660546_eng.pdf.

JECFA (2007a). Steviol glycosides [Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding tentative specifications prepared at the 63rd JECFA (2004)]. In: *Combined Compendium of Food Additive Specifications [Online Edition]. General Specifications for Enzymes Analytical Methods, Volume 4: Analytical Methods, Test Procedures and Laboratory Solutions Used by and Referenced in the Food Specifications*. 68th Meeting, June 19-28, 2007, Geneva, Switz. (FAO JECFA Monographs 4). Rome, Italy: Food and Agriculture Organization of the United Nations. Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA), pp. 61-64. Available at: ftp://ftp.fao.org/docrep/fao/010/a1447e/a1447e.pdf.

JECFA (2007b). Steviol glycosides. In: *68th JECFA - Chemical and Technical Assessment (CTA)*. [Sixty-eighth meeting held June 17-26, 2008]. (Revised by Paul M. Kuznesof, Ph.D., Original prepared by Harriet Wallin for the 63rd JECFA). Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/68/Steviol_glycosides.pdf.

JECFA (2007c). 3.1.9 Steviol glycosides. *Evaluation of Certain Food Additives and Contaminants*. Sixty-eighth Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 19-28, 2007, Geneva, Switz. (WHO Technical Report Series, no 947). Geneva, Switz.: World Health Organization (WHO), pp. 50-54, 206, 215. Available at: http://whqlibdoc.who.int/publications/2007/9789241209472_eng.pdf.

JECFA (2009). Steviol glycosides (addendum). In: *Safety Evaluation of Certain Food Additives and Contaminants*. Sixty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 17-26-29, 2008, Geneva, Switz. (WHO Food Additives Series, vol 60). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO), pp. 183-219. Available at: http://whqlibdoc.who.int/publications/2009/9789241660600_eng.pdf.

JECFA (2010). Steviol glycosides [Prepared at the 73rd JECFA, 2010) and published in FAO JECFA Monographs 10, 2010)]. In: *Combined Compendium of Food Additive Specifications*. (FAO JECFA Monographs 10). 73rd Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 8-17, 2010, Geneva, Switz. Food and Agriculture Organization of the United Nations (FAO), Joint FAO/WHO Expert Committee on Food Additives (JECFA), Rome, Italy. Available at: http://www.fao.org/ag/agn/jecfa-additives/specs/monograph10/additive-442-m10.pdf.

Jensen NS, Canale-Parola E (1985). Nutritionally limited pectinolytic bacteria from the human intestine. Appl Environ Microbiol 50(1):172-173.

Kennelly EJ (2002). Sweet and non-sweet constituents of *Stevia rebaudiana*. In: Kinghorn AD, editor. *Stevia: the Genus Stevia*. (Medicinal and Aromatic Plants—Industrial Profiles, vol 19). London, UK/New York (NY): Taylor and Francis, pp. 68-85.

Kinghorn AD, Kim N-C, Kim DSHL (1999). Terpenoid glycoside sweeteners (Chapter 12). In: Ikan R, editor. *Naturally Occurring Glycosides*. Chicago (IL): Wiley & Sons Ltd., pp. 399-429.

Kobayashi M, Horikawa SH, Degrandi I, Ueno J, Mitsuhashi H (1977). Dulcosides A and B, new diterpene glycosides from *Stevia rebaudiana*. Phytochemistry 16(9):1405-1408.

Kohda H, Kasai R, Yamasaki K, Murakami K, Tanaka O (1976). New sweet diterpene glucosides from *Stevia rebaudiana*. Phytochemistry 15(6):981-983.

Koyama E, Kitazawa K, Ohori Y, Izawa O, Kakegawa K, Fujino A et al. (2003a). In vitro metabolism of the glycosidic sweeteners, stevia mixture and enzymatically modified stevia in human intestinal microflora. Food Chem Toxicol 41(3):359-374.

Koyama E, Sakai N, Ohori Y, Kitazawa K, Izawa O, Kakegawa K et al. (2003b). Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglycone, steviol, in rats and humans. Food Chem Toxicol 41(6):875-883.

Kraemer T, Maurer HH (1994). On the metabolism of the sweetener stevioside in humans. Eur J Pharm Sci 2(1/2):103 [abstract FC12].

Kroyer GT (1999). The low calorie sweetener stevioside: stability and interaction with food ingredients. Lebensm Wiss Technol 32(8):509-512.

Kwok D (2015) [unpublished]. *Pilot In vitro Metabolism of RAF in Male and Female Pooled Human Intestinal Fecal Homogenate under Physiological Anaerobic Conditions [Confidential]*. (BRI Study No.: PUR-2015-002). Prepared by Vancouver (BC): BRI Biopharmaceutical Research Inc. for Negeri Sembilan, Malaysia: PureCircle Ltd.

Maki KC, Curry LL, Reeves MS, Toth PD, McKenney JM, Farmer MV et al. (2008a). Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with type 2 diabetes mellitus. Food Chem Toxicol 46(7, Suppl.):S47-S53.

Maki KC, Curry LL, Carakostas MC, Tarka SM, Reeves MS, Farmer MV et al. (2008b). The hemodynamic effects of rebaudioside A in healthy adults with normal and low-normal blood pressure. Food Chem Toxicol 46(7, Suppl.):S40-S46.

Marie S (1991). Sweeteners. In: Smith J, editor. *Food Additive User's Handbook*. Glasgow, Scotland: Blackie / New York (NY): AVI, pp. 47-74.

Markosyan A, inventor (2013). *Stevia Composition*. US Patent Application US 2013/0324621 A1 [Dec. 5, 2013].

MOHFW (2015). [Steviol]. In: *The Gazette of India, REGD. NO. D. L.-33004/99*. (No. 371, Extraordinary, Part III, Section 4). New Delhi, India: Ministry of Health & Family Welfare (MOHFW), Food Safety and Standards Authority of India (FSSAI). Available at: http://www.fssai.gov.in/Portals/0/Pdf/Gazette_Notification_Steviol.pdf.

Nakayama K, Kasahara D, Yamamoto F (1986). Absorption, distribution, metabolism and excretion of stevioside in rats. Shokuhin Eiseigaku Zasshi [J Food Hyg Soc Jpn] 27(1):1-8.

Nikiforov AI, Eapen AK (2008). A 90-day oral (dietary) toxicity study of rebaudioside A in Sprague-Dawley rats. Int J Toxicol 27(1):65-80.

Nikiforov AI, Rihner MO, Eapen AK, Thomas JA (2013). Metabolism and toxicity studies supporting the safety of rebaudioside D. Int J Toxicol 32(4):261-273.

Ohta M, Sasa S, Inoue A, Tamai T, Fujita I, Morita K et al. (2010). Characterization of novel steviol glycosides from leaves of Stevia rebaudiana Morita. J Appl Glycosci 57(3):199-209.

Prakash I, Dubois GE, Clos JF, Wilkens KL, Fosdick LE (2008). Development of rebiana, a natural, non-caloric sweetener. Food Chem Toxicol 46(7, Suppl.):S75-S82.

Purkayastha S, Pugh G, Lynch B, Roberts A, Kwok D, Tarka SM (2014). *In vitro* metabolism of rebaudioside B, D, and M under anaerobic conditions: comparison with rebaudioside A. Regul Toxicol Pharmacol 68(2):259-268.

Purkayastha S, Bhusari S, Pugh G, Jr., Teng X, Kwok D, Tarka SM (2015). *In vitro* metabolism of rebaudioside E under anaerobic conditions: comparison with rebaudioside A. Regul Toxicol Pharmacol 72(3):646-657.

Purkayastha S, Markosyan A, Prakash I, Bhusari S, Pugh G Jr, Lynch B, Roberts A (2016). Steviol glycosides in purified stevia leaf extract sharing the same metabolic fate. Regul Toxicol Pharmacol 77:125-133.

Renwick AG (2007). Toxicokinetics [section on elimination: excretion via the gut]. In: Hayes W, editor. *Principles and Methods of Toxicology* (5th edition). Philadelphia (PA): Taylor and Francis/CRC Press, p. 188.

Renwick AG, Tarka SM (2008). Microbial hydrolysis of steviol glycosides. Food Chem Toxicol 46(7, Suppl.):S70-S74.

Roberts A, Renwick AG (2008). Comparative toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol in rats. Food Chem Toxicol 46(7, Suppl.):S31-S39.

Sakamoto I, Yamasaki K, Tanaka O (1977a). Application of ^{13}C NMR spectroscopy to chemistry of plant glycosides: rebaudiosides D and E, new sweet diterpene glucosides of *Stevia rebaudiana* Bertoni. Chem Pharm Bull 25(12):3437-3439.

Sakamoto I, Yamasaki K, Tanaka O (1977b). Application of ^{13}C NMR spectroscopy to chemistry of natural glycosides: rebaudioside-C, a new sweet diterpene glycoside of *Stevia rebaudiana*. Chem Pharm Bull 25(4):844-846.

SCF (1985). Reports of the Scientific Committee for Food concerning sweeteners (Opinion expressed by the SCF on 14 September 1984) [Stevioside]. In: *Food Science and Techniques*. (Reports of the Scientific Committee for Food (16th series; EUR 10210 EN). Brussels, Belgium: Commission of the European Communities (EEC), Health & Consumer Protection Directorate-General, Scientific Committee for Food (SCF). Available at: http://aei.pitt.edu/40825/1/16th_food.pdf.

SCF (1999). *Opinion on Stevioside as a Sweetener (Adopted on 17/6/99)*. (CS/ADD/EDUL/167 Final). Brussels, Belgium: European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food (SCF). Available at: http://ec.europa.eu/food/fs/sc/scf/out34_en.pdf.

Simonetti P, Gardana C, Bramati L, Pietta PG (2004). Bioavailability of stevioside from Stevia rebaudiana in humans: preliminary report. In: Geuns JMC, Buyse J, editors. *Safety of Stevioside*: Proceedings of the First Symposium Sponsored by KULeuven, April 16, 2004, Leuven, Belgium. Heverlee, Belgium: Euprint ed., pp. 51-62.

Starratt AN, Kirby CW, Pocs R, Brandle JE (2002). Rebaudioside F, a diterpene glycoside from Stevia rebaudiana. Phytochemistry 59(4):367-370.

Sung LH (2002) [unpublished]. *Unpublished report on pharmacokinetic (PK) studies of T100 sunstevia 95% stevioside in rats*. Unpublished report from Singapore, Singapore: Sunlabel Pte Ltd. Submitted to Geneva, Switz.: World Health Organization (WHO) by Japan: Ministry of Health and Welfare, Japan. Cited In: JECFA, 2006.

Toyoda K, Matsui H, Shoda T, Uneyama C, Takada K, Takahashi M (1997). Assessment of the carcinogenicity of stevioside in F344 rats. Food Chem Toxicol 35(6):597-603.

U.S. FDA (2008a). *Agency Response Letter GRAS Notice No. GRN 000252 [Rebaudioside A purified from Stevia Rebaudiana (Bertoni) Bertoni]*. Submitted by Chicago (IL): Whole Earth Sweetener Company LLC (a wholly owned subsidiary of Merisant Company) to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=252 [December 17, 2008].

U.S. FDA (2008b). *Agency Response Letter GRAS Notice No. GRN 000253 [Rebaudioside A purified from Stevia Rebaudiana (Bertoni) Bertoni]*. Submitted by Wayazata (ST): Cargill, Inc. to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=253 [December 17, 2008].

U.S. FDA (2009a). *Agency Response Letter GRAS Notice No. GRN 000275 [Purified Steviol Glycosides With Rebaudioside A as the Principal Component]*. Submitted by Fort Washington (PA): McNeil Nutritionals, LLC to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=275 [June 11, 2009].

U.S. FDA (2009b). *Agency Response Letter GRAS Notice No. GRN 000278 [Rebaudioside A Purified From Stevia Rebaudiana (Bertoni) Bertoni]*. Submitted by Rancho Santa Margarita (CA): Blue California to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=278 [July 20, 2009].

U.S. FDA (2009c). *Agency Response Letter GRAS Notice No. GRN 000282 [Rebaudioside A Purified From the Leaves of Stevia Rebaudiana (Bertoni) Bertoni]*. Submitted by Bellingham (WA): Sweet Green Fields, LLC to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=282 [August 11, 2009].

U.S. FDA (2009d). *Agency Response Letter GRAS Notice No. GRN 000287 [Purified Steviol Glycosides With Rebaudioside A and Stevioside As the Principal Components]*. Submitted by Gilbert (AZ): Wisdom Natural Brands to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=287 [August 28, 2009].

U.S. FDA (2010a). *Agency Response Letter GRAS Notice No. GRN 000303 [Rebaudioside A purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni]*. Submitted by Frisco (TX) / Erlanger (KY): Sunwin USA, LLC / WILD Flavors to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=303 [March 22, 2010].

U.S. FDA (2010b). *Agency Response Letter GRAS Notice No. GRN 000304 [Purified steviol glycosides with rebaudioside A and stevioside as the principal components]*. Submitted by Submitted by Frisco (TX) / Erlanger (KY): Sunwin USA, LLC / WILD Flavors to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=304 [March 22, 2010].

U.S. FDA (2010c). *Agency Response Letter GRAS Notice No. GRN 000318 [Rebaudioside A purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni]*. Submitted by Naples (FL): Pyure Brands, LLC to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=318 [May 15, 2010].

U.S. FDA (2010d). *Agency Response Letter GRAS Notice No. GRN 000323 [Purified steviol glycosides with rebaudioside A and stevioside as the principal components]*. Submitted by Oak Brook (IL): PureCircle USA, Inc. to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=323 [July 9, 2010].

U.S. FDA (2010e). *Agency Response Letter GRAS Notice No. GRN 000329 [Rebaudioside A purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni]*. Submitted by Vancouver (BC): GLG Life Tech, Ltd. to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=329 [September 9, 2010].

U.S. FDA (2011a). *Agency Response Letter GRAS Notice No. GRN 000337 [Enzyme-modified steviol glycosides preparation (EMSGP)].* Submitted by Bloomingdale (IL): NOW Foods to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=337 [June 17, 2011].

U.S. FDA (2011b). *Agency Response Letter GRAS Notice No. GRN 000348 [Stevioside purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni (stevioside)]*. Submitted by Vancouver (BC): GLG Life Tech, Ltd. to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=348 [July 14, 2011].

U.S. FDA (2011c). *Agency Response Letter GRAS Notice No. GRN 000349 [Purified steviol glycosides with rebaudioside A and stevioside as the principal components]*. Submitted by Vancouver (BC): GLG Life Tech, Ltd. to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=349 [July 14, 2011].

U.S. FDA (2011d). *Agency Response Letter GRAS Notice No. GRN 000354 [Rebaudioside A purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni (rebaudioside A)]*. Submitted by Guilin, China: Guilin Layn Natural Ingredients, Corp. to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=354 [July 15, 2011].

U.S. FDA (2011e). *Agency Response Letter GRAS Notice No. GRN 000365 [Rebaudioside A purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni (rebaudioside A)].* Submitted by El Dorado Hills (CA): BrazTek International Inc., d/b/a Rio Natural) to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=365 [August 18, 2011].

U.S. FDA (2011f). *Agency Response Letter GRAS Notice No. GRN 000367 [Purified steviol glycosides with rebaudioside A and stevioside as the principal components]*. Submitted Quingdao, China: Sinochem Qingdao Co., Ltd. to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=367 [July 8, 2011].

U.S. FDA (2011g). *Agency Response Letter GRAS Notice No. GRN 000369 [Rebaudioside A purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni].* Submitted by Piscataway (NJ): Zhucheng Haotian Pharm Co., Ltd., C/O Shanghai Freemen Americas, LLC to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=369 [October 11, 2011].

U.S. FDA (2011h). *Agency Response Letter GRAS Notice No. GRN 000375 [Enzyme modified steviol glycosides, Tokyo, Japan: Toyo Sugar Refining Co., Ltd. / Tokyo, Japan: Nippon Paper Chemicals Co., Ltd.].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=375 [September 2, 2011].

U.S. FDA (2011i). *Agency Response Letter GRAS Notice No. GRN 000380 [Rebaudioside A purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni (rebaudioside A)].* Submitted by Vancouver (BC): GLG Life Tech Corporation to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=380 [November 28, 2011].

U.S. FDA (2012a). *Agency Response Letter GRAS Notice No. GRN 000388 [Rebaudioside A purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni (rebaudioside A)].* Submitted by Chengdu, Sichuan, China: Chengdu Wagott Pharmaceutical to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=388 [January 9, 2012].

U.S. FDA (2012b). *Agency Response Letter GRAS Notice No. GRN 000389 [Steviol glycosides with stevioside as the principal component].* Submitted by Chengdu, Sichuan, China: Chengdu Wagott Pharmaceutical to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=389 [January 18, 2012].

U.S. FDA (2012c). *Agency Response Letter GRAS Notice No. GRN 000393 [Rebaudioside A purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni (rebaudioside A)].* Submitted by Republic of South Korea: Daepyung Co., Ltd., to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=393 [January 23, 2012].

U.S. FDA (2012d). *Agency Response Letter GRAS Notice No. GRN 000395 [Steviol glycosides with rebaudioside A and stevioside as the principal components].* Submitted by Republic of South Korea: Daepyung Co., Ltd., to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=395 [January 24, 2012].

U.S. FDA (2012e). *Agency Response Letter GRAS Notice No. GRN 000418 [Rebaudioside A purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni (rebaudioside A)].* Submitted by City of Industry (CA): MiniStar International, Inc., to Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=418 [June 7, 2012].

U.S. FDA (2013a). *Agency Response Letter GRAS Notice No. GRN 000473 [Purified steviol glycosides with rebaudioside X as the principal component].* Submitted by Oak Brook (IL): PureCircle Ltd., to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=473 [December 2, 2013].

U.S. FDA (2013b). *Agency Response Letter GRAS Notice No. GRN 000448 [Enzyme-modified steviol glycosides].* Submitted by Republic of South Korea: Daepyung Co., Ltd., to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=448 [May 3, 2013].

U.S. FDA (2013c). *Agency Response Letter GRAS Notice No. GRN 000452 [Enzyme-modified steviol glycosides].* Submitted by Republic of South Korea: Daepyung Co., Ltd., to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=452 [July 1, 2013].

U.S. FDA (2013d). *Agency Response Letter GRAS Notice No. GRN 000456 [Rebaudioside D purified from the leaves of Stevia rebaudiana (Bertoni) Bertoni (rebaudioside D)].* Submitted by Oak Brook (IL): PureCircle USA, Inc., to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=456 [July 1, 2013].

U.S. FDA (2013e). *Agency Response Letter GRAS Notice No. GRN 000461* [Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside A)]. Submitted by Bangkok, Thailand: Almendra Limited to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=461 [August 14, 2013].

U.S. FDA (2013f). *Agency Response Letter GRAS Notice No. GRN 000467 [*Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni]. Submitted by Shangdong, China: Qufu Xiangzhou Stevia Products Co., Ltd., to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=467 [November 25, 2013].

U.S. FDA (2014a). *Agency Response Letter GRAS Notice No. 000493 [High purity steviol glycosides (minimum purity 95%)].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=493 [Date of filing: Dec. 23, 2013; Date of closure: May 30, 2014].

U.S. FDA (2014b). *Agency Response Letter GRAS Notice No. 000512 [High purity Rebaudioside M].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=512 [Date of filing: Apr. 28, 2014; Date of closure: Oct. 24, 2014].

U.S. FDA (2014c). *Agency Response Letter GRAS Notice No. 000516 [Steviol glycosides with rebaudioside A and stevioside as the principal components].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=516 [Date of filing: May 9, 2014; Date of closure: Oct. 30, 2014].

U.S. FDA (2015a). *Agency Response Letter GRAS Notice No. 000536 [High purity rebaudioside C].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=536 [Date of filing: Aug. 18, 2014; Date of closure: Feb. 10, 2015].

U.S. FDA (2015b). *Agency Response Letter GRAS Notice No. 000548 [High purity rebaudioside D].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=548 [Date of filing: Oct. 27, 2014; Date of closure: Apr. 20, 2015].

U.S. FDA (2015c). *Agency Response Letter GRAS Notice No. 000555 [High purity steviol glycosides (minimum purity 95%) consisting primarily of rebaudioside A].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=555 [Date of filing: Dec. 23, 2014; Date of closure: Apr. 20, 2015].

U.S. FDA (2015d). *GRAS Notice No. GRN 000607 [Glucosylated steviol glycosides (minimum purity 80%), Oak Brook (IL): PureCircle Limited]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=607 [Date of filing: Nov 24, 2015; Status of March 15, 2016: Pending].

U.S. FDA (2016a). *GRAS Notice No. GRN 000619 [Purified steviol glycosides with rebaudioside A and stevioside as the principal components, Oak Brook (IL): PureCircle Ltd.].* Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=619 [Date of filing: Feb 2, 2016; Date of closure / FDA's Letter: Pending].

U.S. FDA (2016b). *GRAS Notice No. GRN 000626 [Steviol glycosides produced in Saccharomyces cerevisiae, Wayzata (MN): Cargill, Inc.].* Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=626 [Date of filing: Mar 1, 2016; Date of closure / FDA's Letter: Pending].

U.S. FDA (2016c). *GRAS Notice No. GRN 000632 [Rebaudioside A from Yarrowia lipolytica, Parsippany (NJ): DSM Nutritional Products, LLC].* Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=632 [Date of filing: Mar 18, 2016; Date of closure / FDA's Letter: Pending].

Wallin H (2008). *Steviol Glycosides: Chemical and Technical Assessment (CTA)*. Revised for the 69 [h] Joint FAO/WHO Expert Committee on Food Additives (JECFA) Meeting. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), Food Quality and Standards Service (AGNS). Available at: http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/69/Steviol_glycosides.pdf

Wang LZ, Goh BC, Fan L, Lee HS (2004). Sensitive high-performance liquid chromatography/ mass spectrometry method for determination of steviol in rat plasma. Rapid Commun Mass Spectrom 18(1):83-86.

Wheeler A, Boileau AC, Winkler PC, Compton JC, Prakash I, Jiang X et al. (2008). Pharmacokinetics of rebaudioside A and stevioside after single oral doses in healthy men. Food Chem Toxicol 46(7, Suppl.):S54-S60.

Williams LD, Burdock GA (2009). Genotoxicity studies on a high-purity rebaudioside A preparation. Food Chem Toxicol 47(8):1831-1836.

Wingard RE Jr, Brown JP, Enderlin FE, Dale JA, Hale RL, Seitz CT (1980). Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. Experientia 36(5):519-520.

Wölwer-Rieck U, Tomberg W, Wawrzun A (2010). Investigations on the stability of stevioside and rebaudioside A in soft drinks. J Agric Food Chem 58(23):12216-12220.

Wu C, Venkataraman SK, Nettles BJ, Jaksch F, Rodenburg DL, Alves KM et al. (2012). New glycosides from Stevia rebaudiana. Planta Med 78(11):PJ95 [abstract].

Yamada A, Ohgaki S, Noda T, Shimizu M (1985). Chronic toxicity study of dietary stevia extracts in F344 rats. Shokuhin Eiseigaku Zasshi [J Food Hyg Soc Jpn] 26(2):169-183.