GRAS ASSOCIATES, LLC

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January 30, 2020

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5001 Campus Drive
College Park, MD 20740

Attention: Dr. Susan Carlson
Re: GRAS Notification – *Rebaudioside I*

Dear Dr. Carlson:

GRAS Associates, LLC, acting as the Agent for Blue California, is submitting for FDA review Form 3667 and the enclosed CD, free of viruses, containing a GRAS Notification for *Rebaudioside I*. Along with Blue California's determination of safety, an Expert Panel of qualified persons was assembled to assess the composite safety information of the subject substance with the intended use as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into food in general, other than infant formulas and meat and poultry products. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

William J. Rowe, President
Agent for Blue California
GRAS Associates, LLC
11810 Grand Park Ave
Suite 500
North Bethesda, MD 20852
wrowe@nutrasource.ca

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FOOD ADDITIVE SAFETY

Enclosure: GRAS Notification for Blue California – *Rebaudioside I*

# GRAS Notification

of

## Rebaudioside I

**Food Usage Conditions for General Recognition of Safety**

**on behalf of**

## Blue California

**30111 Tomas**
**Rancho Santa Margarita, CA 92688**

**1/30/20**

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## FOREWORD

Blue California based our Generally Recognized as Safe (GRAS) assessment of the steviol glycoside, Rebaudioside I, primarily on the composite safety information, i.e., scientific procedures with corroboration from history of use. The safety/toxicity of steviol glycosides, history of use of steviol glycosides, and compositional details, specifications, and method of preparation of the subject ingredient were reviewed. In addition, a search of the scientific and regulatory literature was conducted through January 14, 2020, with particular attention paid to adverse reports, as well as those that supported conclusions of safety. Those references that were deemed pertinent to this review are listed in Part 7. The composite safety/toxicity studies, in concert with dietary exposure information, ultimately provide the specific scientific foundation for the GRAS conclusion.

At Blue California's request, GRAS Associates, LLC ("GA") convened an Expert Panel to complete an independent safety evaluation of Blue California's high purity Rebaudioside I (≥95% Reb I) product. Blue California's high purity Rebaudioside I preparation is synthesized from purified *Stevia rebaudiana* extract by a genetically modified yeast and is purified to yield a ≥95% Rebaudioside I finished product. The purpose of the evaluation is to ascertain whether Blue California's Rebaudioside I is generally recognized as safe, i.e., GRAS, under the intended conditions of use. In addition, Blue California has asked GA to act as Agent for the submission of this GRAS notice.

## PART 1. SIGNED STATEMENTS AND CERTIFICATION

### A. Claim of Exclusion from the Requirement for Premarket Approval Pursuant to 21 CFR 170 Subpart E[1]

Blue California has concluded that our high purity Rebaudioside I, referred to as "Reb I" and "BESTEVIA® Rebaudioside I," and which meets the specifications described below, is GRAS in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act (FD&C). This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the following sections. The evaluation accurately reflects the intended conditions of food use for the designated high purity Rebaudioside I (≥ 95%) preparation.

Signed:

Agent for Blue California
William J. Rowe Date: 1/30/2020

[1] See 81 FR 54960, 17 August 2016. Accessible at: https://www.gpo.gov/fdsys/pkg/FR-2016-08-17/pdf/2016-19164.pdf (Accessed 8/23/19)

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GRAS Associates, LLC
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North Bethesda, MD 20852

## B. Name and Address of Responsible Parties

Blue California
30111 Tomas
Rancho Santa Margarita, CA 92688

As the Responsible Party, Blue California accepts responsibility for the GRAS conclusion that has been made for our high purity Rebaudioside I (≥ 95%) preparation, BESTEVIA® Rebaudioside I, as described in the subject safety evaluation; consequently, the purified steviol glycosides preparations having acceptable steviol glycosides compositions which meet the conditions described herein, are not subject to premarket approval requirements for food ingredients.

## C. Common Name and Identity of Notified Substance

The common name of the ingredient to be used on food labels is "high purity Rebaudioside I", which can be also be abbreviated as "Reb I" or "reb I." Blue California also plans to market our high purity Rebaudioside I preparations under the trade name "BESTEVIA® Rebaudioside I."

## D. Conditions of Intended Use in Food

Blue California's BESTEVIA® Rebaudioside I (≥ 95%) preparation is intended for use as a general-purpose sweetener in foods, excluding meat and poultry products and infant formulas, at levels determined by current good manufacturing practices (CGMP).

## E. Basis for GRAS Conclusion

Pursuant to 21 CFR 170.30(a) and (b)[2], Blue California's BESTEVIA® Rebaudioside I (≥ 95%) preparation has been concluded to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

Purified steviol glycosides are not subject to premarket approval requirements of the FD&C Act based on Blue California's conclusion that the substance is GRAS under the conditions of its intended food use.

Blue California certifies, to the best of our knowledge, that this GRAS notice is a complete, representative, and balanced assessment that includes all relevant information, both favorable and

[2] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=170.30 (Accessed 8/21/19)

unfavorable, available and pertinent to the evaluation of the safety and GRAS status of high purity glucosylated steviol glycosides.

### F. Availability of Information

The data and information that serve as the bases for this GRAS notice will be maintained at the offices of Blue California, located at 30111 Tomas, Rancho Santa Margarita, CA 92688, and will be made available during customary business hours.

Blue California certifies that no data or information contained herein are exempt from disclosure under the Freedom of Information Act (FOIA). No non-public, safety-related data were used by the Expert Panel to reach a GRAS conclusion.

## PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

### A. Chemical Identity of Ingredient

"Rebaudioside I" is the common or usual name of the non-nutritive sweetener derived from an extract of *Stevia rebaudiana* Bertoni by genetically modified yeast. The compositional features of the BESTEVIA® Rebaudioside I are described in more detail in this section. "Rebaudioside I," "Reb I," and "reb I" are the terms used by Blue California in referring to the notified substance. The preparation is also marketed as "BESTEVIA® Rebaudioside I."

The general chemistry of steviol glycosides and enzyme modified steviol glycosides has previously been reviewed in a number of GRAS Notices, including those previously submitted by Blue California, specifically GRN 667 (Blue California, 2016), GRN 715 (Blue California, 2017), and GRN 823 (Blue California, 2018).

No known toxins have been identified in stevia or stevia-derived products.

### 1. Chemistry of Rebaudioside I

Rebaudioside I is a minor, naturally occurring steviol glycoside first obtained by Ohta et al. (2010) from the leaves of *Stevia rebaudiana* Morita, which is a selectively-bred cultivar of *Stevia rebaudiana* Bertoni that produces a higher ratio of Rebaudioside A to stevioside than *Stevia rebaudiana* Bertoni. Ohta et al. (2010) reported that Rebaudioside I is present at 0.1% relative to other steviol glycosides detected in *S. rebaudiana* Morita leaves, but no Rebaudioside I was detected in *S. rebaudiana* Bertoni using a high performance liquid chromatography-UV absorbance analysis method.

Subsequently, Prakash et al. (2014) reported that Rebaudioside A can be converted to Rebaudioside I using UDP-glucosyltransferase (UGT) enzymes expressed in *E. coli*.

Similar to the other steviol glycosides, Reb I is an *ent*-kaurane diterpene glycoside with a steviol backbone. Like many of the other naturally occurring steviol glycosides, Reb I is a member of the glucosyl steviol family, which contains only steviol and glucose residues. As with Rebaudioside D, Reb I is composed of steviol and five glucose moieties (Purkayastha et al., 2016).

Rebaudioside I is included in a list of steviol glycosides from *Stevia rebaudiana* Bertoni in Appendix 1 in the 2017 JECFA monograph (FAO, 2017).

**Chemical name:** (4α13-[(O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl ester

**Synonyms:** Rebaudioside I, Reb I, reb I

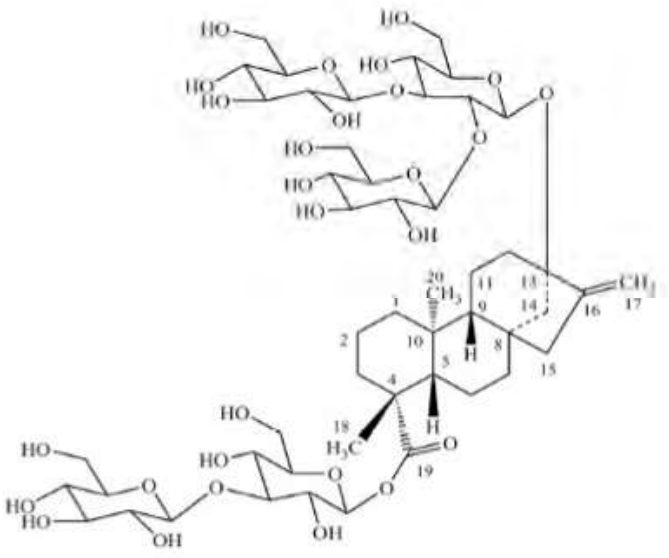
**Chemical formula:** $C_{50}H_{80}O_{28}$

**Molecular weight (MW):** 1129.15

**CAS Number:** 1220616-34-1

The chemical structure of Rebaudioside I, as reported by Prakash et al. (2014), is presented in Figure 1.

**Figure 1. Chemical Structure of Rebaudioside I**



From Prakash et al. (2014)

## 2. Chemistry of the Yeast Vector

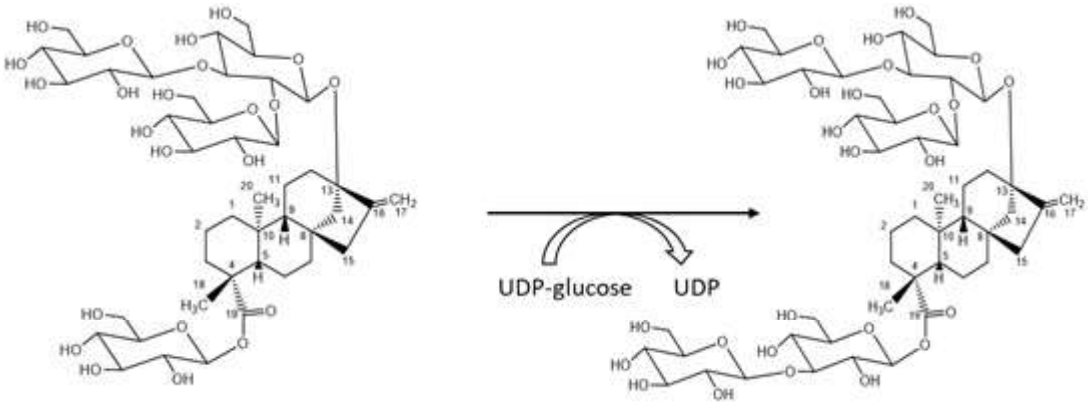
Blue California's manufacturing process for its high purity Reb I preparation uses 5'-diphosphouridine-glucosyltransferase (UGT) enzymes to facilitate catalytic bio-conversion in a process similar to that previously described in GRN 667 for the production of rebaudioside M (Blue California, 2016), GRN 715 for the production of Rebaudioside D (Blue California, 2017), and GRN 823 for the production of Rebaudioside E (Blue California, 2018). The enzymes are produced by a nonpathogenic and nontoxigenic strain of wild-type *Pichia pastoris* from the Saccharomycetaceae family. This strain was originally isolated from harvested plant material, cultured, and studied extensively by other groups, and it has a history of use in food production. It is commonly found in a variety of food products, including cheese and wine.

*P. pastoris* is a unicellular yeast that is widely used in the biotechnology industry. It can be commonly found in nature, and it can grow in a simple, inexpensive medium. Its morphology, physiology, and growth conditions have been widely studied and reported.

The parental strain used by Blue California is closely related to *P. pastoris* ATCC 20864. It is converted to production strains by site-specific DNA integration. A list of relevant references is provided in Appendix 1.

Rebaudioside I is produced from Rebaudioside A via 1,3-19-O-glucose glycosylation, where a sugar moiety is transferred from uridine diphosphate-glucose (UDPG) to the C-3' position of the 19-O-glucose moiety of Rebaudioside A using a mutant of the UGT glycosylation enzyme, UGT76G1. Sucrose synthases (SUS) catalyze the conversion of uridine diphosphate (UDP) to UDPG in the presence of sucrose. Thus, for a glycosylation reaction catalyzed by UGT enzymes, SUS can be used to regenerate UDPG, thereby enhancing the efficiency of such a reaction (Figure 2). For the yeast strain, Blue California transformed the specific UGT and SUS enzymes into yeast cells.

**Figure 2. Biosynthesis Pathway from Rebaudioside A to Rebaudioside I**



## B. Manufacturing Processes

Blue California manufactures high purity rebaudioside I in a process similar to that described for Rebaudioside M in GRN 667 (Blue California, 2016), Rebaudioside D in GRN 715 (Blue California, 2017), and Rebaudioside E in GRN 823 (Blue California, 2018). The multi-step biosynthesis pathway process to manufacture BESTEVIA® Rebaudioside I uses a strain of *P. pastoris* yeast that contains the UGT enzyme, which facilitates the transfer of glucose to small molecules *via* glycosidic bonds.

### 1. Catalytic Bioconversion Process

To produce the enzymes used in the bio-conversion, the glycerol stock of Yeast Cell (carrying the UGT and SUS genes) is removed from the -70°C freezer, thawed to room temperature, and grown in 50 mL yeast culture seed media. After 12 hours, the growing Seed Culture 1 is transferred to 2-L yeast culture seed media as Seed Culture 2. When the cells[3] read $OD_{600}$ = 10, they are transferred to 500-L fermenters. This level 3 Seed Culture is then transferred to a 60-ton production fermenter.

The yeast cells are cultured for 48 hours as described in Blue California's published patent (Mao et al., 2016). After confirming their catalytic activity in a small shaking flask, Yeast Cells are harvested by centrifugation. The cells are then passed through a homogenizer to release enzymes. The enzymes are separated by another centrifugation step and are resuspended in a reaction buffer. For the catalytic reaction needed to convert stevia extract to Reb I, the enzymes are mixed in the reaction buffer in a large 60-ton reaction tank with slow agitation.

Blue California uses a ≥95% steviol glycosides starting material that is derived from *Stevia rebaudiana* leaves. Two extraction techniques can be used to obtain equivalent preparations of ≥95% steviol glycosides starting material: a) hot water extraction or b) aqueous ethanol extraction. The specifications for both raw material steviol glycosides extracts are provided in Table 1.

Manufacturing flow charts and product specifications for the ≥95% steviol glycosides starting materials are provided in Appendix 2.

The ≥95% steviol glycosides extract raw material is fed into the tank containing the enzymes to allow the reaction to proceed. The reaction mixture is then heated to 85°C for 20 minutes to denature the enzymes in the supernatant, which is then removed for down-stream processing.

[3] Blue California uses older, larger cells to perform the measurement.

**Table 1. Specifications for Steviol Glycosides Starting Material**

| Physical & Chemical Parameters | Blue California's Specifications for Steviol Glycosides Starting Material (Hot Water Extraction) Item# ST0301245 | Blue California's Specifications for Steviol Glycosides Starting Material (Aqueous Ethanol Extraction) Item# ST0301238 |
|---|---|---|
| **Appearance Form** | Powder | Powder |
| **Appearance Color** | White | White |
| **Solubility in Water** | Soluble | Soluble |
| **Assay** | ≥ 95% steviol glycosides | ≥ 95% steviol glycosides |
| **Residual Ethanol** | < 5,000 mg/kg | < 5,000 mg/kg |
| **Residual Methanol** | NS | < 200 mg/kg |
| **Loss on Drying** | ≤ 6% | ≤ 6% |
| **pH, 1% Solution** | 4.5-7.0 | 4.5-7.0 |
| **Total Ash** | < 1% | < 1% |
| **Arsenic** | < 1 ppm | < 1 ppm |
| **Lead** | < 1 ppm | < 1 ppm |
| **Cadmium** | < 1 ppm | < 1 ppm |
| **Mercury** | < 1 ppm | < 1 ppm |
| **Total Plate Count** | < 1,000 cfu/g | < 1,000 cfu/g |
| **Total Coliform** | < 10 cfu/g | < 10 cfu/g |
| **Yeast & Mold** | < 100 cfu/g | < 100 cfu/g |
| ***Salmonella*** | Negative | Negative |
| ***Escherichia coli*** | Negative | Negative |

mg – milligram; kg – kilogram; NS – not specified; ppm – parts per million; cfu – colony forming unit; g – gram

## 2. Extraction & Purification

The supernatant from the Catalytic Bioconversion Process, described in Part 2.B.1 above, is loaded onto large columns containing a macro-porous resin. The supernatant flows through the column by gravity and is bound to the resin. The column is then rinsed with a series of buffers. Reb I is eluted with food-grade ethanol a number of times. The exact number of elution cycles required

is determined by the purity of Reb I to be achieved, as each elution results in greater removal of impurities. The eluent is collected and condensed in a wipe-film evaporator. Blue California evaporates out the ethanol, and Reb I remains in aqueous solution.

The condensate is chilled to allow Reb I to crystallize and precipitate from the solution. The wet crystals are collected, washed, and dissolved in ethanol. Blue California uses a recrystallization step to increase the concentration of Reb I, while removing impurities, including other steviol glycosides, such as Stevioside, Reb A, and Reb B, that exhibit higher solubilities than Reb I. Consequently, the other steviol glycosides (i.e., impurities) will remain in solution while Reb I precipitates out first, allowing Blue California to produce a higher purity Reb I product. Activated charcoal is used to purify the final product by adsorbing the non-steviol glycosides impurities. The resulting Reb I product is recrystallized, dried, and processed to the final BESTEVIA® Rebaudioside I product.

The manufacturing process is summarized in a flow chart provided in Figure 3.

All raw materials, processing aids, and additives used to manufacture Reb I are food-grade ingredients permitted by U.S. regulations or have previously been determined to be GRAS for their respective uses, as detailed in Appendix 3.

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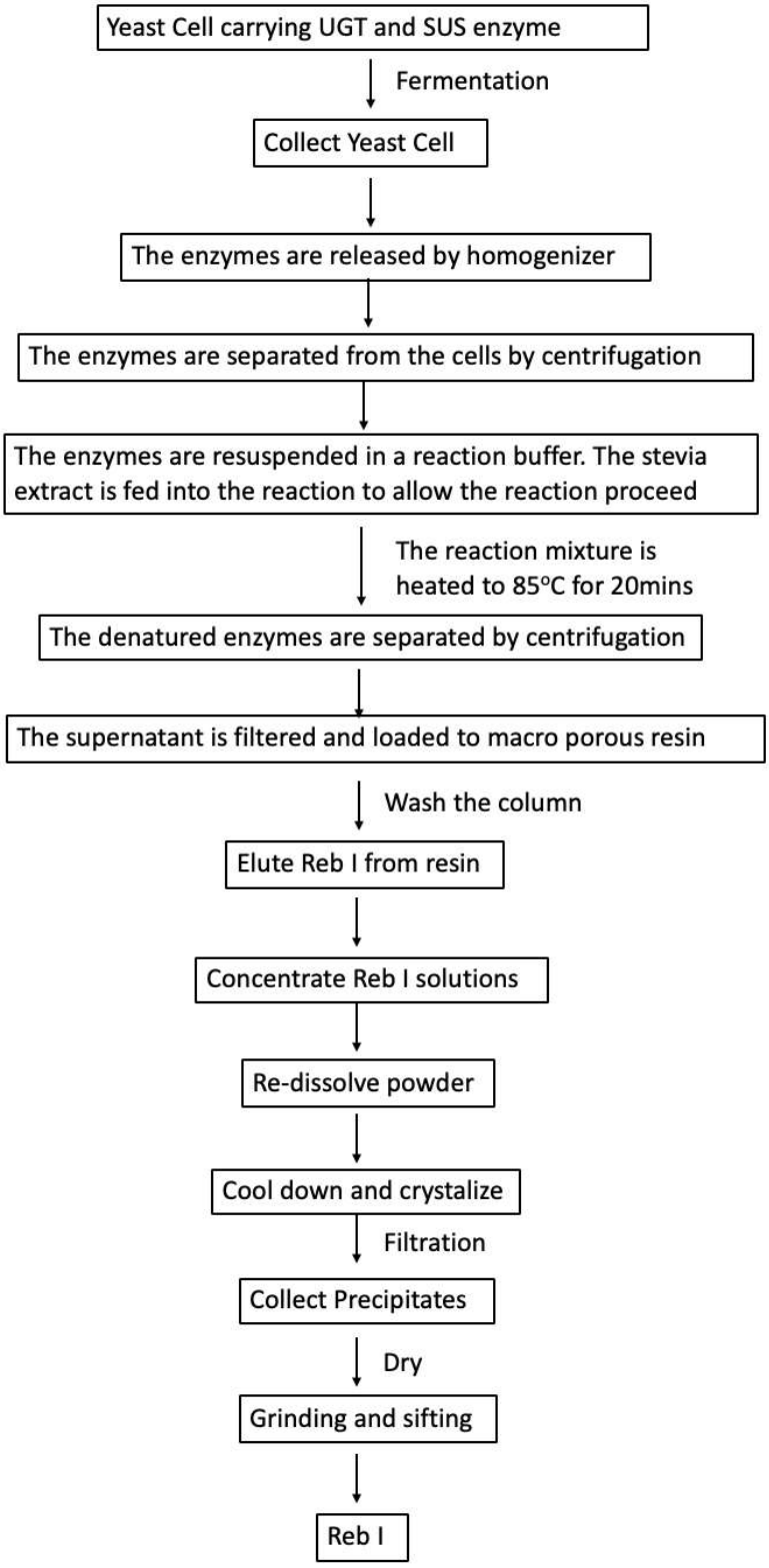
**Figure 3. Flow Chart of Manufacturing Process for Blue California's BESTEVIA® Rebaudioside I**



## C. Product Specifications

### 1. JECFA Specifications for Steviol Glycosides

The compositions of extracts of *Stevia rebaudiana* Bertoni depend upon the compositions of the harvested leaves, which are, in turn, influenced by soil, climate, and the manufacturing process itself (FAO, 2007b).

In the most recent JECFA monograph, published in 2017 (FAO, 2017), steviol glycosides specifications were modified to include a minimum requirement of not less than 95% total steviol glycosides, on a dry basis, "determined as the sum of all compounds containing a steviol backbone conjugated to any number, combination or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose xylose, galactose, arabinose and xylose) occurring in the leaves of *Stevia rebaudiana* Bertoni."

JECFA's 2017 monograph describes steviol glycosides as white-to-yellow powders that are odorless or have a slight characteristic odor and exhibit a sweetness that is 200 - 300 times greater than that of sucrose. The ingredient must consist of a minimum of 95% total steviol glycosides, as defined above. The steviol glycosides are freely soluble in a 50:50 mixture of ethanol and water, and the 1 in 100 solutions exhibit pH values between 4.5 and 7.0. The product should not have more than 1% ash, with no more than a 6% loss on drying at 105 °C after 2 hours. Any residual methanol levels should not exceed 200 mg per kg and ethanol residues should not exceed 5,000 mg per kg. Arsenic and lead levels should not exceed 1 mg per kg. Microbiological criteria have also been established, with specifications of no more than 1,000 colony forming units (cfu) per g total plate count, not more than 200 cfu per g yeasts and molds, and *E. coli* and *Salmonella* negative in 1 g and 25 g, respectively.

Blue California has adopted specifications for our purified steviol glycosides extract starting materials that meet or exceed current JECFA specifications, as demonstrated in Table 2.

### 2. Specifications for Blue California's Rebaudioside I Preparation and Supporting Methods

Blue California has adopted product specifications for its BESTEVIA® Rebaudioside I preparation that meet or exceed JECFA recommendations, while also complying with Food Chemicals Codex (FCC, 2010) specifications for Rebaudioside A as a consumable human food substance. The compositions of five non-consecutive lots of Blue California's BESTEVIA® Rebaudioside I are compared with the JECFA and FCC specifications in Table 3.

## Table 2. Specifications for Steviol Glycosides Starting Material

| Physical & Chemical Parameters | JECFA[a] Specifications Steviol Glycosides | Blue California's Specifications for Steviol Glycosides Starting Material (Hot Water Extraction) Item# ST0301245 | Blue California's Specifications for Steviol Glycosides Starting Material (Aqueous Ethanol Extraction) Item# ST0301238 |
|---|---|---|---|
| **Appearance Form** | Powder | Powder | Powder |
| **Appearance Color** | White to light yellow | White | White |
| **Solubility** | Freely soluble in 50:50 water: ethanol | Soluble | Soluble |
| **Assay** | Not less than 95% total steviol glycosides[b] | ≥ 95% steviol glycosides | ≥ 95% steviol glycosides |
| **Residual Ethanol** | NMT 5,000 mg/kg | < 5,000 mg/kg | < 5,000 mg/kg |
| **Residual Methanol** | NMT 200 mg/kg | NS | < 200 mg/kg |
| **Loss on Drying** | NMT 6.0% | ≤ 6% | ≤ 6% |
| **pH, 1% Solution** | 4.5 - 7.0 | 4.5-7.0 | 4.5-7.0 |
| **Total Ash** | NMT 1% | < 1% | < 1% |
| **Arsenic** | NMT 1 mg/kg | < 1 ppm | < 1 ppm |
| **Lead** | NMT 1 mg/kg | < 1 ppm | < 1 ppm |
| **Cadmium** | NS | < 1 ppm | < 1 ppm |
| **Mercury** | NS | < 1 ppm | < 1 ppm |
| **Total Plate Count** | NMT 1,000 cfu/g | < 1,000 cfu/g | < 1,000 cfu/g |
| **Total Coliform** | NS | < 10 cfu/g | < 10 cfu/g |
| **Yeast & Mold** | NMT 200 cfu/g | < 100 cfu/g | < 100 cfu/g |
| ***Salmonella*** | Negative in 25 g | Negative | Negative |
| ***Escherichia coli*** | Negative in 1 g | Negative | Negative |

NS – not specified; NMT – not more than; ppm – parts per million; cfu – colony forming units; mg – milligram; kg – kilogram; g -- gram

[a] Prepared at 84th JECFA (2017)

[b] Total steviol glycosides as the sum of all compounds containing a steviol backbone conjugated to any number, combination, or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose xylose, galactose, arabinose, and xylose) occurring in the leaves of *Stevia rebaudiana* Bertoni.

## Table 3. Specifications for Blue California's BESTEVIA® Rebaudioside I

| Physical & Chemical Parameters | JECFA[a] Specifications Steviol Glycosides | FCC[b] Specifications Steviol Glycosides | Blue California's Specifications for BESTEVIA® Rebaudioside I | BESTEVIA® Rebaudioside I Representative Lots | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Lot # 783-180510 | Lot # 783-180565 | Lot # 783-180607 | Lot # 78618111226 | Lot # 78618112826 |
| **Appearance Form** | Powder | Powder, flakes, or granules | Powder | Pass | Pass | Pass | Pass | Pass |
| **Appearance Color** | White to light yellow | White or light yellow | White to off white | Pass | Pass | Pass | Pass | Pass |
| **Solubility** | Freely soluble in water:ethanol (50:50) | Freely soluble in water:ethanol (50:50) | NS | 1,000 ppm | 1,000 ppm | 1,000 ppm | 1,000 ppm | 1,000 ppm |
| **Purity (HPLC Area)** | ≥95% Ste iol Glycosides | ≥95% Ste iol Glycosides | ≥95% Reb I | 97.91% | 98.91% | 95.92% | 97.41% | 98.41% |
| **Residual Ethanol** | NMT 5,000 mg/kg | NMT 0.50% | < 1,000 ppm | < 200 ppm | < 200 ppm | < 200 ppm | < 200 ppm | < 200 ppm |
| **Residual Methanol** | NMT 200 mg/kg | NMT 0.020% | < 200 ppm | < 100 ppm | < 100 ppm | < 100 ppm | < 100 ppm | < 100 ppm |
| **Loss on Drying (%)** | NMT 6.0% | NMT 6.0% | ≤ 6% | 1.01% | 1.6% | 1.26% | 1.42% | 1.45% |
| **pH, 1% Solution** | 4.5-7.0 | 4.5-7.0 | 4.5-7.0 | 6.47 | 6.35 | 6.20 | 6.55 | 6.25 |
| **Total Ash (%)** | NMT 1% | NMT 1% | ≤ 1% | 0.13% | 0.10% | 0.15% | 0.12% | 0.09% |
| **Arsenic** | NMT 1 mg/kg | NMT 1 mg/kg | < 0.5 ppm | < 0.5 ppm | < 0.5 ppm | < 0.5 ppm | < 0.02 ppm | < 0.5 ppm |
| **Lead** | NMT 1 mg/kg | NMT 1 mg/kg | < 0.5 ppm | 0.11 ppm | 0.12 ppm | 0.11 ppm | 0.11 ppm | 0.11 ppm |
| **Mercury** | NS | NS | < 0.5 ppm | < 0.1 ppm | < 0.1 ppm | < 0.1 ppm | <0.01 ppm | < 0.1 ppm |
| **Cadmium** | NS | NS | < 0.5 ppm | < 0.25 ppm | < 0.25 ppm | < 0.25 ppm | <0.01 ppm | < 0.25 ppm |
| | | | | | | | | |
| **Total Plate Count (cfu/g, max)** | NMT 1,000 | NS | < 1,000 | < 1,000 | < 1,000 | < 1,000 | < 1,000 | < 1,000 |
| **Total Coliform (cfu/g)** | NS | NS | < 10 | < 3 | < 5 | < 5 | < 3 | < 5 |
| **Yeast & Mold (cfu/g, max)** | NMT 200 | NS | < 100 | < 50 | < 50 | < 50 | < 40 | < 30 |
| ***E. coli* (mpn/g)** | Negative in 1 g | NS | Negative | Negative | Negative | Negative | Negative | Negative |
| ***Salmonella spp.*** | Negative in 25 g | NS | Negative | Negative | Negative | Negative | Negative | Negative |

NS – not specified; NMT – not more than; ppm – parts per million; mg – milligram; kg – kilogram; cfu – colony forming unit; g – grams; mpn – most probable number

[a] Prepared at 84th JECFA (2017)

[b] Steviol glycosides monograph. Food Chemicals Codex (11th Ed.). (FCC, 2018)

It is important to note that individual steviol glycosides have varying levels of solubility in water and water:ethanol solutions. It has been previously reported that pure steviol glycosides display no or low aqueous solubility at high concentrations (Upreti et al., 2011). A study by Celaya et al. (2016) found that Rebaudioside A is poorly soluble in ethanol and water and stevioside is poorly soluble in water, but that the presence of both steviol glycosides together results in higher solubilities for both. While JECFA's most recent steviol glycosides monograph specifies that steviol glycosides are freely soluble in 50:50 water:ethanol solution, this is impractical for high purity preparations of a single steviol glycoside, such as Rebaudioside I, which exhibits lower solubility in water:ethanol solutions.

Based on the solubility definitions provided in Table 4, Blue California's BESTEVIA® Rebaudioside I, which is reported to have a solubility of 1,000 ppm, would be appropriately described as "slightly soluble."

**Table 4. Solubility Definitions[a]**

| Description | Approximate Volume (mL) of Solvent Needed to Dissolve 1 g of Solute |
|---|---|
| **Very soluble** | Less than 1 |
| **Freely soluble** | 1 to 10 |
| **Soluble** | 10 to 30 |
| **Sparingly soluble** | 30 to 100 |
| **Slightly soluble** | 100 to 1,000 |
| **Very slightly soluble** | 1,000 to 10,000 |
| **Practically insoluble** | Greater than 10,000 |

[a] Adapted from Sigma Aldrich (2019)

Blue California analyzes its BESTEVIA® Rebaudioside I preparation by high performance liquid chromatography (HPLC), following the method presented in Appendix 4. In addition to the presentation of key specifications found in Table 3 for comparison with generally accepted purity standards, certificates of analysis for five representative lots of BESTEVIA® Rebaudioside I are provided in Appendix 5. The chromatograms for representative BESTEVIA® Rebaudioside I are provided in Appendix 6. Test reports for analyses of pesticide residues in representative lots of BESTEVIA® Rebaudioside I are located in Appendix 7. The collection of these reports demonstrates that the substance is well characterized and meets the established purity criteria.

## D. Physical or Technical Effect

Blue California determined the relative sweetness of BESTEVIA® Rebaudioside I preparation to be 167 X sweeter than sucrose by organoleptic comparison to 1.0%, 3.0%, and 6.0% sucrose solutions, following the method outlined in Appendix 8.

## E. Stability

### 1. Stability Data on Steviol Glycosides

The stabilities of steviol glycosides and enzyme modified steviol glycosides have previously been reviewed in a number of GRAS Notifications, including GRN 337 (NOW Foods, 2010), GRN 667 (Blue California, 2016), GRN 715 (Blue California, 2017), and GRN 823 (Blue California, 2018).

Stevioside has been reported to be stable over the pH range 3-9 and can be heated at 100°C for 1 hour, but, at pH levels greater than 9, it rapidly decomposes (Kinghorn, 2002). A series of stability studies in food applications was conducted on Stevioside by Kroyer (2010). Solid Stevioside was reported to be stable at up to 120°C for 1 hour. In aqueous solution, Stevioside was reported to be stable at pH levels ranging from 2 to 10 for 2 hours at 60°C. No degradation was observed after 4 months at room temperature for 1 g per L solutions of Stevioside in acetic acid (pH 3.1), citric acid (pH 2.6), and tartaric acid (pH 2.6). A 30% loss of Stevioside was observed in a 1 g per L solution of phosphoric acid (pH 1.6) stored under the same conditions. In addition, degradation was observed in 10 g per L solutions of Stevioside in acetic acid (pH 2.6, 2% loss), citric acid (pH 2.1, 22% loss), tartaric acid (pH 2.1, 33% loss), and phosphoric acid (pH 1.6, 75% loss) after 4 months.

Kroyer (2010) reported no significant changes in the concentration of B-vitamins incubated with Stevioside in aqueous solution at 80°C for 4 hours. A decrease in the degradation rate of vitamin C was observed after 4 hours under the same conditions, indicating that Stevioside provides a protective effect. No stability effects or interactions were observed between mixtures of Stevioside and saccharin, cyclamate, aspartame, acesulfame, and neohesperidin stored at 80°C for 4 hours or room temperature for 4 months. Furthermore, no stability effects or interactions were observed between Stevioside and caffeine in coffee and tea beverages at 80°C for 4 hours. These results indicate that Stevioside is stable under the intended conditions of use.

Previously submitted GRAS notices GRN 252 (Merisant, 2008), GRN 253 (Cargill, 2008), and GRN 304 (Sunwin/WILD, 2010), reported data indicating that Rebaudioside A is stable under the intended conditions of use.

Furthermore, in the 64 GRAS notices for steviol glycosides that have been submitted to FDA and have received “no questions” letters to date, the presented stability data have supported the position that steviol glycosides are stable and well-suited for the intended uses in foods (FDA, 2020).

## 2. Stability Data for Blue California's BESTEVIA® Rebaudioside I

Blue California conducted a 6-month stability study of five lots of BESTEVIA® Rebaudioside I. The samples were stored at 40°C ± 2°C at a relative humidity of 75% ± 5%. BESTEVIA® Rebaudioside I was observed to be stable over the course of the accelerated stability study, as demonstrated in Table 5.

**Table 5. BESTEVIA® Rebaudioside I Storage Stability Data**

| BESTEVIA® Lot# 783-180510 | | | | | |
|---|---|---|---|---|---|
| **Duration** | **Appearance** | **Moisture (%)** | **Rebaudioside I (HPLC %)** | **Total Plate Count (cfu/g)** | ***E. coli* (in 10 g)** |
| t=0 | White Powder | 3.18 | 97.6 | 35 | Absent |
| 1 month | White Powder | 3.15 | 96.9 | 30 | Absent |
| 2 months | White Powder | 3.15 | 96.6 | 25 | Absent |
| 3 months | White Powder | 3.12 | 96.2 | 45 | Absent |
| 6 months | White Powder | 3.14 | 96.74 | 45 | Absent |
| **Average** | **White Powder** | **3.15** | **96.8** | **36** | **Absent** |
| **BESTEVIA® Lot# 78618111226** | | | | | |
| **Duration** | **Appearance** | **Moisture (%)** | **Rebaudioside I (HPLC %)** | **Total Plate Count (cfu/g)** | ***E. coli* (in 10 g)** |
| t=0 | White Powder | 3.56 | 96.2 | 20 | Absent |
| 1 month | White Powder | 3.60 | 96.2 | 25 | Absent |
| 2 months | White Powder | 3.61 | 95.5 | 25 | Absent |
| 3 months | White Powder | 3.60 | 95.3 | 35 | Absent |
| 6 months | White Powder | 3.58 | 95.4 | 35 | Absent |
| **Average** | **White Powder** | **3.59** | **95.7** | **28** | **Absent** |
| **BESTEVIA® Lot# 783-180565** | | | | | |
| **Duration** | **Appearance** | **Moisture (%)** | **Rebaudioside I (HPLC %)** | **Total Plate Count (cfu/g)** | ***E. coli* (in 10 g)** |
| t=0 | White Powder | 3.68 | 97.8 | 50 | Absent |
| 1 month | White Powder | 3.48 | 97.7 | 45 | Absent |
| 2 months | White Powder | 3.31 | 96.1 | 30 | Absent |
| 3 months | White Powder | 3.28 | 95.2 | 40 | Absent |
| 6 months | White Powder | 3.28 | 95.3 | 40 | Absent |
| **Average** | **White Powder** | **3.41** | **96.4** | **41** | **Absent** |

| BESTEVIA® Lot# 783-180607 | | | | | |
|---|---|---|---|---|---|
| **Duration** | **Appearance** | **Moisture (%)** | **Rebaudioside I (HPLC %)** | **Total Plate Count (cfu/g)** | ***E. coli* (in 10 g)** |
| t=0 | White Powder | 3.36 | 95.4 | 10 | Absent |
| 1 month | White Powder | 3.42 | 95.3 | 15 | Absent |
| 2 months | White Powder | 3.50 | 95.5 | 25 | Absent |
| 3 months | White Powder | 3.55 | 95.4 | 10 | Absent |
| 6 months | White Powder | 3.62 | 95.4 | 25 | Absent |
| **Average** | **White Powder** | **3.49** | **95.4** | **17** | **Absent** |
| **BESTEVIA® Lot# 78618112826** | | | | | |
| **Duration** | **Appearance** | **Moisture (%)** | **Rebaudioside I (HPLC %)** | **Total Plate Count (cfu/g)** | ***E. coli* (in 10 g)** |
| t=0 | White Powder | 3.70 | 97.8 | 30 | Absent |
| 1 month | White Powder | 3.68 | 97.6 | 25 | Absent |
| 2 months | White Powder | 3.59 | 96.6 | 20 | Absent |
| 3 months | White Powder | 3.52 | 95.5 | 25 | Absent |
| 6 months | White Powder | 3.55 | 95.1 | 30 | Absent |
| **Average** | **White Powder** | **3.60** | **96.5** | **26** | **Absent** |

HPLC – High-performance liquid chromatography; cfu – colony forming unit; g – gram

The stability data in the scientific literature for stevioside, the JECFA report, and the extensive stability testing for the structurally similar Rebaudioside A as presented by Merisant (GRN 252), Cargill (GRN 253), and Sunwin & WILD Flavors (GRN 304), along with Blue California's stability testing results, support the position that Blue California's BESTEVIA® Rebaudioside I preparation is well-suited for the intended food uses.

## PART 3. DIETARY EXPOSURE

The subject Blue California BESTEVIA® Rebaudioside I (≥95%) preparation is intended to be used as a table top sweetener and general purpose non-nutritive sweetener in various foods other than infant formulas and meat and poultry products, as defined in 21 CFR 170.3(o)(19).[4] The intended use levels will vary by actual food category, but the actual levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts of Blue California's

[4] Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.

high purity Rebaudioside I to be added to foods will not exceed the amounts reasonably required to accomplish the intended technical effect in foods as required by FDA regulation.[5]

## A. Estimate of Dietary Exposure to the Substance

Many scholarly estimates of potential dietary intake replacement of sweeteners, including steviol glycosides, have been published (FSANZ, 2008; WHO, 2003; Renwick, 2008) or submitted to FDA (Merisant, 2008). These are summarized in Appendix 9. In GRAS Notice 301, a simplified estimate was proposed to, and accepted by, FDA based on the estimates of exposure in "sucrose equivalents" (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90$^{th}$ percentile consumer of a sweetener that is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg per kg body weight (bw) per day for any population subgroup.

The estimated sweetness intensity for Blue California's BESTEVIA® Rebaudioside I preparation is approximately 167-fold that of sucrose (Part 2.D). Therefore, the highest 90$^{th}$ percentile consumption by any population subgroup of Blue California's BESTEVIA® Rebaudioside I preparation would consume approximately 5.93 mg per kg steviol glycosides bw per day. Based on an estimate that Reb I preparations consist of approximately 28% steviol equivalents,[6] the consumption would be less than 1.67 mg per kg bw per day on a steviol equivalents basis for any population group. These calculations are summarized in Table 6.

**Table 6. Daily Intake of Sweeteners (in Sucrose Equivalents) & Estimated Daily Intakes of BESTEVIA® Rebaudioside I**

| Population Group | Intakes of Sweeteners (mg sucrose/kg bw/day)[a] | | Calculated Intake of BESTEVIA® Rebaudioside E (mg/kg bw/day)[b] | | Calculated Intake of BESTEVIA® Rebaudioside E as Steviol Equivalents (mg/kg bw/day) | |
|---|---|---|---|---|---|---|
| | Low | High | Low | High | Low | High |
| Healthy Population | 255 | 675 | 1.53 | 4.04 | 0.43 | 1.14 |
| Diabetic Adults | 280 | 897 | 1.68 | 5.37 | 0.47 | 1.51 |
| Healthy Children | 425 | 990 | 2.54 | 5.93 | 0.72 | 1.67 |
| Diabetic Children | 672 | 908 | 4.02 | 5.44 | 1.13 | 1.53 |

[a] From Renwick (2008)
[b] Calculated by dividing the sucrose intake by the minimum average relative sweetness value of 167 for BESTEVIA® Rebaudioside I

[5] See 21 CFR 182.1(b)(1).
[6] Calculated as percent of molecular weight of Steviol to molecular weight of Rebaudioside I

The values in Table 6 assume Blue California's BESTEVIA® Rebaudioside I preparation constitutes the entire sweetener market, which makes these estimates extremely conservative since the likelihood of that occurrence is minimal. For the general healthy adult population, the estimated maximum intake of purified steviol glycosides is 4.04 mg per kg bw per day, or 1.14 mg per kg steviol equivalents. For healthy children, the estimated maximal intake is 5.93 mg per kg bw per day, or 1.67 mg per kg as steviol equivalents. In all population groups, the estimated daily intake of purified steviol glycosides, expressed as steviol equivalents, is well below the JECFA-established acceptable daily intake (ADI) of 4.0 mg per kg bw per day steviol equivalents.

### B. Estimated Dietary Exposure to Any Other Substance That is Expected to be Formed In or On Food

This section is not applicable to Blue California's BESTEVIA® Rebaudioside I product, which would be chemically stable under the proposed conditions of use.

### C. Dietary Exposure to Contaminants or Byproducts

While a recent publication by Kumari et al. (2016) investigated the Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Antioxidant Capacity (TAC) of *S. rebaudiana* leaf --- and the observed activity has been attributed to naturally-occurring phytochemicals such as phenolics, flavonoids, and pigments in the plant --- the study has minimal relevance with regard to the safety considerations of highly purified stevia extract, of which ≥95% consists of the most familiar steviol glycosides and their glucosylated steviol glycosides. These phytochemical contaminants, if present, are in low amounts and were likely similarly present in purified test materials that were used in the toxicology studies summarized in Appendix 10.

Furthermore, no concerns regarding dietary exposure to contaminants or byproducts have been raised by expert regulatory bodies, including the World Health Organization/Joint FAO/WHO Expert Committee on Food Additives (WHO/JECFA), European Food Safety Authority (EFSA), Food Standards Australia New Zealand (FSANZ), and FDA, since JECFA's first steviol glycosides review was performed in 2000 (WHO, 2000).

## PART 4. SELF-LIMITING LEVELS OF USE

It has been well-documented in the published literature that the use of steviol glycosides is self-limiting due to organoleptic factors and consumer taste considerations (Kochikyan et al., 2006; Carakostas et al., 2008; Brandle et al., 1998; Prakash et al., 2008; Gupta et al., 2016; Gerwig et al., 2016). These organoleptic factors include bitterness and astringency, as well as a lingering metallic aftertaste (Gerwig et al., 2016).

# PART 5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

## A. Other Information on Dietary Exposure

### 1. History of Traditional Medicinal and Human Food Use

Stevia has been used as a traditional medicine and sweetener by native Guarani tribes for centuries (Esen, 2016; Gerwig et al., 2016; Brusick, 2008; Brandle et al., 1998). Hawke (2003) reported that stevia is commonly used as a treatment for type 2 diabetes in South America. However, therapeutic doses of 1 gram per person per day or more were reported to be necessary to achieve the desired effects (Gregersen et al., 2004).

For about 30 years, consumers in Japan and Brazil, where stevia has long been approved as a food additive, have been using stevia extracts as non-caloric sweeteners (Raintree, 2012). It was previously reported that 40% of the artificial sweetener market in Japan had been stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999). Use of steviol glycosides as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand, and use as a natural health product is permitted in Canada. It has wide use in China and Japan in food and in dietary supplements. In 2005, it was estimated that sales of stevia in the US reached $45 million (Newsday, 2006).

NewHope360 reported that the global market for stevia in 2014 was $347 million and is expected to increase to $565.2 million by 2020. In addition, consumption was expected to increase from 2014 levels of 5,100.6 tons to 8,506.9 tons by 2020 (NewHope360, 2015).

Most recently, Nutritional Outlook reported that Mintel data indicated a 48% increase in stevia-containing products over the last five years (Decker and Prince, 2018).

## B. Summary of Regulatory History of Enzyme Modified Steviol Glycosides

Stevia-derived sweeteners are permitted as food additives in South America and in several countries in Asia, including China, Japan, and Korea. In recent years, these sweeteners have received food usage approvals in Mexico, Australia, New Zealand, Switzerland, France, Peru, Uruguay, Colombia, Senegal, Russia, Malaysia, Turkey, Taiwan, Thailand, Israel, Canada, and Hong Kong (EFSA, 2010; Watson, 2010; Health Canada, 2012). In the United States, steviol glycosides have been used as a dietary supplement since 1995 (Geuns, 2003).

A brief overview of the most recent regulatory activity regarding steviol glycosides is presented below in Part 5.B. Sections 1-5; a more detailed historical overview is provided in Appendix 11.

### 1. U.S. Regulatory History

Based on available information from FDA's GRAS Notice Inventory website (FDA, 2020) as of January 13, 2020, FDA has issued 64 "no questions" letters on GRAS notices on Rebaudioside A,

Rebaudioside D, Rebaudioside M, Rebaudioside E, or steviol glycosides, including those undergoing enzyme modification.

In addition, the Flavor and Extract Manufacturers Association (FEMA) includes 20 steviol glycosides preparations, as detailed in Appendix 11, including FEMA No. 7937 for Rebaudioside I 95%, on their GRAS lists.

## 2. Canadian Regulatory History

On November 30, 2012, Health Canada published its final clearance for use of steviol glycosides as a sweetener in foods (Health Canada, 2012). In March 2014, Health Canada updated the List of Permitted Sweeteners (Lists of Permitted Food Additives) to include steviol glycosides in applications as a table-top sweetener and as an ingredient in a variety of foods, beverages, baked goods, meal replacement bars, condiments, and confectionary and gums (Health Canada, 2014). On January 15, 2016, Health Canada approved the use of Rebaudioside M as a high-intensity sweetener under the same conditions as the previously approved steviol glycosides (Health Canada, 2016).

Health Canada's Food Directorate updated its List of Permitted Sweeteners to allow for the use of steviol glycosides as a sweetener in "unstandardized snack bars," including granola bars, cereal bars, fiber bars, and protein isolate-based bars (Health Canada, 2017a). Health Canada (2017b) also modified the List of Permitted Sweeteners to include "all the steviol glycosides in the *Stevia rebaudiana* Bertoni plant (stevia plant)."

In April 2019, Health Canada's Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides from *Stevia rebaudiana* Bertoni in canned fruit products (Health Canada, 2019). Most recently, Health Canada's Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides derived from *Saccharomyces cerevisiae* strains CD15380 and CD15407 at the same maximum levels of use as steviol glycosides derived from *Stevia rebaudiana* Bertoni (Health Canada, 2019).

## 3. European Regulatory History

An amendment to the European Union (EU) food additives regulation 231/2012, which became active on November 3, 2016, removed the previous requirement for stevia blends to contain at least 75% Reb A or Stevioside. In addition, the updated regulation ---(EU) 2016/1814---now permits the following steviol glycosides in stevia blends: Stevioside, Rebaudiosides A, B, C, D, E, F and M, Steviolbioside, Rubusoside, and Dulcoside (Searby, 2016).

In 2017, JECFA updated the steviol glycosides specifications to include a minimum requirement of not less than 95% total steviol glycosides, on a dry basis, "determined as the sum of all compounds containing a steviol backbone conjugated to any number, combination or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose, xylose, galactose, arabinose and xylose)

occurring in the leaves of *Stevia rebaudiana* Bertoni." Microbiological criteria were also established, with specifications of no more than 1,000 cfu per g total plate count, not more than 200 CFU per g yeasts and molds, and *E. coli* and *Salmonella* negative in 1 g and 25 g, respectively (FAO, 2017).

The European Food Safety Authority (EFSA, 2010) Panel of Food Additives and Nutrient Sources reviewed an application for glucosylated steviol glycoside preparations for use as a new food additive. The Panel concluded that the data supplied by the applicant were "insufficient to assess the safety" of the glucosylated steviol glycosides preparation. It should be noted that no safety concerns were raised in a more recent review by the EFSA Panel where their decision was based on the "limited" data provided in the dossier submitted by the applicant (EFSA, 2018).

### 4. Asian Regulatory History

No regulatory updates have been identified in recent years. The Asian regulatory history for steviol glycosides through 2014 is presented in Appendix 11.

### 5. Other Regulatory History

FSANZ called for submissions on permitting all minor steviol glycosides extracted from stevia leaf to be included in the definition of steviol glycosides in the Food Standards Code, noting that "[no] evidence was found to suggest that the proposed changes pose any public health and safety concerns." The submission period ended on December 19, 2016 (FSANZ, 2016b). Subsequently, on February 8, 2017, FSANZ approved a draft variation of the definition of steviol glycosides to include all steviol glycosides present in the *Stevia rebaudiana* leaf (FSANZ, 2017).

In 2018, FSANZ called for comments on the production of Reb M using enzymes derived from genetically modified yeast. The comment period closed on August 31, 2018 (FSANZ, 2018b). Subsequently, on October 31, 2018, FSANZ approved a draft variation to include a reference to the production method (FSANZ, 2018a).

Most recently, FSANZ called for comments on the production of steviol glycosides produced with enzymes derived from genetically modified strains of *E. coli* (FSANZ, 2019a). The comment period closed on October 8, 2019 with five comments, all of which supported the draft document. The approval report was issued on December 20, 2019 (FSANZ, 2019b).

## PART 6. NARRATIVE

The biological, toxicological, and clinical effects of stevia and steviol glycosides have been extensively reviewed (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002). Additionally---and as noted earlier---the national and international regulatory agencies have thoroughly reviewed the safety of stevia and its glycosides. Most notably, over the years, JECFA has evaluated purified

steviol glycosides multiple times (WHO, 2000; WHO, 2006; WHO, 2007; WHO, 2008), and their findings have been summarized in Part 5.B.3. FSANZ (2008) also evaluated steviol glycosides for use in food. The JECFA reviews, as well as the other reviews completed before 2008, primarily focused on mixtures of steviol glycosides. These studies are summarized in Appendix 12.

Since the JECFA evaluation (WHO, 2008), FDA has received and not objected to over sixty GRAS notifications for steviol glycosides or enzyme modified steviol glycosides, many of which were discussed by Perrier et al. (2018). In each case, FDA has agreed with the conclusions that steviol glycosides are GRAS based largely on the 0-4 mg per kg bw per day ADI on a steviol equivalence basis that was established by JECFA. A recent publication by Roberts et al. (2016) indicates that the ADI could be higher, as discussed further in Appendix 9. Among the GRAS notifications submitted to FDA, several assessed purified preparations of Rebaudioside A, and they were supported by additional toxicology and clinical studies that are summarized in Appendix 10. As of January 14, 2020, FDA has issued 64 “no questions” letters in response to GRAS notices on Rebaudioside A, Rebaudioside D, Rebaudioside M, Rebaudioside E, or steviol glycosides, including those undergoing enzyme treatment (FDA, 2020).

Because of their sweetness characteristics, steviol glycosides have viable uses as a non-nutritive sweetener in foods.[7] Periodic reviews by JECFA over the years indicate the progression of knowledge on the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple investigations with *in vivo* and *in vitro* models, which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years, as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic, and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably, the initially reported concerns related to the effects of stevia leaves or crude extracts on fertility were refuted by the well-designed reproductive studies with purified steviol glycosides. All other concerns failed to manifest themselves at the doses employed in the long-term rat studies.

As discussed in Appendix 12 and elsewhere, at its 51st meeting, JECFA determined that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), to establish a temporary ADI of 0 - 2 mg per kg bw per day with an adequate margin of safety (Toyoda et al.,

[7] It has also been reported that steviol glycosides may have pharmacological properties that can be used to treat certain disease conditions such as hypertension and type 2 diabetes. Chatsudthipong and Muanprasat (2009), as well as others, have published reviews where they note that such therapeutic applications have not been firmly established as being due to steviol glycosides. The reviewers point out that the effects occur at higher doses than would be used for sweetening purposes. Furthermore, many effects noted in older studies may have been due to impurities in preparations that do not meet the contemporary purity specifications established by JECFA for use as a sweetener. If oral doses of steviol glycosides impart pharmacological effects, such effects would undoubtedly occur due to actions of the principal metabolite, steviol, but the pharmacological effects of steviol have not been comprehensively investigated.

1997). The Committee also critically reviewed the lack of carcinogenic response in well-conducted studies. These studies validated the Committee's conclusion that the *in vitro* mutagenic activity of steviol did not present a risk of carcinogenic effects *in vivo* and, therefore, all common steviol glycosides that likely share the same basic metabolic and excretory pathway and that use high purity preparations of various steviol glycosides, are safe as sugar substitutes. Subsequently, the additional clinical data reviewed by JECFA allowed the Committee to establish a permanent ADI of 0 - 4 mg per kg bw per day (based on steviol equivalents).

In 2017, JECFA published a safety evaluation of a number of food additives, including steviol glycosides (WHO, 2017). The JECFA Committee reviewed information supporting the safety of a *Yarrowia lipolytica* fermentation-produced Rebaudioside A, which included a 90-day rat toxicity study and two *in vitro* genotoxicity studies, as well as *in vitro* colonic microflorae hydrolysis studies in several steviol glycosides, toxicokinetic studies of stevioside in humans and rats, and literature published since the 69$^{th}$ meeting.

The Committee noted that the most recent short-term toxicity studies were consistent with those reviewed at or prior to the 69$^{th}$ meeting, and that the new toxicokinetic study in humans did not have a large enough subject pool to provide reliable toxicokinetic estimates to derive an update ADI for steviol glycosides. The Committee confirmed the current ADI of 0 - 4 mg per kg bw steviol. In addition, the Committee prepared new "tentative" specifications for steviol glycosides, which were expanded to include "any mixture of steviol glycosides compounds derived from *S. rebaudiana* Bertoni" while retaining the requirement that the total percentage of steviol glycosides is ≥95% (WHO, 2017).

Blue California critically reviewed the JECFA assessments and agrees with the calculation of the ADI for steviol glycosides.

Several published and unpublished studies (summarized in Appendix 10) on purified preparations of Rebaudioside A showed an absence of toxicological effects in rats (Curry and Roberts, 2008; Nikiforov and Eapen, 2008) and dogs (Eapen, 2008) in subchronic studies, and an absence of reproductive (Curry et al., 2008; Sloter, 2008a) and developmental effects (Sloter, 2008b) in rats. Most notably, pharmacokinetic studies in rats (Roberts and Renwick, 2008) and humans (Wheeler et al., 2008) showed that purified Rebaudioside A follow the same pathway of being degraded to steviol by intestinal bacteria with subsequent rapid glucosylation and elimination in urine and feces.

Blue California concluded that these studies on Rebaudioside A strengthen the argument that all steviol glycosides that follow the same metabolic pathway are safe at the JECFA established ADI.

Blue California has also reviewed the findings from human clinical studies. Blue California noted that the clinical effects of steviol glycosides reported in humans occurred in patients with either elevated blood glucose or blood pressure (or both). JECFA called for studies in individuals who are neither hypertensive nor diabetic (WHO, 2006). The supplemental data presented to JECFA and

also published by Barriocanal et al. (2008) demonstrate the lack of pharmacological effects of steviol glycosides at 11 mg per kg bw per day in normal individuals, or approximately slightly more than 4 mg per kg bw on the basis of steviol equivalents (Barriocanal et al., 2008). Clinical studies on purified Rebaudioside A showed an absence of effects on blood pressure (Maki et al., 2008a) and blood glucose levels (Maki et al., 2008b) at doses slightly higher than the exposures expected in food. Blue California concludes that there will be no effects on blood pressure and glucose metabolism in humans at the doses of steviol glycosides expected from its use in food as a non-nutritive sweetener.

Two previously published studies summarized in Appendix 10 raised a potential concern regarding the toxicological effects of steviol glycosides. In one study, DNA damage was seen in a variety of organs as assessed by Comet assay in rats given drinking water containing 4 mg per mL steviol glycosides for up to 45 days (Nunes et al., 2007a). Several experts in the field have since questioned the methodology used in this study (Geuns, 2007; Williams, 2007; Brusick, 2008). Blue California has reviewed the cited publications, along with the responses made by the authors (Nunes et al., 2007b; Nunes et al., 2007c), and concurs with the challenges to the methodology utilized by (Nunes et al., 2007a), thereby discounting the validity and relevance of this study.

In another study with stevioside in rats, tartrate-resistant alkaline phosphatase (TRAP) levels were measured and found to be significantly decreased at doses as low as 15 mg per kg bw (Awney et al., 2011). TRAP is an enzyme that is expressed by bone-resorbing osteoclasts, inflammatory macrophages, and dendritic cells. This enzyme was not measured in any previous toxicology studies on steviol glycosides, nor has it been adequately vetted for application in toxicological studies. Critical reviews of this study by Carakostas (2012) and Waddell (2011) revealed a poor study design that included: insufficient numbers of animals; group-housing with the potential for stress-related changes; unreliable access to steviol *via* drinking water resulting in suspect dosing calculations in group-housed cages; no indication of fasting prior to blood collection (which affects many chemistry and hematological values); no urine collection; and no histopathological evaluations for confirmation of findings beyond the controls. Additionally, the report did not adequately describe mean or individual organ weight data, and it lacked comparison of study findings against laboratory historical control data.

Urban et al. (2013) examined the extensive genotoxicity database on steviol glycosides because some concern was expressed in two other publications (Brahmachari et al., 2011; Tandel, 2011) in which the authors concluded that additional testing is necessary to adequately address the genotoxicity profile (Urban et al., 2013). The review aimed to address this matter by evaluating the specific genotoxicity studies of concern, while evaluating the adequacy of the database that includes more recent genotoxicity data not noted in these publications. The results of this literature review showed that the current database of *in vitro* and *in vivo* studies for steviol glycosides is robust and does not indicate that either Stevioside or Rebaudioside A is genotoxic. This finding, combined with a paucity of evidence for neoplasm development in rat bioassays, establishes the safety of all steviol glycosides with respect to their genotoxic/carcinogenic potential.

In addition, a paper by Shannon et al. (2016) raises a possible concern of endocrine disruption by steviol. Blue California reviewed the publication and noted that the effects on progesterone production and on the action of progesterone (both antagonistic and agonistic) were observed *in vitro* in sperm cells. Blue California concludes that it is difficult to translate *in vitro* concentrations to local concentrations *in vivo* at receptors and that no adverse effects were observed in well-conducted reproductive toxicology studies. Therefore, this study does not alter Blue California's opinion that steviol glycosides preparations are generally recognized as safe. A summary of this study is provided in Appendix 10.

Philippaert et al. (2017) demonstrated that Stevioside, Rebaudioside A, and steviol potentiate the activity of transient receptor potential cation channel subfamily melastatin member 5 (TRPM5), a $Ca^{2+}$-activated cation channel that is expressed in type II taste receptor cells and pancreatic β-cells. The authors found that the steviol glycosides increased the perception of bitter, sweet, and umami tastes and enhanced glucose-induced insulin secretion in a TRPM5-dependent manner. Furthermore, *in vivo* studies indicated that daily consumption of stevioside prevents high-fat-induced diabetic hyperglycemia development in wild-type mice. No adverse events or animal deaths were discussed.

A commercially available steviol glycoside extract (>99%, composition and brand unknown) was used to investigate genotoxicity in human peripheral blood lymphocytes. Uçar et al. (2017) observed no significant differences in chromosomal aberration induction or micronuclei between the control and treatment groups at 24 and 48 h. These data support previous findings that steviol glycosides are not genotoxic.

Panagiotou et al. (2018) observed that steviol and steviol glycosides exert glucocorticoid receptor-mediated effects in human leukemic T-cells (Jurkat cells) but not in normal human peripheral blood mononuclear cells, which they concluded was due to a cell-type specific manner of glucocorticoid receptor-modulation.

Thøgersen et al. (2018) investigated the effect of Rebaudioside A, Stevioside, and steviol on porcine cytochrome p450 (CYP) expression and activity to assess their potential food-drug interactions in the IPEC-J2 cell line, which is a non-transformed cell line derived from intestinal porcine epithelial cells and in primary hepatocytes. The authors reported that there were no changes in CYP messenger ribonucleic acid (mRNA) expression following treatment of IPEC-J2 cells with Rebaudioside A, Stevioside, and steviol compared with control. Treatment of primary hepatocytes resulted in increases in CYP329 mRNA at low concentrations of Rebaudioside A and steviol, and at all concentrations of Stevioside. The authors reported that while treatment with the steviol glycosides tested over 24 hours resulted in minor (< 2.0 fold) increases in CYP3A29 mRNA expression, "no direct effect on CYP activity" was observed. The authors concluded that Rebaudioside A, Stevioside, and steviol are unlikely to cause a food-drug interaction but noted that the study could not predict long term effects and effects *in vivo*.

In a study that addressed the genotoxic activity of stevia (Svetia™, purity not reported[8]), human lymphocytes were treated with 5% and 0.5% Svetia™ for 2 hours. No statistically significant difference in genetic damage was observed in the 0.5% treatment concentration compared with the negative control, while the 5% treatment concentration resulted in a statistically significant difference ($P<0.0001$) compared with the control, with a decrease in migration average. The authors described the effect as being beneficial. Human lymphocytes treated with 10% Svetia™ demonstrated significant ($P<0.0001$) genotoxic activity compared to the control; however, at treatment concentrations of 0.05%, 0.5%, and 5% Svetia™, a significant ($P<0.0001$) decrease in average migration of DNA was observed compared with the control. The authors conclude that these results demonstrate the absence of genotoxicity at concentrations up to 5% Svetia™ (Silva et al., 2018). It should be noted that these observations are inconsistent with data reported by (Nunes et al., 2007a); however, as discussed above, the validity and importance of the Nunes et al. study has been discounted given the questions surrounding the methodology.

Recently, a case report was published by Tangkiatkumjai et al. (2019) indicating a probable interaction between a stevia product and etoricoxib, a selective inhibitor of cyclooxygenase-2 (COX-2) resulting in declined kidney function. A 47-year-old Thai woman developed acute kidney injury (AKI) after reporting consumption of stevia (as a Thai-registered food product stevia extract or as a stevia tea) with 90 mg etoricoxib, 2 to 3 times a week over the course of 6 months. No information was provided regarding the purity of the stevia extract or how the tea was prepared directly from the leaves, and it was reported that the subject purchased etoricoxib without a prescription. The subject displayed mild hyponatremia and hyperkalemia, which were associated with renal tubular dysfunction, as well as elevated serum creatinine. Serum creatinine and electrolytes returned to normal when stevia and etoricoxib use was discontinued. Using a modified Naranjo algorithm, a causal relationship between stevia and AKI was determined to be probable. The authors suggest that the interaction between stevia and etoricoxib was linked to AKI, and that consumers should not use stevia products while taking COX-2 inhibitors or non-steroidal anti-inflammatory drugs (NSAIDs).

Blue California reviewed the Tangkiatkumjai et al. (2019) publication and notes the absence of sufficient detail regarding the purity, source, and dosage levels of the stevia preparations consumed by the subject. Furthermore, Blue California concludes that it is impractical to compare the safety of un-characterized stevia preparations with a high purity steviol glycoside material. Given the lack of similar case studies, the paucity of critical details of the stevia ingested, and the history of safety in use of high purity steviol glycosides preparations, this study does not alter Blue California's opinion that high purity steviol glycosides preparations are generally recognized as safe.

[8] While the purity of the material used for the study was not reported by Silva et al. (2018), a search of the manufacturer's website (www.svetia.us, accessed 1/29/20) indicates that the trademarked material is a blend of cane sugar and 97% pure Reb A.

Gu et al. (2019) investigated the *in vitro* effects of steviol glucuronide, the metabolite excreted into urine after ingestion of steviol glycosides, on insulin secretion from mouse pancreatic islets. The insulinotropic action of their metabolic end product, steviol glucuronide, only operates at high glucose level, but disappears at low glucose concentration, which is indicative of improved glycemic control with a good safety profile.

Kurek and Krejpcio (2019) reviewed the functional properties and biological effects of *Stevia rebaudiana* Bertoni and steviol glycosides. The authors did not identify any safety concerns with regard to the use of steviol glycosides in conventional foods.

Pham et al. (2019) reviewed the acute effects of nutritive and non-nutritive sweeteners, including steviol glycosides, on postprandial hypotension. The authors did not identify any publications regarding the acute effects of steviol glycosides on postprandial blood pressure and did not identify any safety concerns regarding the use of steviol glycosides in foods.

Blue California reviewed the recent steviol glycosides publications by Zhou et al. (2019), Wang and Wu (2019), and Sánchez-Delgado et al. (2019) and did not find any safety concerns with respect to the use of Rebaudioside I in foods under the proposed uses.

Blue California agrees with the safety conclusions of the 64 GRAS Expert Panels in the notifications for steviol glycosides previously submitted to FDA that resulted in "no questions" responses from FDA, JECFA (WHO, 2006; WHO, 2008), and Renwick (2008) that a sufficient number of good quality health and safety studies exist to support the determination that purified preparations of steviol glycosides, when added to food at levels up to full replacement of sucrose on a sweetness equivalency basis, meet FDA's definition of safe.

Blue California concludes that it is reasonable to apply the JECFA ADI of 4 mg per kg bw per day for steviol glycosides (expressed on a steviol basis) to BESTEVIA® Rebaudioside I. Therefore, with the steviol equivalence values shown in Table 5, Blue California concludes that, for the general population, the estimated maximum daily intake of Blue California's BESTEVIA® Rebaudioside I is 5.93 mg per kg bw or 1.67 mg per kg expressed as steviol equivalents. Based upon these calculations, the intake of Blue California's BESTEVIA® Rebaudioside I as described herein safely aligns with the 4 mg per kg bw per day ADI expressed as steviol equivalents as determined by JECFA.

Blue California's BESTEVIA® Rebaudioside I preparations contains not less than 95% Rebaudioside I. While Rebaudioside I has not been observed in *Stevia rebaudiana* Bertoni, it was first identified in the closely-related subspecies *Stevia rebaudiana* Morita. However, Rebaudioside I is listed in Appendix 1 in the 2017 JECFA monograph as a recognized steviol glycoside (FAO, 2017) and has been determined to be GRAS by FEMA (Cohen et al., 2019).

Given the structural similarities with Rebaudioside A, Stevioside, and other steviol glycosides, and considering analogous metabolic pathways for all these substances, the safety data on stevia and

its other components have a direct bearing on the present safety assessment for BESTEVIA® Rebaudioside I. This is further supported by over a decade and a half of scientific studies on the safety of these substances, along with the fact that the major regulatory bodies view the results of toxicology studies on either Stevioside or Rebaudioside A as applicable to the safety assessment of all known steviol glycosides, since all are metabolized and excreted by similar pathways, with steviol being the common metabolite for each. The foundational safety of Reb A, other steviol glycosides and steviol has been summarized, with key studies summarized in Appendix 10 and Appendix 12.

In addition, Blue California affirms that BESTEVIA® Rebaudioside I is manufactured under CGMP conditions with raw materials and processing aids that meet the appropriate food grade regulations. Blue California has established sufficient rigorous product specifications based upon FCC and JECFA monographs---which are consistent with other steviol glycosides on the market---and has demonstrated batch-to-batch consistency against these specifications.

Furthermore, Blue California has reviewed this safety information and has concluded that BESTEVIA® Rebaudioside I preparation is generally recognized as safe for the proposed uses.

## A. GRAS Criteria

FDA defines “safe” or “safety” as it applies to food ingredients as:

> “…reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use.”[9]

Amplification is provided in that the conclusion of safety is to include probable consumption of the substance in question, the cumulative effect of the substance and appropriate safety factors. It is FDA’s operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that:

> “…General recognition of safety requires common knowledge, throughout the expert scientific community knowledgeable about the safety of substances directly or indirectly added to food, that there is reasonable certainty that the substance is not harmful under the conditions of its intended use.”
>
> “‘Common knowledge’ can be based on either “scientific procedures” or on experience based on common use of a substance in food prior to January 1, 1958.”[10]

[9] See 21 CFR 170.3 (e)(i) and 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 9/8/18).

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following component elements:[11]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive. General recognition of safety through scientific procedures shall be based upon the application of generally available and accepted scientific data, information, or methods, which ordinarily are published, as well as the application of scientific principles, and may be corroborated by the application of unpublished scientific data, information, or methods.

The apparent imprecision of the terms "appreciable," "at the time," and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety in this or any other area (Lu, 1988; Renwick, 1990; Rulis and Levitt, 2009).

As noted below, this safety assessment to ascertain GRAS status for high purity steviol glycosides for the specified food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

## B. Expert Panel Findings on Safety of Blue California's BESTEVIA® Rebaudioside I

An evaluation of the safety and GRAS status of the intended use of Blue California's BESTEVIA® Rebaudioside I has been conducted by an Expert Panel convened by GRAS Associates; the Panel consisted of Robert Kapp, Ph.D., Fellow Academy of Toxicological Sciences (ATS), Fellow Royal Society of Biology (FRSB) & European Registered Toxicologist (ERT, UK); Kara Lewis, Ph.D.; and Katrina Emmel, Ph.D., as Panel Chair. The Expert Panel reviewed Blue California's dossier as well as other publicly available information available to them. The individuals who served as Expert Panelists are qualified to evaluate the safety of foods and food ingredients by merit of scientific training and experience.

The GRAS Expert Panel report is provided in Appendix 13.

[10] See 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 8/26/19).
[11] See Footnote 1.

## C. Common Knowledge Elements for GRAS Conclusions

The first common knowledge element for a GRAS conclusion requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The second common knowledge element for a GRAS conclusion requires that consensus exists within the broader scientific community.

### 1. Public Availability of Scientific Information

The majority of the studies reviewed on steviol glycosides and steviol have been published in the scientific literature as summarized in Appendix 10 and Appendix 12. Most of the literature relied upon by JECFA has also been published---most importantly, the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website.

With regard to the safety documentation, the key pharmacokinetic data establish that steviol glycosides are not absorbed through the gastrointestinal (GI) tract, *per se*; they are converted to steviol by bacteria normally present in the large intestine, and the steviol is absorbed but rapidly metabolized and excreted (Gardana et al., 2003; Koyama et al., 2003b). The action of bacteria in the large intestine is directly supported by the published study that showed that steviol glycosides can be converted to steviol in the large intestine by normal anaerobic GI flora as demonstrated by an *in vitro* study in fecal homogenates (Koyama et al., 2003b; Renwick and Tarka, 2008).

The ADI for steviol glycosides has been set largely based on a published chronic study in rats (Toyoda et al., 1997) and several published clinical studies that report no pharmacological effects in humans at doses several fold higher than the ADI (Barriocanal et al., 2006; Barriocanal et al., 2008; Wheeler et al., 2008). As mentioned previously, Roberts et al. (2016) noted that the ADI could be higher using a chemical-specific adjustment factor (CSAF), as defined by the WHO in 2005, determined by comparative studies in rats and humans, which they conclude can justify an ADI value of 6-16 mg per kg bw per day for steviol glycosides.

The toxicity of the metabolite, steviol, has been well reviewed in the published literature (Geuns, 2003; WHO, 2006; Urban et al., 2013; Brusick, 2008). There is a general consensus that steviol is not toxic and does not pose a safety risk following human consumption.

In addition, there is a large, publicly available, collection of GRNs regarding steviol glycosides on FDA’s website.

## 2. Scientific Consensus

The second common knowledge element for a GRAS conclusion requires that there be a basis to conclude that consensus exists among qualified scientists about the safety of the substance for its intended use.

A number of well-respected regulatory agencies, including JECFA, EFSA, FSANZ, the Switzerland Office of Public Health, and Health Canada, as well as numerous well-respected individual scientists, have indicated that steviol glycosides are safe for human consumption at doses in the range of the JECFA ADI (FAO, 2010; EFSA, 2010; FSANZ, 2008; Switzerland Federal Office of Public Health, 2008; Health Canada, 2012; Xili et al., 1992; Toyoda et al., 1997; Geuns, 2003; Williams, 2007). Since December 2008, 64 GRAS notices have been submitted to FDA for highly purified stevia-derived sweetener products, and FDA detailed reviews have consistently yielded "no questions" letters.

In summary, a compelling case can be made that scientific consensus exists regarding the safety of steviol glycosides when of sufficiently high purity. The central role of conversion to steviol and subsequent elimination with these naturally occurring steviol glycosides extends to the manner in which the various steviol glycosides molecules are metabolized and eliminated from the body. While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, Blue California believes that a wide consensus does exist in the scientific community to support a GRAS conclusion as evidenced by several publications (Carakostas, 2012; Geuns, 2007; Urban et al., 2013; Waddell, 2011; Williams, 2007; Brusick, 2008) that refute safety concerns expressed by a minority of scientists. Roberts et al. (2016) suggests that the ADI could be higher than has been previously accepted by the scientific community.

## D. Conclusion

In consideration of the aggregate safety information available on naturally occurring steviol glycosides, Blue California concludes that BESTEVIA® Rebaudioside I as defined in the subject notification is safe for use as a general purpose non-nutritive sweetener in foods other than infant formulas and meat and poultry products. The JECFA ADI for steviol glycosides of 4 mg per kg bw per day (as steviol equivalents) can be applied to Blue California's BESTEVIA® Rebaudioside I preparation. Based on published dietary exposure data for other approved sweeteners and adjusting for relative sweetness intensity, intake was estimated for healthy non-diabetic children and adults, and diabetic children and adults with the following findings.

The worst-case estimated intakes of Blue California's BESTEVIA® Rebaudioside I preparation for several population groups summarized in Part 3.A are no greater than 1.67 mg per kg steviol equivalents per bw per day, which is well below the ADI of 4 mg per kg bw expressed as steviol equivalents as established by JECFA. The dietary levels from anticipated food consumption are

not likely to exceed the ADI when BESTEVIA® Rebaudioside I is used as a general non-nutritive sweetener.

Accordingly, BESTEVIA® Rebaudioside I as produced by Blue California and declared within the subject notification meets FDA's definition of safety in that there is "reasonable certainty of no harm under the intended conditions of use" as described herein and, therefore, is generally recognized as safe (GRAS).

## PART 7. LIST OF SUPPORTING DATA AND INFORMATION IN THE GRAS NOTICE.

## A. References

**1. List of Acronyms**

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| ADME | Absorption, Distribution, Metabolism and Excretion |
| AKI | Acute kidney injury |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| ATS | Academy of Toxicological Sciences |
| AUC | Area under the plasma-concentration time curve |
| AVA | Agri-food and Veterinary Authority of Singapore |
| BP | Blood pressure |
| bw | Body Weight |
| CFR | Code of Federal Regulations |
| cfu | Colony Forming Unit |
| CGMP | Current Good Manufacturing Practice |
| COX-2 | Cyclooxygenase-2 |
| CSAF | Chemical-Specific Adjustment Factor |
| CYP | Cytochrome P450 |
| DBP | Diastolic blood pressure |
| DNA | Deoxyribonucleic Acid |
| EDI | Estimated daily intake |
| EFSA | European Food Safety Authority |
| EU | European Union |
| FCC | Food Chemicals Codex |
| FD&C | Federal Food Drug and Cosmetics Act |
| FDA | Food and Drug Administration |
| FEMA | Flavor Extract Manufacturers Association |
| FOIA | Freedom of Information Act |
| FRSB | Fellow Royal Society of Biology |
| FSANZ | Food Standards Australia New Zealand |
| FSSAI | Food Safety and Standards Authority of India |
| g | Gram |
| GA | GRAS Associates |
| GEMS | Global Environment Monitoring System |
| GGT | Gamma-glutamyltransferase |

| | |
|---|---|
| GI | Gastrointestinal |
| GRAS | Generally Recognized as Safe |
| HbA1c | Glycated hemoglobin |
| IADSA | International Alliance of Dietary/Food Supplement Associations |
| INS | International Numbering System |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| kg | Kilogram |
| L | Liter |
| $LD_{50}$ | Median lethal dose |
| mg | Milligram |
| Mins | Minutes |
| mL | Milliliter |
| MPL | Maximum permitted level |
| mpn | Most probable number |
| mRNA | Messenger ribonucleic acid |
| MW | Molecular Weight |
| ng | Nanogram |
| NHANES | National Health and Nutrition Examination Surveys |
| NHPs | Natural Health Products |
| NMT | Not more than |
| NOAEL | No observed adverse effect level |
| NOEL | No observed effect level |
| NS | Not specified |
| NSAIDs | Nonsteroidal anti-inflammatory drugs |
| $OD_{600}$ | Optical density at 600 nm |
| OECD | Organisation for Economic Co-operation and Development |
| ppm | Parts per million |
| Reb A | Rebaudioside A |
| Reb M | Rebaudioside M |
| SBP | Systolic blood pressure |
| SUS | Sucrose synthases |
| TAC | Total antioxidant capacity |
| TFC | Total flavonoid content |
| $T_{max}$ | Time to maximum plasma concentration |
| TPC | Total phenolic content |
| TRAP | Tartrate-resistant alkaline phosphatase |
| TRPM5 | Transient receptor potential cation channel subfamily melastatin member 5 |
| U.S. | United States |
| UDP | Uridine diphosphate |
| UDPG | Uridine diphosphate-glucose |
| UDS | Unscheduled DNA synthesis |
| ug | Microgram |
| UGT | Uridine 5’-diphosphouridine-glucosyltransferase |
| WHO | World Health Organization |
| WHO/JECFA | World Health Organizaiton/Joint FAO/WHO Expert Committee on Food Additives |

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## B. Appendices

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# Appendix 1 List of Scientific Publications Regarding the Synthesis of Rebaudioside I

1. Bioconversion of Rebaudioside I from Rebaudioside A. Molecules (2014) 19:17345-17355; (enzymatic bioconversion).

2. Functional genomics uncovers three glucosyltransferases involved in the synthesis of the major sweet glucosides of Stevia rebaudiana. The Plant Journal (2005) 41:56–67 (identification of UGTs (UGT76G1, UGT74G1 and UGT85C2) involve in steviol glycoside biosynthesis from stevia).

3. Synthesis of rebaudioside-A by enzymatic transglycosylation of stevioside present in the leaves of Stevia rebaudiana Bertoni. Food Chemistry (2016) 200:154–158 (Conversion of stevioside to Reb A by stevia leaf crude protein).

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# Appendix 2 Steviol Glycosides Raw Material Flow Charts and Specifications

**Appendix 2.1 Steviol Glycosides Aqueous Extract Raw Material Manufacturing Flow Chart**

**Appendix 2.2 Steviol Glycosides Aqueous Extract Raw Material Specifications**

**Appendix 2.3 Steviol Glycosides Aqueous Ethanol Extract Raw Material Manufacturing Flow Chart**

**Appendix 2.4 Steviol Glycosides Aqueous Ethanol Extract Raw Material Specifications**

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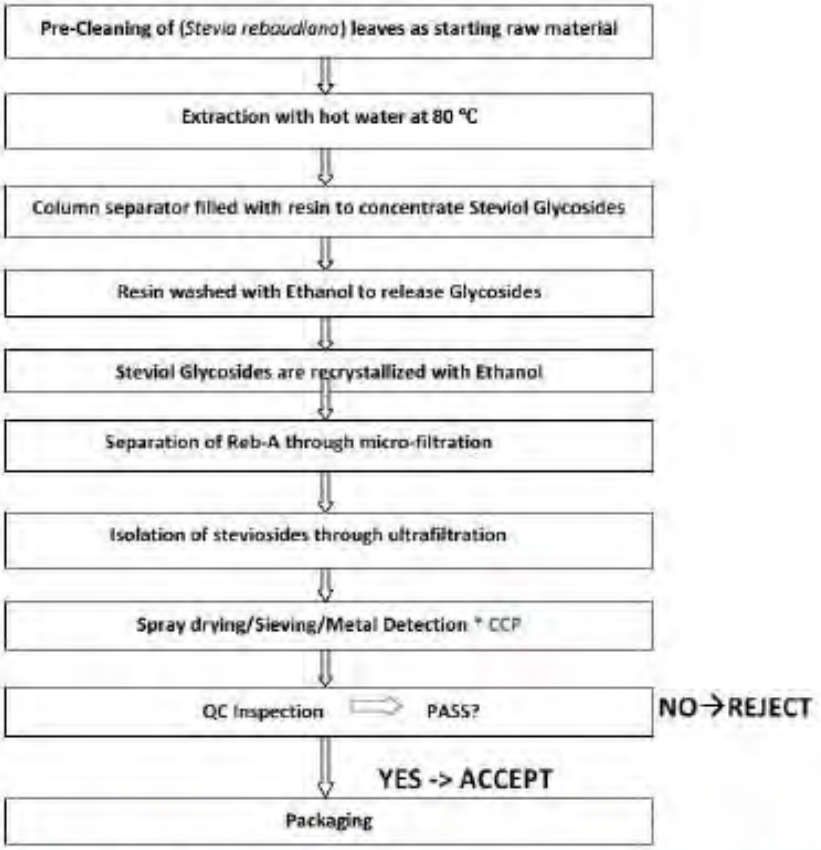
## Appendix 2.1 Steviol Glycosides Aqueous Extract Raw Material Manufacturing Flow Chart

Blue California
NATURE - SCIENCE - INNOVATION

A Perfect Blend of Science and Nature

Product Name: **Steviol Glycosides 95%**

### PROCESS DIAGRAM




* CCP: Metal detection. Physical Hazard: Metal impurities. Criterion: Metal equipment used in production line, such as mesh screens. In order to prevent chronic intoxication by metal impurities, product has to go through magnet and metal detector.

Corporate Headquarters
30111 Tomas, Rancho Santa Margarita, CA 92688 Tel: 949-635-1990 Fax: 949-635-1984
Website: www.bluecal-ingredients.com

## Appendix 2.2 Steviol Glycosides Aqueous Extract Raw Material Specifications

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

### PRODUCT SPECIFICATION

**Product: Steviol Glycosides 95% (*Stevia rebaudiana, leaves*)**
**Item# ST0301245**

| | | | |
|---|---|---|---|
| **Country of Origin:** | **China** | **Shelf life:** | **2 Years** |
| **Grade:** | **Food** | **Extraction Solvent:** | **Water** |

| ATTRIBUTES | SPECIFICATION | METHODS |
|---|---|---|
| APPEARANCE | WHITE POWDER | VISUAL |
| FOREIGN MATTER | ABSENT | VISUAL |
| ODOR | CHARACTERISTIC | OLFACTORY |
| TASTE | CHARACTERISTIC | GUSTATORY |
| **STEVIOL GLYCOSIDES** | **≥ 95%** | **HPLC** |
| **SOLUBILITY IN WATER** | **SOLUBLE** | **USP** |
| LOSS ON DRYING | ≤ 6% | USP |
| HEAVY METALS | < 10 ppm | USP |
| LEAD | < 1 ppm | ICP-MS |
| ARSENIC | < 1 ppm | ICP-MS |
| CADMIUM | < 1 ppm | ICP-MS |
| MERCURY | < 1 ppm | ICP-MS |
| Ethanol | < 5000 mg/kg | USP |
| pH | 4.5-7.0 | USP |
| ASH | < 1% | USP |
| BULK DENSITY | > 0.2 g/ml | USP |
| TAP DENSITY | ≥ 0.3 g/ml | USP |
| PARTICLE SIZE: | > 95% through Mesh #80 Sieve | USP |
| TOTAL PLATE COUNT | < 1,000 cfu/gm | AOAC |
| TOTAL COLIFORM | < 10 cfu/gm | AOAC |
| YEAST AND MOLDS | < 100 cfu/gm | AOAC |
| E. COLI: | NEGATIVE | AOAC |
| SALMONELLA | NEGATIVE | AOAC |

*Approved by:* [redacted] *( QC Manager )* *Revision date: 10-15-2018*

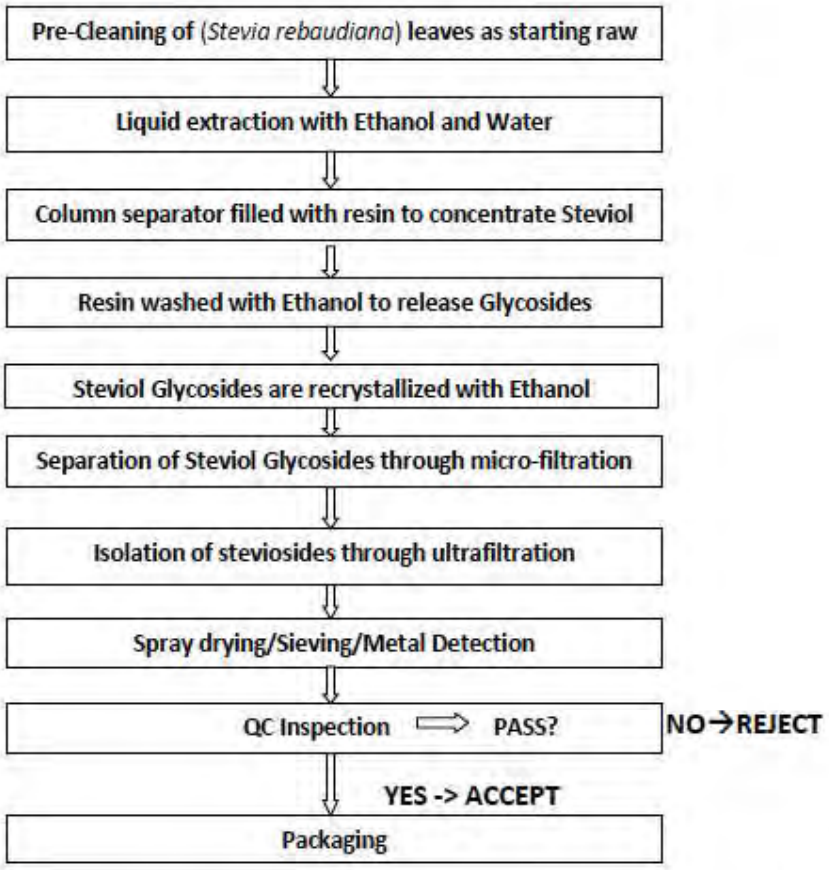
## Appendix 2.3 Steviol Glycosides Aqueous Ethanol Extract Raw Material Manufacturing Flow Chart

Blue California
NATURE + SCIENCE + INNOVATION

A Perfect Blend of Science and Nature

Product Name: **Stevia Extract 95%**

### PROCESS DIAGRAM




**Corporate Headquarters**
30111 Tomas, Rancho Santa Margarita, CA 92688 Tel: 949-635-1990 Fax: 949-635-1984
Website: www.bluecal-ingredients.com

## Appendix 2.4 Steviol Glycosides Aqueous Ethanol Extract Raw Material Specifications

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

### PRODUCT SPECIFICATION

**Product: Stevia Extract 95% (*Stevia rebaudiana, leaves*)**
**Item# ST0301238**

**Country of Origin:** China **Shelf life:** 2 Years
**Grade:** Food

| ATTRIBUTES | SPECIFICATION | METHODS |
|---|---|---|
| APPEARANCE | WHITE POWDER | VISUAL |
| FOREIGN MATTER | ABSENT | VISUAL |
| ODOR | CHARACTERISTIC | OLFACTORY |
| TASTE | CHARACTERISTIC | GUSTATORY |
| **STEVIOL GLYCOSIDES** | **≥ 95%** | **HPLC** |
| **SOLUBILITY IN WATER** | **SOLUBLE** | **USP** |
| LOSS ON DRYING | ≤ 6% | USP |
| HEAVY METALS | < 10 ppm | USP |
| LEAD | < 1 ppm | ICP-MS |
| ARSENIC | < 1 ppm | ICP-MS |
| CADMIUM | < 1 ppm | ICP-MS |
| MERCURY | < 1 ppm | ICP-MS |
| Methanol | < 200 mg/kg | GC |
| Ethanol | < 5000 mg/kg | GC |
| pH | 4.5-7.0 | USP |
| ASH | < 1% | USP |
| BULK DENSITY | > 0.2 g/ml | USP |
| TAP DENSITY | ≥ 0.3 g/ml | USP |
| PARTICLE SIZE: | > 95% through Mesh #80 Sieve | USP |
| TOTAL PLATE COUNT | < 1,000 cfu/gm | AOAC |
| TOTAL COLIFORM | < 10 cfu/gm | AOAC |
| YEAST AND MOLDS | < 100 cfu/gm | AOAC |
| E. COLI: | NEGATIVE | AOAC |
| SALMONELLA | NEGATIVE | AOAC |

*Approved by:* *( QC Manager )* *Revision date: 11-20-2018*

# Appendix 3 Regulatory Status of Raw Materials and Processing Aids Used in the Manufacture of BESTEVIA® Rebaudioside I

| Raw Material | Use | Regulatory Status | |
|---|---|---|---|
| | | 21 CFR | Approved Uses |
| Yeast extract | Fermentation and Culture Media | 184.1983 | Used as a flavoring agent and adjuvant at levels not to exceed 5% in food. |
| Yeast Peptone | Fermentation and Culture Media | 184.1553 | Peptones are GRAS affirmed for use as processing aids |
| Glycerol | Fermentation and Culture Media | | GRAS; standard material used within food industry |
| Potassium phosphate | Fermentation Medium | | GRAS; standard materials used within enzyme industry |
| Ferric chloride | Fermentation Medium | 184.1297 | Used as a nutrient supplement and processing aid with no limitation other than cGMP |
| Ammonia | Fermentation Medium | 184.1139 | Used as a leavening agent, pH control agent, surface- finished agent, and boiler water additive with no limitation other than cGMP |
| Sucrose/ sugar | Reaction Medium | | GRAS; standard material used within food industry |
| UDP-glucose | Reaction Medium | | FDA's approval of UDP-Glucose is noted in GRAS notice # 00045, GRN 000626 and GRN 000106 |
| Ethanol | Elution solvent Crystallization | 182.1 | GRAS when used in accordance with cGMP<br>JECFA specifications for steviol glycosides specify a level of not more than 5,000 ppm for ethanol residues |
| Methanol | Fermentation media and Crystallization | 182.1 | GRAS when used in accordance with cGMP<br>JECFA specifications for steviol glycosides specify a level of not more than 200 ppm for methanol residues |
| Activated charcoal | Decolorizing agent | | GRAS; standard material used within the food industry |
| Microporous resin | Purification | | Used in accordance with §173.25 |

# Appendix 4 Analytical Method

Please refer to the Appendix 4 document, provided as a separate file.

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# Appendix 5 Certificates of Analysis for Multiple Lots of BESTEVIA® Rebaudioside I

**Appendix 5.1 BESTEVIA® Rebaudioside I Lot 783-180510**

**Appendix 5.2 BESTEVIA® Rebaudioside I Lot 783-180565**

**Appendix 5.3 BESTEVIA® Rebaudioside I Lot 783-180607**

**Appendix 5.4 BESTEVIA® Rebaudioside I Lot 78618111226**

**Appendix 5.5 BESTEVIA® Rebaudioside I Lot 78618112826**

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## Appendix 5.1 BESTEVIA® Rebaudioside I Lot 783-180510

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

# CERTIFICATE OF ANALYSIS

**Product: BESTEVIA® Rebaudioside I 95%**
**Item#: BE1707831**

| | | | |
|---|---|---|---|
| **Lot No:** | **783-180510** | **Original Manufacturer:** | **Blue California Co.** |
| **Date of Manufacturing:** | **June 14-2018** | **Expiration/Re-test date:** | **June 14-2020** |
| **QC acceptance date:** | **July 22-2018** | | |

**This product has NOT been treated by Irradiation or ETO**

| ATTRIBUTES | SPECIFICATION | METHODS | RESULTS |
|---|---|---|---|
| APPEARANCE | White to off white powder | VISUAL | PASS |
| FOREIGN MATTER | ABSENT | VISUAL | PASS |
| ODOR | CHARACTERISTIC | OLFACTORY | PASS |
| TASTE | CHARACTERISTIC | GUSTATORY | PASS |
| **REBAUDIOSIDE I** | **≥ 95%** | **HPLC** | **97.91%** |
| LOSS ON DRYING | ≤ 6% | USP 34 | 1.01% |
| HEAVY METALS | < 10 ppm | USP 34 | PASS |
| ARSENIC | < 0.5 ppm | ICP-MS | < 0.5 ppm |
| CADMIUM | < 0.5 ppm | ICP-MS | < 0.25 ppm |
| LEAD | < 0.5 ppm | ICP-MS | 0.11 ppm |
| MERCURY | < 0.5 ppm | ICP-MS | < 0.1 ppm |
| ETHANOL | < 1,000 ppm | USP 34 | < 200 ppm |
| METHANOL | < 200 ppm | USP 34 | < 100 ppm |
| ASH | ≤ 1% | USP 34 | 0.13% |
| SOLUBILITY | 50:50 (Water:Ethanol) | JECFA | 1000 ppm |
| PH | 4.5-7.0 | USP 34 | 6.47 |
| BULK DENSITY | ≥ 0.15 g/ml | USP 34 | PASS |
| TAP DENSITY | ≥ 0.30 g/ml | USP 34 | PASS |
| PARTICLE SIZE: | > 90% through Mesh #80 Sieve | USP 34 | PASS |
| TOTAL PLATE COUNT | < 1,000 cfu/gm | AOAC | < 1,000 cfu/gm |
| TOTAL COLIFORM | < 10 cfu/gm | AOAC | < 3 cfu/gm |
| YEAST AND MOLDS | < 100 cfu/gm | AOAC | < 50 cfu/gm |
| E. COLI: | NEGATIVE | AOAC | NEGATIVE |
| SALMONELLA | NEGATIVE | AOAC | NEGATIVE |
| SHELF LIFE | 2 YEARS | HPLC | PASS |

*Approved by:* [redacted] *( QC Manager )* *Revision date: 09-17-2019*

## Appendix 5.2 BESTEVIA® Rebaudioside I Lot 783-180565

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

# CERTIFICATE OF ANALYSIS

**Product: BESTEVIA® Rebaudioside I 95%**
**Item#: BE1707831**

| | | | |
|---|---|---|---|
| **Lot No:** | **783-180565** | **Original Manufacturer:** | **Blue California Co.** |
| **Date of Manufacturing:** | **May 10-2018** | **Expiration/Re-test date:** | **May 10-2020** |
| **QC acceptance date:** | **May 22-2018** | | |

**This product has NOT been treated by Irradiation or ETO**

| ATTRIBUTES | SPECIFICATION | METHODS | RESULTS |
|---|---|---|---|
| APPEARANCE | White to off white powder | VISUAL | PASS |
| FOREIGN MATTER | ABSENT | VISUAL | PASS |
| ODOR | CHARACTERISTIC | OLFACTORY | PASS |
| TASTE | CHARACTERISTIC | GUSTATORY | PASS |
| **REBAUDIOSIDE I** | **≥95%** | **HPLC** | **98.91%** |
| LOSS ON DRYING | ≤6% | USP 34 | 1.6% |
| HEAVY METALS | < 10 ppm | USP 34 | PASS |
| ARSENIC | < 0.5 ppm | ICP-MS | < 0.5 ppm |
| CADMIUM | < 0.5 ppm | ICP-MS | < 0.25 ppm |
| LEAD | < 0.5 ppm | ICP-MS | 0.12 ppm |
| MERCURY | < 0.5 ppm | ICP-MS | < 0.1 ppm |
| ETHANOL | < 1,000 ppm | USP 34 | < 200 ppm |
| METHANOL | < 200 ppm | USP 34 | < 100 ppm |
| ASH | ≤1% | USP 34 | 0.10% |
| SOLUBILITY | 50:50 (Water:Ethanol) | JECFA | 1000 ppm |
| PH | 4.5-7.0 | USP 34 | 6.35 |
| BULK DENSITY | ≥0.15 g/ml | USP 34 | PASS |
| TAP DENSITY | ≥0.30 g/ml | USP 34 | PASS |
| PARTICLE SIZE: | > 90% through Mesh #80 Sieve | USP 34 | PASS |
| TOTAL PLATE COUNT | < 1,000 cfu/gm | AOAC | < 1,000 cfu/gm |
| TOTAL COLIFORM | < 10 cfu/gm | AOAC | < 5 cfu/gm |
| YEAST AND MOLDS | < 100 cfu/gm | AOAC | < 50 cfu/gm |
| E. COLI: | NEGATIVE | AOAC | NEGATIVE |
| SALMONELLA | NEGATIVE | AOAC | NEGATIVE |
| SHELF LIFE | 2 YEARS | HPLC | PASS |

*Approved by:* [redacted] *( QC Manager )* *Revision date: 09-17-2019*

## Appendix 5.3 BESTEVIA® Rebaudioside I Lot 783-180607

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

# CERTIFICATE OF ANALYSIS

**Product: BESTEVIA® Rebaudioside I 95%**
**Item#: BE1707831**

| | | | |
|---|---|---|---|
| **Lot No:** | **783-180607** | **Original Manufacturer:** | **Blue California Co.** |
| **Date of Manufacturing:** | **June 06-2018** | **Expiration/Re-test date:** | **June 06-2020** |
| **QC acceptance date:** | **June 18-2018** | | |

**This product has NOT been treated by Irradiation or ETO**

| ATTRIBUTES | SPECIFICATION | METHODS | RESULTS |
|---|---|---|---|
| APPEARANCE | White to off white powder | VISUAL | PASS |
| FOREIGN MATTER | ABSENT | VISUAL | PASS |
| ODOR | CHARACTERISTIC | OLFACTORY | PASS |
| TASTE | CHARACTERISTIC | GUSTATORY | PASS |
| **REBAUDIOSIDE I** | **≥ 95%** | **HPLC** | **95.92%** |
| LOSS ON DRYING | ≤ 6% | USP 34 | 1.26% |
| HEAVY METALS | < 10 ppm | USP 34 | PASS |
| ARSENIC | < 0.5 ppm | ICP-MS | < 0.5 ppm |
| CADMIUM | < 0.5 ppm | ICP-MS | < 0.25 ppm |
| LEAD | < 0.5 ppm | ICP-MS | 0.11 ppm |
| MERCURY | < 0.5 ppm | ICP-MS | < 0.1 ppm |
| ETHANOL | < 1,000 ppm | USP 34 | < 200 ppm |
| METHANOL | < 200 ppm | USP 34 | < 100ppm |
| ASH | ≤ 1% | USP 34 | 0.15% |
| SOLUBILITY | 50:50 (Water:Ethanol) | JECFA | 1000 ppm |
| PH | 4.5-7.0 | USP 34 | 6.20 |
| BULK DENSITY | ≥ 0.15 g/ml | USP 34 | PASS |
| TAP DENSITY | ≥ 0.30 g/ml | USP 34 | PASS |
| PARTICLE SIZE: | > 90% through Mesh #80 Sieve | USP 34 | PASS |
| TOTAL PLATE COUNT | < 1,000 cfu/gm | AOAC | < 1,000 cfu/gm |
| TOTAL COLIFORM | < 10 cfu/gm | AOAC | < 5 cfu/gm |
| YEAST AND MOLDS | < 100 cfu/gm | AOAC | < 50 cfu/gm |
| E. COLI: | NEGATIVE | AOAC | NEGATIVE |
| SALMONELLA | NEGATIVE | AOAC | NEGATIVE |
| SHELF LIFE | 2 YEARS | HPLC | PASS |

*Approved by:* [redacted] *( QC Manager )* *Revision date: 09-17-2019*

## Appendix 5.4 BESTEVIA® Rebaudioside I Lot 78618111226

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

# CERTIFICATE OF ANALYSIS

**Product: BESTEVIA® Rebaudioside I 95%**
**Item#: BE1707831**

| | | | |
|---|---|---|---|
| **Lot No:** | **78618111226** | **Original Manufacturer:** | **Blue California Co.** |
| **Date of Manufacturing:** | **November 12-2018** | **Expiration/Re-test date:** | **November 12-2020** |
| **QC acceptance date:** | **January 10-2019** | | |

**This product has NOT been treated by Irradiation or ETO**

| ATTRIBUTES | SPECIFICATION | METHODS | RESULTS |
|---|---|---|---|
| APPEARANCE | White to off white powder | VISUAL | PASS |
| FOREIGN MATTER | ABSENT | VISUAL | PASS |
| ODOR | CHARACTERISTIC | OLFACTORY | PASS |
| TASTE | CHARACTERISTIC | GUSTATORY | PASS |
| **REBAUDIOSIDE I** | **≥95%** | **HPLC** | **97.41%** |
| LOSS ON DRYING | ≤6% | USP 34 | 1.42% |
| HEAVY METALS | <10 ppm | USP 34 | PASS |
| ARSENIC | <0.5 ppm | ICP-MS | <0.02 ppm |
| CADMIUM | <0.5 ppm | ICP-MS | <0.01ppm |
| LEAD | <0.5 ppm | ICP-MS | 0.11 ppm |
| MERCURY | <0.5 ppm | ICP-MS | <0.01 ppm |
| ETHANOL | <1,000 ppm | USP 34 | <200 ppm |
| METHANOL | <200 ppm | USP 34 | <100 ppm |
| ASH | ≤1% | USP 34 | 0.12% |
| SOLUBILITY | 50:50 (Water:Ethanol) | JECFA | 1000 ppm |
| PH | 4.5-7.0 | USP 34 | 6.55 |
| BULK DENSITY | ≥0.15 g/ml | USP 34 | PASS |
| TAP DENSITY | ≥0.30 g/ml | USP 34 | PASS |
| PARTICLE SIZE: | >90% through Mesh #80 Sieve | USP 34 | PASS |
| TOTAL PLATE COUNT | <1,000 cfu/gm | AOAC | <1,000 cfu/gm |
| TOTAL COLIFORM | <10 cfu/gm | AOAC | <3 cfu/gm |
| YEAST AND MOLDS | <100 cfu/gm | AOAC | <40 cfu/gm |
| E. COLI: | NEGATIVE | AOAC | NEGATIVE |
| SALMONELLA | NEGATIVE | AOAC | NEGATIVE |
| SHELF LIFE | 2 YEARS | HPLC | PASS |

*Approved by:* [redacted] *( QC Manager )* *Revised date: 09-17-2019*

## Appendix 5.5 BESTEVIA® Rebaudioside I Lot 78618112826

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

# CERTIFICATE OF ANALYSIS

**Product: BESTEVIA® Rebaudioside I 95%**
**Item#: BE1707831**

| | | | |
|---|---|---|---|
| **Lot No:** | **78618112826** | **Original Manufacturer:** | **Blue California Co.** |
| **Date of Manufacturing:** | **November 28-2018** | **Expiration/Re-test date:** | **November 28-2020** |
| **QC acceptance date:** | **January 07-2019** | | |

**This product has NOT been treated by Irradiation or ETO**

| ATTRIBUTES | SPECIFICATION | METHODS | RESULTS |
|---|---|---|---|
| APPEARANCE | White to off white powder | VISUAL | PASS |
| FOREIGN MATTER | ABSENT | VISUAL | PASS |
| ODOR | CHARACTERISTIC | OLFACTORY | PASS |
| TASTE | CHARACTERISTIC | GUSTATORY | PASS |
| **REBAUDIOSIDE I** | **≥95%** | **HPLC** | **98.41%** |
| LOSS ON DRYING | ≤6% | USP 34 | 1.45% |
| HEAVY METALS | < 10 ppm | USP 34 | PASS |
| ARSENIC | < 0.5 ppm | ICP-MS | < 0.5 ppm |
| CADMIUM | < 0.5 ppm | ICP-MS | < 0.25 ppm |
| LEAD | < 0.5 ppm | ICP-MS | 0.11 ppm |
| MERCURY | < 0.5 ppm | ICP-MS | < 0.1 ppm |
| ETHANOL | < 1,000 ppm | USP 34 | < 200 ppm |
| METHANOL | < 200 ppm | USP 34 | < 100 ppm |
| ASH | ≤1% | USP 34 | 0.09% |
| SOLUBILITY | 50:50 (Water:Ethanol) | JECFA | 1000 ppm |
| PH | 4.5-7.0 | USP 34 | 6.25 |
| BULK DENSITY | ≥0.15 g/ml | USP 34 | PASS |
| TAP DENSITY | ≥0.30 g/ml | USP 34 | PASS |
| PARTICLE SIZE: | > 90% through Mesh #80 Sieve | USP 34 | PASS |
| TOTAL PLATE COUNT | < 1,000 cfu/gm | AOAC | < 1,000 cfu/gm |
| TOTAL COLIFORM | < 10 cfu/gm | AOAC | < 5 cfu/gm |
| YEAST AND MOLDS | < 100 cfu/gm | AOAC | < 30 cfu/gm |
| E. COLI: | NEGATIVE | AOAC | NEGATIVE |
| SALMONELLA | NEGATIVE | AOAC | NEGATIVE |
| SHELF LIFE | 2 YEARS | HPLC | PASS |

*Approved by:* [redacted] *( QC Manager )* *Revision date: 09-17-2019*

# Appendix 6 Analytical Chromatograms for Multiple Production Lots of BESTEVIA® Rebaudioside I

**Appendix 6.1 BESTEVIA® Rebaudioside I Lot 783-180510**

**Appendix 6.2 BESTEVIA® Rebaudioside I Lot 783-180565**

**Appendix 6.3 BESTEVIA® Rebaudioside I Lot 783-180607**

**Appendix 6.4 BESTEVIA® Rebaudioside I Lot 78618111226**

**Appendix 6.5 BESTEVIA® Rebaudioside I Lot 78618112826**

Note: The following key identifies corresponding analytical sample and production batch numbers

1. Eurofins sample **740-2019-06120037**, Reb I, Powder, Lot # 78618112826

2. Eurofins sample **740-2019-06120038**, Reb I, Powder, Lot # 783-180510

3. Eurofins sample **740-2019-06120039**, Reb I, Powder, Lot # 78618111226

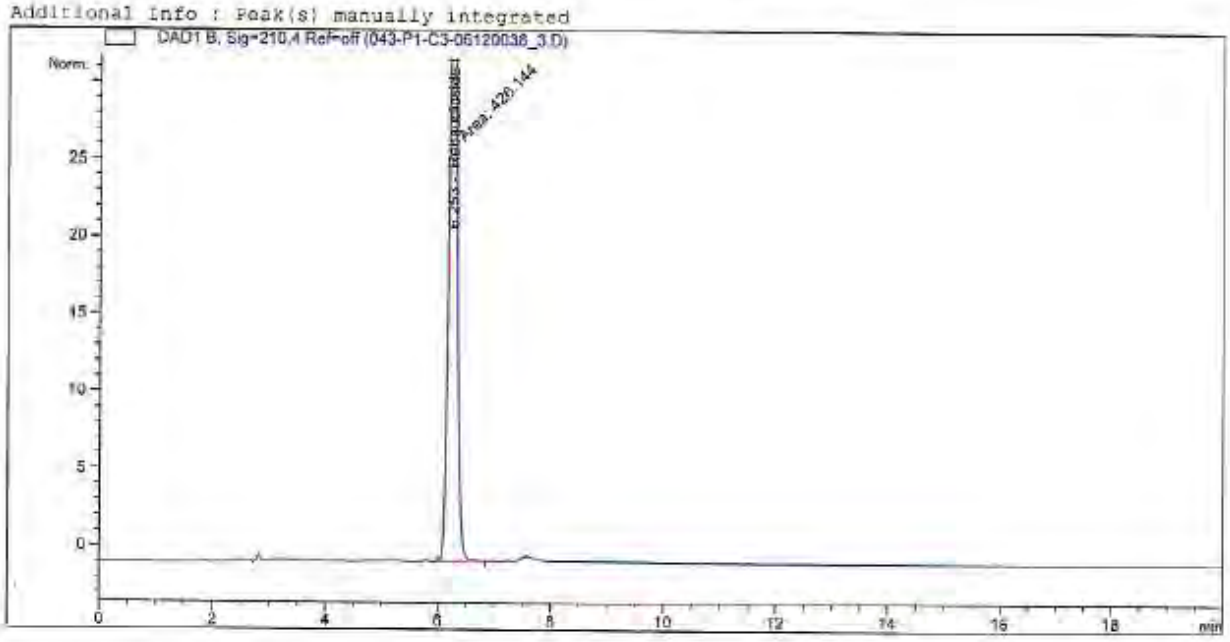
4. Eurofins sample **740-2019-06120040**, Reb I, Powder, Lot # 783-180565

5. Eurofins sample **740-2019-06120041**, Reb I, Powder, Lot # 783-180607

## Appendix 6.1 BESTEVIA® Rebaudioside I Lot 783-180510

Sample Name: 06120038_3

```
Acq. Operator   : SYSTEM                          Seq. Line :  43
Acq. Instrument : HPLC24                           Location :  P1-C3
Injection Date  : 6/25/2019 7:44:51 PM                Inj :  1
                                                  Inj Volume : 5.000 µl
Sequence File   : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\kk262_LC_2019_z1.S
Acq. Method     : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\KK262 offline.M
Last changed    : 6/25/2019 4:24:40 PM by SYSTEM
Analysis Method : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\KK262 offline.M (
                  Sequence Method)
Last changed    : 7/11/2019 11:35:41 PM by SYSTEM
Method Info     : KK262 JECFA Steviol Glycoside Method Workstation LC24

Additional Info : Peak(s) manually integrated
```

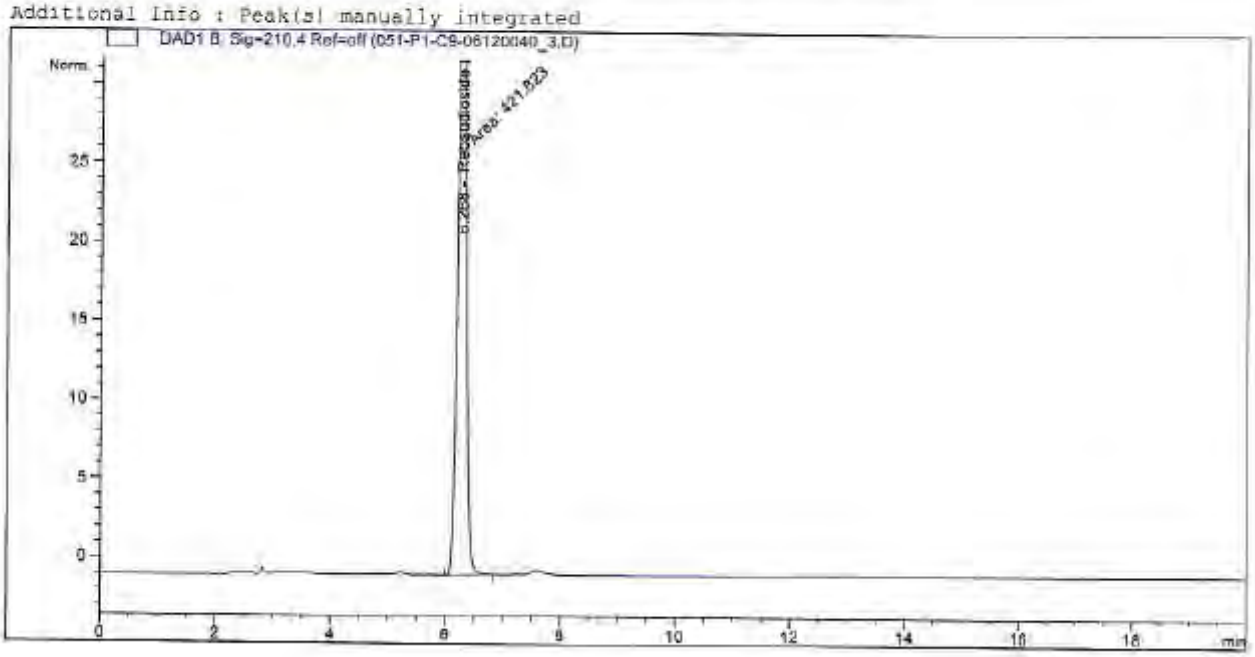



```
                        ESTD Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      7/11/2019 11:35:41 PM
Multiplier            :      1.0000
Dilution              :      40.0000
Sample Amount:                :     20.40000  [%]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=210,4 Ref=off

RetTime  Type      Area      Amt/Area    Amount    Grp   Name
 [min]           |mAU*s|                   %
--------|------|----------|----------|----------|--|------------------
  6.253 MM      426.14413 1.13885e-3  95.159964     Rebaudioside I

Totals :                              95.159964
```

## Appendix 6.2 BESTEVIA® Rebaudioside I Lot 783-180565

Sample Name: 06120040_3

```
Acq. Operator    : SYSTEM                                  Seq. Line :  51
Acq. Instrument  : HPLC24                                   Location :  P1-C9
Injection Date   : 6/25/2019 11:53:52 PM                        Inj :  1
                                                          Inj Volume : 5.000 µl
Sequence File    : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\kk262_LC_2019_z1.S
Acq. Method      : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\KK262 offline.M
Last changed     : 6/25/2019 10:01:05 PM by SYSTEM
Analysis Method  : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\KK262 offline.M (
                   Sequence Method)
Last changed     : 7/11/2019 11:35:41 PM by SYSTEM
Method Info      : KK262 JECFA Steviol Glycoside Method Workstation LC24

Additional Info : Peak(s) manually integrated
```

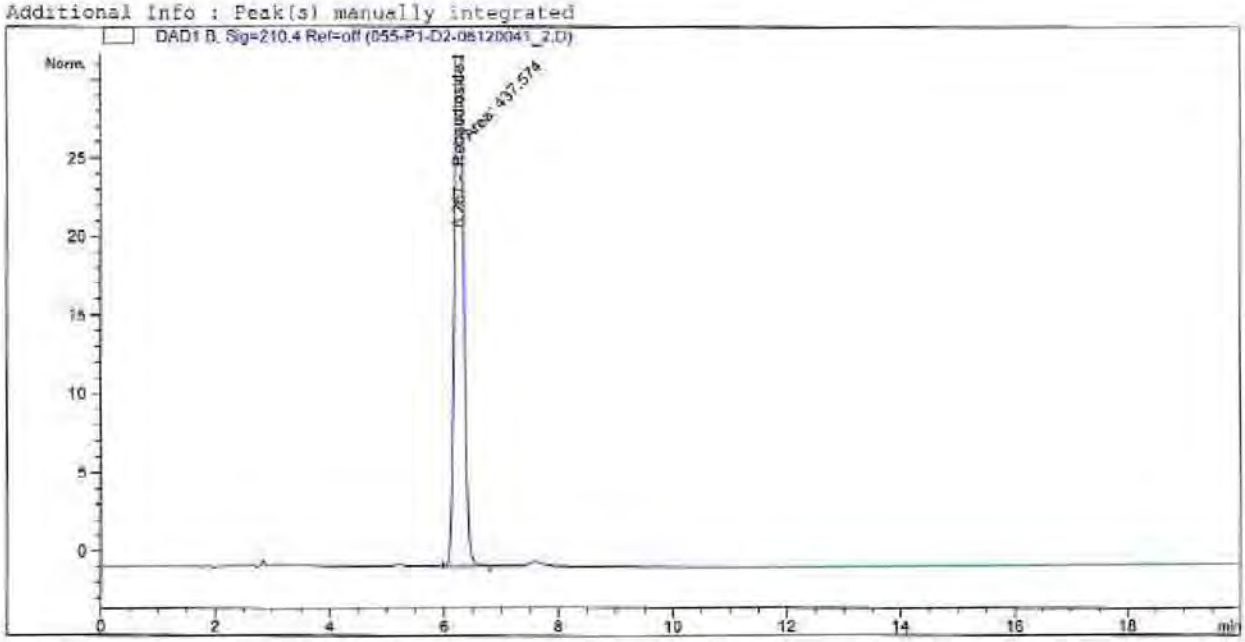



```
                          ESTD Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      7/11/2019 11:35:41 PM
Multiplier            :      1.0000
Dilution              :      40.0000
Sample Amount:                     20.41000  [%]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=210,4 Ref=off

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          [mAU*s]                   %
-------|------|----------|----------|----------|--|------------------
  6.268 MM      421.82336 1.33805e-3  94.148964     Rebaudioside I

Totals :                              94.148964
```

## Appendix 6.3 BESTEVIA® Rebaudioside I Lot 783-180607

Sample Name: 08120041_2

```
Acq. Operator   : SYSTEM                          Seq. Line :  55
Acq. Instrument : HPLC24                           Location :  P1-D2
Injection Date  : 6/26/2019 1:58:26 AM                 Inj :  1
                                                  Inj Volume : 5.000 µl
Sequence File   : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\kk262_LC_2019_z1.S
Acq. Method     : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\KK262 offline.M
Last changed    : 6/25/2019 10:01:05 PM by SYSTEM
Analysis Method : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\KK262 offline.M (
                  Sequence Method)
Last changed    : 7/11/2019 11:35:41 PM by SYSTEM
Method Info     : KK262 JECFA Steviol Glycoside Method Workstation LC24

Additional Info : Peak(s) manually integrated
```

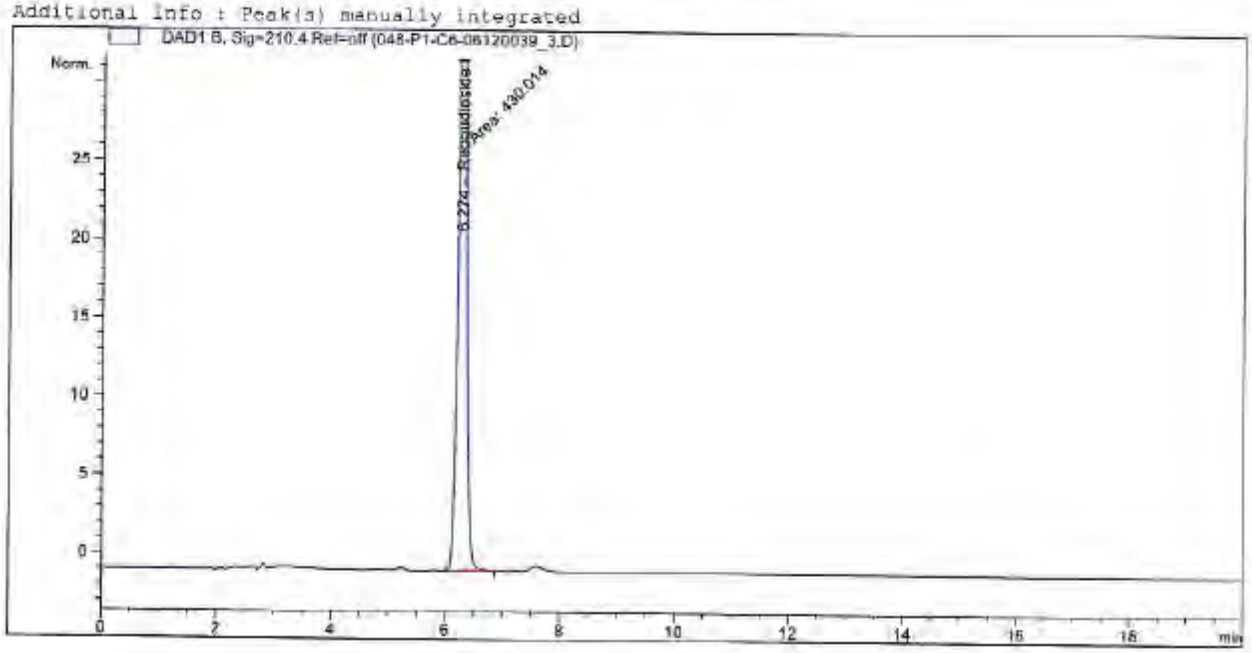



```
                          ESTD Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      7/11/2019 11:35:41 PM
Multiplier            :      1.0000
Dilution              :      40.0000
Sample Amount:                :     20.96000  [%]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=210,4 Ref=off

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [mAU*s]                  %
-------|------|----------|----------|----------|--|------------------
  6.267 MM      437.57367 1.13805e-3  95.101600    Rebaudioside I

Totals :                              95.101600
```

## Appendix 6.4 BESTEVIA® Rebaudioside I Lot 78618111226

Sample Name: 06120039_3

```
Acq. Operator   : SYSTEM                                  Seq. Line :  48
Acq. Instrument : HPLC24                                   Location :  P1-C6
Injection Date  : 6/25/2019 10:20:33 PM                        Inj :  1
                                                       Inj Volume : 5.000 µl
Sequence File   : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\kk262_LC_2019_z1.S
Acq. Method     : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\KK262 offline.M
Last changed    : 6/25/2019 10:01:05 PM by SYSTEM
Analysis Method : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\KK262 offline.M (
                  Sequence Method)
Last changed    : 7/11/2019 11:35:41 PM by SYSTEM
Method Info     : KK262 JECFA Steviol Glycoside Method Workstation LC24

Additional Info : Peak(s) manually integrated
```

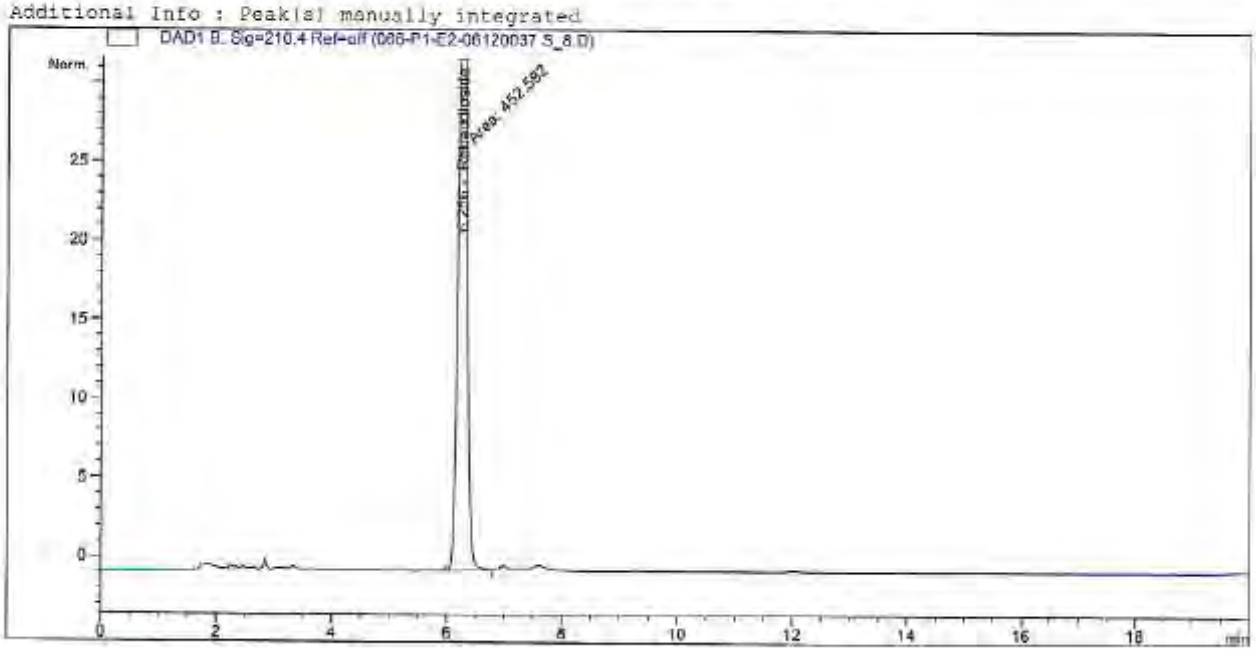



```
                          ESTD Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      7/11/2019 11:35:41 PM
Multiplier            :      1.0000
Dilution              :     40.0000
Sample Amount:                :     20.60000  [%]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=210,4 Ref=off

RetTime  Type      Area      Amt/Area     Amount    Grp   Name
 [min]           [mAU*s]                    %
-------|------|----------|----------|----------|--|------------------
  6.274 MM       430.01407 1.13885e-3  95.091865     Rebaudioside I

Totals :                               95.091865
```

## Appendix 6.5 BESTEVIA® Rebaudioside I Lot 78618112826

Sample Name: 06120037 S_8

```
Acq. Operator   : SYSTEM                          Seq. Line :  66
Acq. Instrument : HPLC24                           Location :  P1-E2
Injection Date  : 6/26/2019 7:40:56 AM                 Inj :  1
                                                  Inj Volume : 5.000 µl
Sequence File   : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\kk262_LC_2019_z1.S
Acq. Method     : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\KK262 offline.M
Last changed    : 6/25/2019 10:01:05 PM by SYSTEM
Analysis Method : C:\Chem32\1\Data\kk262_LC_2019_z1 2019-06-24 21-56-06\KK262 offline.M (
                  Sequence Method)
Last changed    : 7/11/2019 11:35:41 PM by SYSTEM
Method Info     : KK262 JECFA Steviol Glycoside Method Workstation LC24

Additional Info : Peak(s) manually integrated
```




```
                        ESTD Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      7/11/2019 11:35:41 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      5.57550e-1  [%]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=210,4 Ref=off

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]           [mAU*s]                  %
-------|------|----------|----------|----------|--|------------------
  6.256 MM      452.59238 1.13885e-3  92.446685     Rebaudioside I

Totals :                              92.446685
```

# Appendix 7 Pesticide Testing Reports for BESTEVIA® Rebaudioside I

**Appendix 7.1 BESTEVIA® Rebaudioside I Lot 783-180510**

**Appendix 7.2 BESTEVIA® Rebaudioside I Lot 783-180565**

**Appendix 7.3 BESTEVIA® Rebaudioside I Lot 783-180607**

**Appendix 7.4 BESTEVIA® Rebaudioside I Lot 78618111226**

**Appendix 7.5 BESTEVIA® Rebaudioside I Lot 78618112826**

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## Appendix 7.1 BESTEVIA® Rebaudioside I Lot 783-180510

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 7570
Tel.+1 707 792 7300

July 15, 2019

Hadi Omrani
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

**CERTIFICATE OF ANALYSIS**
AR-19-KK-008363-01
Batch #: EUCAPE-00109704

**Sample Identification:**
Sample #: 740-2019-01290009
Description: BESTEVIA® Reb I 95%, Powder, Lot #783-180510
Condition: Acceptable
Date Received: January 29, 2019

**QA12C: Pesticides - USP 561 Screen (USP 42)**
**Method Reference:** USP 561
**Completed:** 02/13/2019

| | Result | Theoretical Level |
|---|---|---|
| Acephate | <0.1 mg/kg | |
| Alachlor | <0.02 mg/kg | |
| Aldrin and Dieldrin (sum of) | <0.02 mg/kg | |
| Azinphos-ethyl | <0.02 mg/kg | |
| Azinphos-methyl | <0.05 mg/kg | |
| Bromophos-ethyl | <0.02 mg/kg | |
| Bromophos-methyl | <0.02 mg/kg | |
| Bromopropylate | <0.05 mg/kg | |
| Chlordane (sum of cis-, trans- and Oxychlordane) | <0.05 mg/kg | |
| Chlorfenvinphos | <0.02 mg/kg | |
| Chlorpyrifos-ethyl | <0.02 mg/kg | |
| Chlorpyrifos-methyl | <0.02 mg/kg | |
| Chlorthal-dimethyl | <0.01 mg/kg | |
| Cyfluthrin (sum of) | <0.1 mg/kg | |
| Cyhalothrin, lambda-(incl. Cyhalothrin, gamma-) | <0.02 mg/kg | |
| Cypermethrin and isomers (sum of) | <0.1 mg/kg | |
| DDT (total) | <0.02 mg/kg | |
| Deltamethrin | <0.1 mg/kg | |
| Diazinon | <0.02 mg/kg | |
| Dichlofluanid | <0.02 mg/kg | |
| Dichlorvos | <0.02 mg/kg | |
| Dicofol | <0.02 mg/kg | |
| Dimethoate/Omethoate (sum) | <0.1 mg/kg | |
| Endosulfan (sum of isomers and endo. sulfate) | <0.02 mg/kg | |
| Endrin | <0.02 mg/kg | |
| Ethion | <0.02 mg/kg | |
| Etrimfos | <0.05 mg/kg | |
| Fenchlorphos (sum) | <0.1 mg/kg | |
| Fenitrothion | <0.02 mg/kg | |
| Fenpropathrin | <0.03 mg/kg | |
| Fensulfothion (sum of parent, -oxons and sulfones) | <0.05 mg/kg | |

eurofins Sample #: 740-2019-01290009

Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA
92688

**QA12C: Pesticides - USP 561 Screen (USP 42)**
**Method Reference:** USP 561
**Completed:** 02/13/2019

| | Result | Theoretical Level |
|---|---|---|
| Fenthion (sum of fenthion, -oxons, -sulfones) | <0.05 mg/kg | |
| Fenvalerate | <0.2 mg/kg | |
| Flucythrinate | <0.05 mg/kg | |
| Fluvalinate, tau- | <0.05 mg/kg | |
| Fonofos | <0.02 mg/kg | |
| Heptachlor (heptachlor+ cis-, trans- h. epoxide | <0.03 mg/kg | |
| Hexachlorobenzene | <0.01 mg/kg | |
| Hexachlorocyclohexane isomers (other than gamma) | <0.02 mg/kg | |
| Lindane (gamma-HCH) | <0.01 mg/kg | |
| Malathion and malaoxon (sum of) | <0.02 mg/kg | |
| Mecarbam | <0.05 mg/kg | |
| Methacriphos | <0.05 mg/kg | |
| Methamidophos | <0.05 mg/kg | |
| Methidathion | <0.02 mg/kg | |
| Methoxychlor | <0.05 mg/kg | |
| Mirex | <0.01 mg/kg | |
| Monocrotophos | <0.1 mg/kg | |
| Parathion-ethyl and Paraoxon-ethyl (sum of) | <0.2 mg/kg | |
| Parathion-methyl and Paraoxon-methyl (sum of) | <0.2 mg/kg | |
| Pendimethalin | <0.1 mg/kg | |
| Pentachloranisole | <0.01 mg/kg | |
| Permethrin and isomers (sum of) | <0.2 mg/kg | |
| Phosalone | <0.04 mg/kg | |
| Phosmet | <0.05 mg/kg | |
| Piperonyl butoxide (PBO) | <1 mg/kg | |
| Pirimiphos-ethyl | <0.05 mg/kg | |
| Pirimiphos-methyl (incl. N-desethyl-) | <0.1 mg/kg | |
| Procymidone | <0.1 mg/kg | |
| Profenofos | <0.1 mg/kg | |
| Prothiofos | <0.05 mg/kg | |
| Pyrethrum (sum of cinerins, jasmolins, pyrethrins) | <3 mg/kg | |
| Quinalphos | <0.05 mg/kg | |
| Quintozene (sum quintozene,pentachloraniline,MPPS) | <0.1 mg/kg | |
| S 421 | <0.02 mg/kg | |
| Tecnazene | <0.05 mg/kg | |
| Tetradifon | <0.05 mg/kg | |
| Vinclozolin | <0.05 mg/kg | |

*[Method performed by an outsource lab.]*

## Appendix 7.2 BESTEVIA® Rebaudioside I Lot 783-180565

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 7570
Tel.+1 707 792 7300

July 15, 2019

Hadi Omrani
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

**CERTIFICATE OF ANALYSIS**
AR-19-KK-008365-01
Batch #: EUCAPE-00109704

**Sample Identification:**
Sample #: 740-2019-01290011
Description: BESTEVIA® Reb I 95%, Powder, Lot #783-180565
Condition: Acceptable
Date Received: January 29, 2019

**QA12C: Pesticides - USP 561 Screen (USP 42)**
**Method Reference:** USP 561
**Completed:** 02/11/2019

| | Result | Theoretical Level |
|---|---|---|
| Acephate | <0.1 mg/kg | |
| Alachlor | <0.02 mg/kg | |
| Aldrin and Dieldrin (sum of) | <0.02 mg/kg | |
| Azinphos-ethyl | <0.02 mg/kg | |
| Azinphos-methyl | <0.05 mg/kg | |
| Bromophos-ethyl | <0.02 mg/kg | |
| Bromophos-methyl | <0.02 mg/kg | |
| Bromopropylate | <0.05 mg/kg | |
| Chlordane (sum of cis-, trans- and Oxychlordane) | <0.05 mg/kg | |
| Chlorfenvinphos | <0.02 mg/kg | |
| Chlorpyrifos-ethyl | <0.02 mg/kg | |
| Chlorpyrifos-methyl | <0.02 mg/kg | |
| Chlorthal-dimethyl | <0.01 mg/kg | |
| Cyfluthrin (sum of) | <0.1 mg/kg | |
| Cyhalothrin, lambda-(incl. Cyhalothrin, gamma-) | <0.02 mg/kg | |
| Cypermethrin and isomers (sum of) | <0.1 mg/kg | |
| DDT (total) | <0.02 mg/kg | |
| Deltamethrin | <0.1 mg/kg | |
| Diazinon | <0.02 mg/kg | |
| Dichlofluanid | <0.02 mg/kg | |
| Dichlorvos | <0.02 mg/kg | |
| Dicofol | <0.02 mg/kg | |
| Dimethoate/Omethoate (sum) | <0.1 mg/kg | |
| Endosulfan (sum of isomers and endo. sulfate) | <0.02 mg/kg | |
| Endrin | <0.02 mg/kg | |
| Ethion | <0.02 mg/kg | |
| Etrimfos | <0.05 mg/kg | |
| Fenchlorphos (sum) | <0.1 mg/kg | |
| Fenitrothion | <0.02 mg/kg | |
| Fenpropathrin | <0.03 mg/kg | |
| Fensulfothion (sum of parent, -oxons and sulfones) | <0.05 mg/kg | |

eurofins

Sample #: 740-2019-01290011

Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA
92688

**QA12C: Pesticides - USP 561 Screen (USP 42)**
**Method Reference:** USP 561
**Completed:** 02/11/2019

| | Result | Theoretical Level |
|---|---|---|
| Fenthion (sum of fenthion, -oxons, -sulfones) | <0.05 mg/kg | |
| Fenvalerate | <0.2 mg/kg | |
| Flucythrinate | <0.05 mg/kg | |
| Fluvalinate, tau- | <0.05 mg/kg | |
| Fonofos | <0.02 mg/kg | |
| Heptachlor (heptachlor+ cis-, trans- h. epoxide | <0.03 mg/kg | |
| Hexachlorobenzene | <0.01 mg/kg | |
| Hexachlorocyclohexane isomers (other than gamma) | <0.02 mg/kg | |
| Lindane (gamma-HCH) | <0.01 mg/kg | |
| Malathion and malaoxon (sum of) | <0.02 mg/kg | |
| Mecarbam | <0.05 mg/kg | |
| Methacriphos | <0.05 mg/kg | |
| Methamidophos | <0.05 mg/kg | |
| Methidathion | <0.02 mg/kg | |
| Methoxychlor | <0.05 mg/kg | |
| Mirex | <0.01 mg/kg | |
| Monocrotophos | <0.1 mg/kg | |
| Parathion-ethyl and Paraoxon-ethyl (sum of) | <0.2 mg/kg | |
| Parathion-methyl and Paraoxon-methyl (sum of) | <0.2 mg/kg | |
| Pendimethalin | <0.1 mg/kg | |
| Pentachloranisole | <0.01 mg/kg | |
| Permethrin and isomers (sum of) | <0.2 mg/kg | |
| Phosalone | <0.04 mg/kg | |
| Phosmet | <0.05 mg/kg | |
| Piperonyl butoxide (PBO) | <1 mg/kg | |
| Pirimiphos-ethyl | <0.05 mg/kg | |
| Pirimiphos-methyl (incl. N-desethyl-) | <0.1 mg/kg | |
| Procymidone | <0.1 mg/kg | |
| Profenofos | <0.1 mg/kg | |
| Prothiofos | <0.05 mg/kg | |
| Pyrethrum (sum of cinerins, jasmolins, pyrethrins) | <3 mg/kg | |
| Quinalphos | <0.05 mg/kg | |
| Quintozene (sum quintozene,pentachloraniline,MPPS) | <0.1 mg/kg | |
| S 421 | <0.02 mg/kg | |
| Tecnazene | <0.05 mg/kg | |
| Tetradifon | <0.05 mg/kg | |
| Vinclozolin | <0.05 mg/kg | |

*[Method performed by an outsource lab.]*

## Appendix 7.3 BESTEVIA® Rebaudioside I Lot 783-180607

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 7570
Tel.+1 707 792 7300

July 15, 2019

Hadi Omrani
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

**CERTIFICATE OF ANALYSIS**
AR-19-KK-008366-01
Batch #: EUCAPE-00109704

**Sample Identification:**

Sample #: 740-2019-01290012
Description: BESTEVIA® Reb I 95%, Powder, Lot #783-180607
Condition: Acceptable
Date Received: January 29, 2019

**QA12C: Pesticides - USP 561 Screen (USP 42)**
**Method Reference:** USP 561
**Completed:** 02/11/2019

| | Result | Theoretical Level |
|---|---|---|
| Acephate | <0.1 mg/kg | |
| Alachlor | <0.02 mg/kg | |
| Aldrin and Dieldrin (sum of) | <0.02 mg/kg | |
| Azinphos-ethyl | <0.02 mg/kg | |
| Azinphos-methyl | <0.05 mg/kg | |
| Bromophos-ethyl | <0.02 mg/kg | |
| Bromophos-methyl | <0.02 mg/kg | |
| Bromopropylate | <0.05 mg/kg | |
| Chlordane (sum of cis-, trans- and Oxychlordane) | <0.05 mg/kg | |
| Chlorfenvinphos | <0.02 mg/kg | |
| Chlorpyrifos-ethyl | <0.02 mg/kg | |
| Chlorpyrifos-methyl | <0.02 mg/kg | |
| Chlorthal-dimethyl | <0.01 mg/kg | |
| Cyfluthrin (sum of) | <0.1 mg/kg | |
| Cyhalothrin, lambda-(incl. Cyhalothrin, gamma-) | <0.02 mg/kg | |
| Cypermethrin and isomers (sum of) | <0.1 mg/kg | |
| DDT (total) | <0.02 mg/kg | |
| Deltamethrin | <0.1 mg/kg | |
| Diazinon | <0.02 mg/kg | |
| Dichlofluanid | <0.02 mg/kg | |
| Dichlorvos | <0.02 mg/kg | |
| Dicofol | <0.02 mg/kg | |
| Dimethoate/Omethoate (sum) | <0.1 mg/kg | |
| Endosulfan (sum of isomers and endo. sulfate) | <0.02 mg/kg | |
| Endrin | <0.02 mg/kg | |
| Ethion | <0.02 mg/kg | |
| Etrimfos | <0.05 mg/kg | |
| Fenchlorphos (sum) | <0.1 mg/kg | |
| Fenitrothion | <0.02 mg/kg | |
| Fenpropathrin | <0.03 mg/kg | |
| Fensulfothion (sum of parent, -oxons and sulfones) | <0.05 mg/kg | |

eurofins

Sample #: 740-2019-01290012

Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA
92688

**QA12C: Pesticides - USP 561 Screen (USP 42)**
**Method Reference:** USP 561
**Completed:** 02/11/2019

| | Result | Theoretical Level |
|---|---|---|
| Fenthion (sum of fenthion, -oxons, -sulfones) | <0.05 mg/kg | |
| Fenvalerate | <0.2 mg/kg | |
| Flucythrinate | <0.05 mg/kg | |
| Fluvalinate, tau- | <0.05 mg/kg | |
| Fonofos | <0.02 mg/kg | |
| Heptachlor (heptachlor+ cis-, trans- h. epoxide | <0.03 mg/kg | |
| Hexachlorobenzene | <0.01 mg/kg | |
| Hexachlorocyclohexane isomers (other than gamma) | <0.02 mg/kg | |
| Lindane (gamma-HCH) | <0.01 mg/kg | |
| Malathion and malaoxon (sum of) | <0.02 mg/kg | |
| Mecarbam | <0.05 mg/kg | |
| Methacriphos | <0.05 mg/kg | |
| Methamidophos | <0.05 mg/kg | |
| Methidathion | <0.02 mg/kg | |
| Methoxychlor | <0.05 mg/kg | |
| Mirex | <0.01 mg/kg | |
| Monocrotophos | <0.1 mg/kg | |
| Parathion-ethyl and Paraoxon-ethyl (sum of) | <0.2 mg/kg | |
| Parathion-methyl and Paraoxon-methyl (sum of) | <0.2 mg/kg | |
| Pendimethalin | <0.1 mg/kg | |
| Pentachloranisole | <0.01 mg/kg | |
| Permethrin and isomers (sum of) | <0.2 mg/kg | |
| Phosalone | <0.04 mg/kg | |
| Phosmet | <0.05 mg/kg | |
| Piperonyl butoxide (PBO) | <1 mg/kg | |
| Pirimiphos-ethyl | <0.05 mg/kg | |
| Pirimiphos-methyl (incl. N-desethyl-) | <0.1 mg/kg | |
| Procymidone | <0.1 mg/kg | |
| Profenofos | <0.1 mg/kg | |
| Prothiofos | <0.05 mg/kg | |
| Pyrethrum (sum of cinerins, jasmolins, pyrethrins) | <3 mg/kg | |
| Quinalphos | <0.05 mg/kg | |
| Quintozene (sum quintozene,pentachloraniline,MPPS) | <0.1 mg/kg | |
| S 421 | <0.02 mg/kg | |
| Tecnazene | <0.05 mg/kg | |
| Tetradifon | <0.05 mg/kg | |
| Vinclozolin | <0.05 mg/kg | |

*[Method performed by an outsource lab.]*

## Appendix 7.4 BESTEVIA® Rebaudioside I Lot 78618111226

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 7570
Tel.+1 707 792 7300

July 15, 2019

Hadi Omrani
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

**CERTIFICATE OF ANALYSIS**
AR-19-KK-008364-01
Batch #: EUCAPE-00109704

**Sample Identification:**
Sample #: 740-2019-01290010
Description: BESTEVIA® Reb I 95%, Powder, Lot #78618111226
Condition: Acceptable
Date Received: January 29, 2019

**QA12C: Pesticides - USP 561 Screen (USP 42)**
**Method Reference:** USP 561
**Completed:** 02/11/2019

| | Result | Theoretical Level |
|---|---|---|
| Acephate | <0.1 mg/kg | |
| Alachlor | <0.02 mg/kg | |
| Aldrin and Dieldrin (sum of) | <0.02 mg/kg | |
| Azinphos-ethyl | <0.02 mg/kg | |
| Azinphos-methyl | <0.05 mg/kg | |
| Bromophos-ethyl | <0.02 mg/kg | |
| Bromophos-methyl | <0.02 mg/kg | |
| Bromopropylate | <0.05 mg/kg | |
| Chlordane (sum of cis-, trans- and Oxychlordane) | <0.05 mg/kg | |
| Chlorfenvinphos | <0.02 mg/kg | |
| Chlorpyrifos-ethyl | <0.02 mg/kg | |
| Chlorpyrifos-methyl | <0.02 mg/kg | |
| Chlorthal-dimethyl | <0.01 mg/kg | |
| Cyfluthrin (sum of) | <0.1 mg/kg | |
| Cyhalothrin, lambda-(incl. Cyhalothrin, gamma-) | <0.02 mg/kg | |
| Cypermethrin and isomers (sum of) | <0.1 mg/kg | |
| DDT (total) | <0.02 mg/kg | |
| Deltamethrin | <0.1 mg/kg | |
| Diazinon | <0.02 mg/kg | |
| Dichlofluanid | <0.02 mg/kg | |
| Dichlorvos | <0.02 mg/kg | |
| Dicofol | <0.02 mg/kg | |
| Dimethoate/Omethoate (sum) | <0.1 mg/kg | |
| Endosulfan (sum of isomers and endo. sulfate] | <0.02 mg/kg | |
| Endrin | <0.02 mg/kg | |
| Ethion | <0.02 mg/kg | |
| Etrimfos | <0.05 mg/kg | |
| Fenchlorphos (sum) | <0.1 mg/kg | |
| Fenitrothion | <0.02 mg/kg | |
| Fenpropathrin | <0.03 mg/kg | |
| Fensulfothion (sum of parent, -oxons and sulfones) | <0.05 mg/kg | |

eurofins

Sample #: 740-2019-01290010

Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA
92688

**QA12C: Pesticides - USP 561 Screen (USP 42)**
**Method Reference:** USP 561
**Completed:** 02/11/2019

| | Result | Theoretical Level |
|---|---|---|
| Fenthion (sum of fenthion, -oxons, -sulfones) | <0.05 mg/kg | |
| Fenvalerate | <0.2 mg/kg | |
| Flucythrinate | <0.05 mg/kg | |
| Fluvalinate, tau- | <0.05 mg/kg | |
| Fonofos | <0.02 mg/kg | |
| Heptachlor (heptachlor+ cis-, trans- h. epoxide | <0.03 mg/kg | |
| Hexachlorobenzene | <0.01 mg/kg | |
| Hexachlorocyclohexane isomers (other than gamma) | <0.02 mg/kg | |
| Lindane (gamma-HCH) | <0.01 mg/kg | |
| Malathion and malaoxon (sum of) | <0.02 mg/kg | |
| Mecarbam | <0.05 mg/kg | |
| Methacriphos | <0.05 mg/kg | |
| Methamidophos | <0.05 mg/kg | |
| Methidathion | <0.02 mg/kg | |
| Methoxychlor | <0.05 mg/kg | |
| Mirex | <0.01 mg/kg | |
| Monocrotophos | <0.1 mg/kg | |
| Parathion-ethyl and Paraoxon-ethyl (sum of) | <0.2 mg/kg | |
| Parathion-methyl and Paraoxon-methyl (sum of) | <0.2 mg/kg | |
| Pendimethalin | <0.1 mg/kg | |
| Pentachloranisole | <0.01 mg/kg | |
| Permethrin and isomers (sum of) | <0.2 mg/kg | |
| Phosalone | <0.04 mg/kg | |
| Phosmet | <0.05 mg/kg | |
| Piperonyl butoxide (PBO) | <1 mg/kg | |
| Pirimiphos-ethyl | <0.05 mg/kg | |
| Pirimiphos-methyl (incl. N-desethyl-) | <0.1 mg/kg | |
| Procymidone | <0.1 mg/kg | |
| Profenofos | <0.1 mg/kg | |
| Prothiofos | <0.05 mg/kg | |
| Pyrethrum (sum of cinerins, jasmolins, pyrethrins) | <3 mg/kg | |
| Quinalphos | <0.05 mg/kg | |
| Quintozene (sum quintozene,pentachloraniline,MPPS) | <0.1 mg/kg | |
| S 421 | <0.02 mg/kg | |
| Tecnazene | <0.05 mg/kg | |
| Tetradifon | <0.05 mg/kg | |
| Vinclozolin | <0.05 mg/kg | |

*[Method performed by an outsource lab.]*

## Appendix 7.5 BESTEVIA® Rebaudioside I Lot 78618112826

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 7570
Tel.+1 707 792 7300

July 15, 2019

Hadi Omrani
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

**CERTIFICATE OF ANALYSIS**
AR-19-KK-008367-01
Batch #: EUCAPE-00109704

**Sample Identification:**
Sample #: 740-2019-01290013
Description: BESTEVIA® Reb I 95%, Powder, Lot #78618112826
Condition: Acceptable
Date Received: January 29, 2019

**QA12C: Pesticides - USP 561 Screen (USP 42)**
**Method Reference:** USP 561
**Completed:** 02/11/2019

| | Result | Theoretical Level |
|---|---|---|
| Acephate | <0.1 mg/kg | |
| Alachlor | <0.02 mg/kg | |
| Aldrin and Dieldrin (sum of) | <0.02 mg/kg | |
| Azinphos-ethyl | <0.02 mg/kg | |
| Azinphos-methyl | <0.05 mg/kg | |
| Bromophos-ethyl | <0.02 mg/kg | |
| Bromophos-methyl | <0.02 mg/kg | |
| Bromopropylate | <0.05 mg/kg | |
| Chlordane (sum of cis-, trans- and Oxychlordane) | <0.05 mg/kg | |
| Chlorfenvinphos | <0.02 mg/kg | |
| Chlorpyrifos-ethyl | <0.02 mg/kg | |
| Chlorpyrifos-methyl | <0.02 mg/kg | |
| Chlorthal-dimethyl | <0.01 mg/kg | |
| Cyfluthrin (sum of) | <0.1 mg/kg | |
| Cyhalothrin, lambda-(incl. Cyhalothrin, gamma-) | <0.02 mg/kg | |
| Cypermethrin and isomers (sum of) | <0.1 mg/kg | |
| DDT (total) | <0.02 mg/kg | |
| Deltamethrin | <0.1 mg/kg | |
| Diazinon | <0.02 mg/kg | |
| Dichlofluanid | <0.02 mg/kg | |
| Dichlorvos | <0.02 mg/kg | |
| Dicofol | <0.02 mg/kg | |
| Dimethoate/Omethoate (sum) | <0.1 mg/kg | |
| Endosulfan (sum of isomers and endo. sulfate) | <0.02 mg/kg | |
| Endrin | <0.02 mg/kg | |
| Ethion | <0.02 mg/kg | |
| Etrimfos | <0.05 mg/kg | |
| Fenchlorphos (sum) | <0.1 mg/kg | |
| Fenitrothion | <0.02 mg/kg | |
| Fenpropathrin | <0.03 mg/kg | |
| Fensulfothion (sum of parent, -oxons and sulfones) | <0.05 mg/kg | |

eurofins Sample #: 740-2019-01290013

Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA
92688

**QA12C: Pesticides - USP 561 Screen (USP 42)**
**Method Reference:** USP 561
**Completed:** 02/11/2019

| | Result | Theoretical Level |
|---|---|---|
| Fenthion (sum of fenthion, -oxons, -sulfones) | <0.05 mg/kg | |
| Fenvalerate | <0.2 mg/kg | |
| Flucythrinate | <0.05 mg/kg | |
| Fluvalinate, tau- | <0.05 mg/kg | |
| Fonofos | <0.02 mg/kg | |
| Heptachlor (heptachlor+ cis-, trans- h. epoxide | <0.03 mg/kg | |
| Hexachlorobenzene | <0.01 mg/kg | |
| Hexachlorocyclohexane isomers (other than gamma) | <0.02 mg/kg | |
| Lindane (gamma-HCH) | <0.01 mg/kg | |
| Malathion and malaoxon (sum of) | <0.02 mg/kg | |
| Mecarbam | <0.05 mg/kg | |
| Methacriphos | <0.05 mg/kg | |
| Methamidophos | <0.05 mg/kg | |
| Methidathion | <0.02 mg/kg | |
| Methoxychlor | <0.05 mg/kg | |
| Mirex | <0.01 mg/kg | |
| Monocrotophos | <0.1 mg/kg | |
| Parathion-ethyl and Paraoxon-ethyl (sum of) | <0.2 mg/kg | |
| Parathion-methyl and Paraoxon-methyl (sum of) | <0.2 mg/kg | |
| Pendimethalin | <0.1 mg/kg | |
| Pentachloranisole | <0.01 mg/kg | |
| Permethrin and isomers (sum of) | <0.2 mg/kg | |
| Phosalone | <0.04 mg/kg | |
| Phosmet | <0.05 mg/kg | |
| Piperonyl butoxide (PBO) | <1 mg/kg | |
| Pirimiphos-ethyl | <0.05 mg/kg | |
| Pirimiphos-methyl (incl. N-desethyl-) | <0.1 mg/kg | |
| Procymidone | <0.1 mg/kg | |
| Profenofos | <0.1 mg/kg | |
| Prothiofos | <0.05 mg/kg | |
| Pyrethrum (sum of cinerins, jasmolins, pyrethrins) | <3 mg/kg | |
| Quinalphos | <0.05 mg/kg | |
| Quintozene (sum quintozene,pentachloraniline,MPPS) | <0.1 mg/kg | |
| S 421 | <0.02 mg/kg | |
| Tecnazene | <0.05 mg/kg | |
| Tetradifon | <0.05 mg/kg | |
| Vinclozolin | <0.05 mg/kg | |

*[Method performed by an outsource lab.]*

# Appendix 8 Relative Sweetness Intensity Method

SWEETNESS EQUIVALENCY OF BESTEVIA REB-I

INTRODUCTION:

Sucrose, more commonly known as table sugar, is the standard by which sugar substitutes are compared to in terms of taste, texture, and caloric values. Bestevia-Reb I, a trademarked product produced by Blue California, is made by enzymatic bioconversion of steviol glycosides extracted from the stevia leaf, in order to create a non-caloric sweetener that can be used in similar applications to sucrose.

PURPOSE:

To determine the sweetness equivalence of Bestevia-Reb I (Rebaudioside I) produced by Blue California in comparison to sucrose.

TEST SAMPLES:

Samples of BESTEVIA-Reb I and Sucrose were prepared in water at room temperature respectively for comparison.

EQUIPMENT & MATERIALS:

Bestevia-Reb I

Sucrose

Purified water

Analytical Scale

1000ml beakers

Glass stirrers

Plastic cups

PROCEDURE:

1. 6 participants were pre-screened for taste acuity prior to completing the taste panel
2. Sensory evaluation of Reb I was performed using sucrose as a control. The sucrose sample purchased from Sigma-Aldrich and prepared control samples at three different concentrations of 1.0%, 3.0%, and 6.0% sucrose in purified water (w/v) at room temperature.
3. The Bestevia-E at 300ppm for sensory evaluation was prepared by adding corresponding mass into a 1000 mL of bottled water.
4. The mixture was stirred at room temperature until complete dissolved.
5. The Bestevia-Reb I sample was evaluated against several control sucrose samples at 1.0%, 3.0%, and 6.0% by a panel of 6 volunteers.

RESULTS:
All the value from tasters were averaged and converted to the sweetness equivalency comparing to sucrose. The blind results showed consistent results among volunteers. The result indicates that the Bestevia-Reb I is 167 times sweeter to sucrose.

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# Appendix 9 Estimated Daily Intake Levels of Steviol Glycosides Preparations

**Food Uses as Addressed by JECFA, EFSA, FSANZ & Others**

JECFA reviewed various estimates of possible daily intake of steviol glycosides (WHO, 2006). Merisant (2008) also listed intended use levels of Rebaudioside A for various food applications in their GRAS notice. Cargill (2008) estimated the possible daily intake of Rebaudioside A assuming the use levels would be comparable to aspartame and (Renwick, 2008). BioVittoria (2009) used an exposure estimate of "sucrose equivalents" and the sweetness intensity of Luo Han Guo fruit extract.

## A. Estimated Daily Intake

Using different approaches, JECFA (WHO, 2006), Merisant (2008), and Cargill (2008) estimated daily intakes (EDIs) ranging from 1.3 – 4.7 mg per kg bw per day.

## B. JECFA

- JECFA (WHO, 2006) evaluated information on exposure to steviol glycosides as submitted by Japan, China and the European Commission by the Scientific Committee on Food. They used the Global Environment Monitoring System (GEMS)/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1.
- The intakes ranged from 1.3 mg per kg bw per day with the African diet to 3.5 mg per kg bw per day with the European diet. Exposures to steviol glycosides assumed full replacement of all dietary sugars in the diets for Japan and the US.
- JECFA concluded that the replacement estimates were highly conservative. Furthermore, the calculated dietary exposures were overestimates and would probably be 20 – 30% of these values, or 1.0 - 1.5 mg per kg bw per day on a steviol basis or 3.0 – 4.5 mg per kg bw per day for Rebaudioside A, based on the molecular weight adjustment.

## C. EFSA

- On January 13, 2011, EFSA revised its dietary exposure assessment of steviol glycosides. For high consumers, revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg per kg bw (steviol equivalent). For European children aged 1-14 years, revised intake estimates ranged from 1.7 to 16.3 mg per kg bw per day, and for adults, the range was reported to be from 5.6 to 6.8 mg per kg bw per day (EFSA, 2011b).

## D. FSANZ

- FSANZ (2008) estimated the steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario. The estimated exposure to Rebaudioside A ranged from 0.3 – 1.0 mg per kg bw per day for a consumer at the mean and 0.5 – 1.5 mg per kg bw per day for a consumer in the 90$^{th}$ percentile. FSANZ concluded that there were no safety concerns for either adults or children.
- In 2009, Cargill applied to FSANZ to increase the maximum usage levels of steviol glycosides in the high-volume food categories with increased usage levels by presenting market share analyses that overestimate actual intake while remaining well below the generally accepted ADI.
- FSANZ (2010) accepted the increased usage levels as requested from Cargill since no public health and safety issues were identified.

## E. MERISANT

- Merisant (2008) utilized food consumption survey data from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) to determine the estimated daily intake from the proposed uses of Rebaudioside A.
- On a per user basis, the mean and 90$^{th}$ precentile daily consumption levels of Rebaudioside A were estimated as 2.0 and 4.7 mg per kg bw per day, respectively.
- On a steviol equivalent basis, the Merisant estimates were calculated to be 0.7 and 1.6 mg per kg bw per day, respectively.
- On December 17, 2008, Merisant (2008) received a “no questions” letter from FDA for the use of Rebaudioside A using NHANES food consumption data.

## F. CARGILL

- Cargill (2008) estimated dietary intake figures for rebaudioside A by assuming that use levels of Rebaudioside A would be comparable to those of aspartame in the US via post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008).
- On December 17, 2008, Cargill (2008) received a “no questions” letter from FDA for the use of Rebaudioside A using comparative aspartame data.
- On May 13, 2011, FSANZ approved a Cargill application to increase the allowed maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages and flavored soy beverages up to 200 mg per kg and in plain soy beverages up to 100 mg per kg (FSANZ, 2011).

## G. BIOVITTORIA

- BioVittoria Ltd. (2009) used an exposure estimate of “sucrose equivalents” and the sweetness intensity of any particular sweetener based upon data published by Renwick (2008).
- These data resulted in a maximum of 9.9 mg per kg bw per day for any population.
- BioVittoria (2010) received a “no questions” letter from FDA for the use of Luo Han Guo fruit extract using Renwick’s “sucrose equivalents.”

## H. Other Publications

- Roberts et al. (2016) suggested that a higher ADI is justified based on metabolic factors to reduce the 100X safety factor. A chemical-specific adjustment factor (CSAF), as defined by the WHO in 2005, is determined by comparative studies in rats and humans.
    - A CSAF that is less than the standard 100X safety factor will result in an increase in the ADI, independent of the no observed adverse effect level (NOAEL).
    - The authors determined that using a CSAF can justify an ADI value of 6-16 mg per kg bw per day for steviol glycosides, depending on whether area under the plasma-concentration time curve (AUC) or $C_{max}$ data are used when considering the 1,000 mg per kg bw per day NOAEL (which is equivalent to 400 mg per kg bw per day of steviol) for stevioside reported by Toyoda et al. (1997).

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# Appendix 10 Studies on Steviol Glycosides Preparations

## PART 1. Preparations that are Primarily Mixtures of Stevioside & Rebaudioside A

### A. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

#### 1. Animal Studies

- Various animal studies show stevioside is not readily absorbed from the GI tract:
  - Rats – Wingard Jr. et al. (1980); Nakayama et al. (1986); Koyama et al. (2003b);
  - Chickens – Geuns et al. (2003b);
  - Hamsters – Hutapea et al. (1999); and
  - Pigs – Geuns et al. (2003a)
- *In vitro* metabolism studies show steviol glycosides are transformed to steviol which is better absorbed in rats and humans (Geuns, 2003; Koyama et al., 2003b; Gardana et al., 2003; Wang et al., 2004).
- Koyama et al. (2003b) showed steviol can be converted to various glucuronides.
- Excretion of metabolites of stevioside after oral doses has been shown in urine and feces in rats (Sung, 2002) and hamsters (Hutapea et al., 1999).
- Oral doses in pigs led to the detection of metabolites in feces but not in urine (Geuns et al., 2003a).
- Koyama et al. (2003b) published an *in vitro* study where α-glucosylated steviol glycosides were degraded by fecal microflora to steviol glycosides. These glycosides are subsequently hydrolyzed to the aglycone, steviol, demonstrating that the metabolic fate of α-glucosylated steviol glycosides follows that of non-modified steviol glycosides.
- Due to the similarities in metabolic fate, the safety of α-glucosylated steviol glycosides can be established based on studies conducted with non-modified steviol glycosides.
- Since the individual steviol glycosides show similar pharmacokinetics in rats and humans, the results of toxicology studies on individual steviol glycosides are applicable to the safety of steviol glycosides in general.

#### 2. Human Studies

- Geuns et al. (2006) measured blood, urine, and fecal metabolites in 10 healthy subjects who received 3 doses of 250 mg of purified stevioside (>97%) three times a day for 3 days:
  - Free steviol was detected in feces but not in blood or urine. Steviol glucuronide was detected in blood, urine, and feces. Approximately 76% of the total steviol equivalents dosed were recovered in urine and feces.
  - The authors concluded that there was complete conversion of Stevioside in the colon to steviol, which was absorbed and rapidly converted to the glucuronide.

- Renwick and Tarka (2008) reviewed studies on microbial hydrolysis of steviol glycosides and concluded that Stevioside and Rebaudioside A are not absorbed directly but are converted to steviol by gut microbiota in rats and in humans. This hydrolysis occurs more slowly for Rebaudioside A than for Stevioside.

## B. Acute Toxicity Studies

A summary of the acute toxicity of Stevioside (96% pure) is presented in Table 10.1.

**Table 10.1. Acute Toxicity of Stevioside (Purity 96%) Given Orally to Rodents**

| Species | Sex | $LD_{50}$ (g/kg bw) | Reference |
|---|---|---|---|
| **Mouse** | Male and Female | >15 | Toskulkac et al. (1997) |
| **Mouse** | Male | > 2 | Medon et al. (1982) |
| **Rat** | Male and Female | >15 | Toskulkac et al. (1997) |
| **Hamster** | Male and Female | >15 | Toskulkac et al. (1997) |

No lethality was noted within 14 days after the administration, and no clinical signs of toxicity, or morphological or histopathological changes were found, indicating that Stevioside is essentially nontoxic in acute oral exposures.

## C. Subchronic Toxicity Studies

- Aze et al. (1990) added stevioside at 0, 0.31, 0.62, 1.25, 2.5, and 5% to the diets of F344 rats for 13 weeks and reported no adverse effects. The apparent NOAEL was >5% dietary Stevioside.
- Mitsuhashi (1976) added up to 7% Stevioside to the diets of F344 rats for 3 months and reported no adverse effects.
- Akashi and Yokoyama (1975) dosed rats with up to 2,500 mg per kg bw per day for 3 months and reported no adverse effects.
- The Awney et al. (2011) study revealed toxicity in rats dosed at 15 and 1,500 mg per kg, which resulted in a NOAEL of 15 mg per kg per day. This study is considered to be an outlier in critical reviews by Carakostas (2012) and Waddell (2011) for the following reasons:
    - Insufficient number of animals;
    - Animals were group housed leaving unreliable drinking water quantification;
    - No evidence of fasting before blood collection;
    - No urinalyses;
    - No histopathological confirmation of effects;
    - No organ weight data;
    - No laboratory historical control comparisons; and
    - Use of tartrate-resistant alkaline phosphatase (TRAP) enzyme, which has not been properly vetted for application on toxicological studies;

In summary, the data presented by Awney et al. (2011) are probably not representative of changes due to the subchronic dietary administration of steviol glycosides because of overall inadequate study design and reliance on the findings of the untested enzyme TRAP.

## D. Chronic Toxicity Studies

- Toyoda et al. (1997) added Stevioside (96.5%) to the diets of F344 rats at 0, 2.5, and 5% for 104 weeks. The authors reported dose-dependent reductions in body weight gains decreased in both sexes. Kidney weights were significantly lower in 5% males; ovary, kidney and brain weights were significantly increased in 5% females and there were decreased survival rates in males receiving 5%. However, Stevioside was not carcinogenic at any level. The apparent NOAEL was the dietary level of 2.5%.
- Xili et al. (1992) added Stevioside (86%) to the diets of F344 rats at 0, 0.2, 0.6, and 1.2% for 3 months and report no adverse effects. The calculated NOAEL was 794 mg per kg bw per day (high dose – 1.2%).
- Yamada et al. (1985) added Stevioside to the diets of F344 rats at 0.1, 0.3, and 1.0% with 95.2% steviol (75% Stevioside/16% Rebaudioside) for 22 months in males and 24 months in females. Differences were noted in some parameters; however, the authors concluded that after 2 years of exposure, there were no significant changes that could be attributed to the administration of Stevioside and reported no adverse effects. The calculated NOAEL was 550 mg per kg bw per day.
- No treatment-related increase in tumor incidence was seen in any of these studies.

## E. Reproductive & Developmental Toxicity Studies

- No effects on pregnancy or developmental parameters were observed in Swiss albino mice administered Stevioside or aqueous stevia extract at doses of 500 and 800 mg per kg bw per day for 15 days to female mice (Kumar and Oommen, 2008).
- No effect on fertility or reproductive parameters was seen in a three-generation study in hamsters at doses of 90% Stevioside at 0, 500, 1,000, and 2,500 mg per kg bw per day (Yodyingyuad and Bunyawong, 1991). The NOAEL was determined to be 2,500 mg per kg bw per day.
- No effects were observed in rats at doses of 96% Stevioside dosed at 0, 0.15, 0.75, or 3% (equivalent to 2,000 mg per kg bw per day). The NOAEL was determined to be 2,000 mg per kg bw per day (Mori et al., 1981).
- No teratogenic effects were observed in an additional rat study that was reviewed by Geuns (2003) in which pregnant female Wistar rats were administered Stevioside (95.6%) at 0, 250, 500 or 1,000 mg per kg bw per day for 10 days (Usami et al., 1994). The NOAEL was determined to be 1,000 mg per kg bw per day.
- In rat studies, dried stevia leaves were administered at 0.67 g per mL in 2 mL doses twice per day for 60 days (Oliveira-Filho et al., 1989). The only difference due to treatment was

seminal vesicle weight, which fell to 60% compared with control. No treatment-related adverse effects were noted.

- In experimental studies in rats, crude stevia leaf extract (5%) was administered to female rats at 0 or 5% for 12 days. The female rats were subsequently mated with untreated males for the last 6 days, making a total of 18 days of exposure for the females (Planas and Kuć, 1968). Fertility was reduced to 21% of the fertility of control rats and remained reduced during the 50- to 60-day recovery period. The study report did not discuss histological examinations, weights of organs, blood analysis, urine chemistry, or necropsy.
- The use of *S. rebaudiana* as an oral contraceptive has been reported by indigenous populations in Paraguay (Planas and Kuć, 1968; Schvartaman et al., 1977).
- A developmental study of 90% steviol in hamsters at 0, 250, 500, 750, or 1,000 mg per kg bw per day on days 6-10 of gestation resulted in a significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12, respectively) at the three highest doses. No dose-dependent teratogenic effects were observed. The no observed effect level (NOEL) was 250 mg per kg bw per day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

## F. Mutagenicity & Genotoxicity Studies

The following key mutagenicity studies have been conducted on stevia extracts and Stevioside and are negative for mutagenic responses:

- Bacterial mutagenicity studies negative for mutagenic response:
  - Matsui et al. (1996)
  - Suttajit et al. (1993)
  - Klongpanichpak et al. (1997)
  - Matsui et al. (1996)
  - Pezzuto et al. (1985)
  - Medon et al. (1982)
- Mouse lymphoma (L5178Y/TK+/) study negative for mutagenic response:
  - Oh et al. (1999)
- Chromosome aberration studies negative for mutagenic response:
  - Human lymphocytes – Suttajit et al. (1993)
  - Chinese hamster lung fibroblasts – Nakajima (2000a); Ishidate et al. (1984)
- DNA damage (Comet assay) negative for mutagenic response:
  - Sekihashi et al. (2002)
  - Sasaki et al. (2002)
- Mouse bone marrow/liver micronucleus studies negative for mutagenic response:
  - Oh et al. (1999)

One study was found to be positive and was conducted by (Nunes et al., 2007a). The Nunes study revealed toxicity and was conducted on rat liver, brain, and spleen on rats dosed 4 mg per mL steviol glycosides in drinking water (estimated 80 to 500 mg per kg bw per day) for 45 days. There were positive findings in all tissues – notably the liver. This study is considered to be an outlier in critical reviews conducted by Geuns (2007), Williams (2007), and Brusick (2008). These critiques were responded to by the authors (Nunes et al., 2007b; Nunes et al., 2007c). However, the consensus appears to be that (Nunes et al., 2007a) used flawed methodology and improperly interpreted data as a positive response.

- In two separate reviews by Carakostas et al. (2008) and Brusick (2008), the recent research on Rebaudioside A was summarized and combined with the body of knowledge on Stevioside. These authors noted the following:
    - Steviol glycosides, Rebaudioside A, and Stevioside are not genotoxic *in vitro.*
    - Steviol glycosides, Rebaudioside A, and Stevioside have not been shown to be genotoxic *in vivo* in well-conducted assays.
    - The (Nunes et al., 2007a) study was improperly interpreted as positive.
    - Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- Urban et al. (2013) examined the genotoxicity database on steviol glycosides concluding that the current database of *in vitro* and *in vivo* studies for steviol glycosides is robust and does not indicate that either Stevioside or Rebaudioside A is genotoxic.

## G. Clinical Studies & Other Reports in Humans

In several studies, pharmacological and biochemical effects of crude extracts of stevia leaves and purified steviol glycosides have been investigated. The effects noted included glucose uptake, insulin secretion, and blood pressure. In South America, Stevioside is used as a treatment for type 2 diabetes. These effects were key concerns for JECFA. In 2006, JECFA summarized the available clinical studies of Stevioside and further studies were recommended (WHO, 2006). Subsequently, several additional studies were conducted, and in 2009, JECFA again reviewed these new studies (WHO, 2009). JECFA's summaries of the key studies are included in Table 10.2.

**Table 10.2. Human Studies with Stevioside Preparations**

| Author/ Year | Substance Tested | Total Daily Dose | Population Characteristics | Study Design and Duration | Noted Effects Safety parameter Results |
|---|---|---|---|---|---|
| Curi et al. (1986) | Aqueous extracts *S. rebaudiana* leaves | 5 g at 6 h intervals for 3 days = 20 g/day | 16 healthy patients – extract/ 6 healthy patients – arabinose | 3-day glucose tolerance in healthy adults | The extract of *Stevia rebaudiana* increased glucose tolerance. The extract decreased plasma glucose levels during the test and after overnight fasting in all volunteers. |

| Author/ Year | Substance Tested | Total Daily Dose | Population Characteristics | Study Design and Duration | Noted Effects Safety parameter Results |
|---|---|---|---|---|---|
| Chan et al. (2000) | Stevioside (purity not stated) | 750 mg (11 mg per kg bw/day) | 60 hypertensive Chinese men and woman aged 28-75) + 46 patients were given placebo. | Multicenter randomized, double-blind, placebo-controlled for 12 months | 3 months: mean systolic and diastolic BP decreased and continued through the 12 months. Minor side effects occurred with 2 test group and 1 placebo group patient withdrawing. Other side effects were minor and resolved. |
| Hsieh et al. (2003) | Stevioside (purity not stated) | 1,500 mg (21 mg/kg bw/day) | 85 hypertensive Chinese men and woman aged 20-75) + 89 patients were given placebo. | Multicenter randomized, double-blind, placebo-controlled for 24 months | Mean systolic and diastolic blood pressures were decreased commencing from about 1 week after the start of treatment. At 2 years test group patients had ↓ in incidence of left ventricular hypertrophy. 3 patients withdrew. Other side effects were minor and resolved. |
| Anonymous (2004a) | Steviol extract: (~73% Stevioside ~24% Reb A) | 100 mg (3.3 mg/kg bw/day) | 48 hyperlipidemic volunteers (24/24) | Randomized, double-blind, placebo-controlled for 3 months | Analyses of serum concentrations of triglycerides, liver-derived enzymes, and glucose indicated no adverse effects. 3 patients withdrew. No adverse side effects were reported. |
| Anonymous (2004b) | Steviol extract: (~73% Stevioside ~24% Reb A) | 3.25, 7.5, and 5 mg/kg bw/day | 12 patients per test group | Randomized, double-blind, placebo-controlled for 30 days | No adverse responses in blood and urine biochemical parameters |
| Gregersen et al. (2004) | Stevioside - 91% + 9% other stevia glycosides | Single dose 1 g Stevioside or 1 g starch | 12 patients with type 2 diabetes total | Acute paired cross-over study | ↓18% glucose concentr io : Systolic and diastolic blood pressure unchanged. No adverse effects |
| Temme et al. (2004) | Stevioside 97% | 750 mg/kg bw/day (288 mg/kg bw steviol) | 4 male 5 female healthy patients | Short term study – 3 days | Blood chemistry, blood pressure and urinalyses were unremarkable |
| Barriocanal et al. (2006) | Stevioside - 64.5% + 18.9% Reb A | 750 mg/kg bw/day | Type 1 (n=8) + Type 2 (n=15) diabetics + non-diabetics (n=15) + matching controls - placebo | Double-blind, placebo-controlled trial study for 3 months | Blood chemistry, glycated hemoglobin (HbA1c), blood pressure and urinalyses were unremarkable. No adverse effects |
| Barriocanal et al. (2008) | Stevioside - >92% | 250 mg/kg bw/day | Type 1, Type 2, placebo controls | Randomized, double-blind, placebo-controlled for 3 months | No changes in systolic BP, diastolic BP, glucose, or glycated hemoglobin from baseline. No adverse effects |
| Ferri et al. (2006) | Stevioside (purity not stated) | 3.75 (7 weeks), 7.5 (11 weeks), 15 (6 weeks) + placebo (24 weeks) mg/kg bw/day | Patients with mild hypertension | Randomized 24-week study | No changes in systolic BP, or diastolic BP. No adverse effects |

| Author/ Year | Substance Tested | Total Daily Dose | Population Characteristics | Study Design and Duration | Noted Effects Safety parameter Results |
|---|---|---|---|---|---|
| Silva et al. (2006) | Stevioside: 70% | Equivalent to 1.04 mg steviol/kg bw/day + placebo | 49 Mild hyperlipidemic patients: Stevioside group (n=24) placebo controls (n=25) | Placebo-controlled double-blind trial for 90 days | No effects of treatment on ALT, AST, or GGT were found. No relevant adverse effects were noted. |
| Jeppesen et al. (2006) | Stevioside (purity not stated) | 1,500 mg/kg bw/day or maize starch placebo | 55 patients with Type 2 diabetes: | Randomized, double blinded, placebo-controlled study | No effects on the HbA1c fasting blood glucose levels, lipids, or blood pressure |

Pham et al. (2019) reviewed the acute effects of nutritive and non-nutritive sweeteners, including steviol glycosides, on postprandial hypotension. The authors did not identify any publications regarding the acute effects of steviol glycosides on postprandial blood pressure. However, they noted that the majority of published studies evaluated long-term steviol glycosides intake on blood pressure, with results indicating that steviol glycosides "may be effective" in decreasing systolic and diastolic blood pressure in hypertensive (Hsieh et al., 2003; Chan et al., 2000), but not hypotensive or normotensive (Maki et al., 2008a; Barriocanal et al., 2008), subjects.

## PART 2. Preparations That Are Primarily Rebaudioside A

### A. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

### 1. Animal Studies

Studies investigating the ADME of extracts from stevia are available on Stevioside, Rebaudioside A, and other steviol glycosides. Data evaluating the absorption and fate of these extracts from various animal species and humans indicate that one can extrapolate these results from rats to humans.

- Studies investigating the hydrolysis of steviol glycosides by intestinal microflora have demonstrated that both Stevioside and Rebaudioside A are hydrolyzed to steviol following *in vitro* incubation with various cecal microflora (Wingard Jr. et al., 1980; Hutapea et al., 1997; Gardana et al., 2003; Geuns et al., 2003a).
- *In vitro* hydrolysis of Rebaudioside A to steviol was found to be slower than that of Stevioside (Koyama et al., 2003a).
    - The major pathway for Rebaudioside A is conversion to Stevioside with a minor pathway of conversion to Rebaudioside B prior to being ultimately converted to steviol. Stevioside is further converted to steviolbioside, steviolmonosides, and finally steviol, with glucose being released with each subsequent hydrolysis.

- Roberts and Renwick (2008) identified free steviol (82 to 86%), steviol, glucuronide (10 to 12%), and two unidentified metabolites (5-6%) in rat plasma following treatment with either Stevioside or Rebaudioside A eight hours post oral administration. Steviol $T_{max}$ for plasma was noted within 30 minutes of oral administration as opposed to Rebaudioside A, which has a $T_{max}$ of 2 to 8 hours.
    - Following Rebaudioside A treatment, significant amounts of unchanged Rebaudioside A (29% in males and 19% in females) and Stevioside (3% in males and 4% in females) were excreted in the feces.
    - Urinary excretion accounted for less than 2% of the administered dose
    - Steviol was the predominant component found in plasma samples after oral administration of Rebaudioside A, Stevioside, and steviol in rats. The majority of all samples were found to be excreted rapidly---primarily in the feces---within 48 hours.
    - The predominant compound detected in the bile was steviol glucuronide, while the prominent material in the intestine was steviol.
    - The authors concluded that the overall data on toxicokinetics and metabolism indicate that Rebaudioside A and Stevioside are handled in an almost identical manner in the rat after oral dosing.
- Wheeler et al. (2008) assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of Rebaudioside A and Stevioside.
    - Following administration of Rebaudioside A or Stevioside, steviol glucuronide appeared in the plasma of all subjects, with median $T_{max}$ values of 12.0 and 8.00 hours post-dose, respectively.
    - Administration of Rebaudioside A resulted in a significantly (~22%) lower steviol glucuronide geometric mean $C_{max}$ value (1,472 ng per mL) than administration of Stevioside (1,886 ng per mL). The geometric mean $AUC_{0-t}$ value for steviol glucuronide after administration of Rebaudioside A (30,788 ng*h per mL) was approximately 10% lower than after administration of stevioside (34,090 ng*h per mL).
    - The authors concluded that Rebaudioside A and Stevioside underwent similar metabolic and elimination pathways in humans, with steviol glucuronide excreted primarily in the urine and steviol in the feces.
    - No safety concerns were noted as determined by reporting of adverse events, laboratory assessments of safety, or vital signs.
- Sloter (2008a) examined the potential of Rebaudioside A toxicity in rats up to 2,000 mg per kg bw per day
    - Low levels of Rebaudioside A were detected in peripheral blood of rats post administration of 2,000 mg per kg bw per day.
    - Mean plasma concentrations of Rebaudioside A of 0.6 µg per mL in plasma resulting in an estimated absorbed dose of 0.02% based on amounts calculated from urine collection.

- Mean fecal Rebaudioside A and measured hydrolysis products, expressed as Total Rebaudioside A Equivalents, compared to daily administered dose results in an estimated dose recovery of approximately 84%.

**2. Subchronic Toxicity Studies**

- Curry and Roberts (2008) added up to 100,000 ppm of Rebaudioside A (97%) to the diets of Wistar rats for 13 weeks and reported no treatment-related adverse effects. Hence, the NOAEL was reported to be 9,938 mg per kg males and 11,728 mg per kg females – the highest level of treatment.
- Rebaudioside A (99.25%) was added to the diets of CRL:CD(SD) rats for 90 days at target doses of 500, 1,000, and 2,000 mg per kg bw per day with no treatment-related effects. The NOAEL was determined to be ≥2,000 mg per kg (Eapen, 2007; Nikiforov and Eapen, 2008).
- Eapen (2008) added Rebaudioside A (97.5%) to the diets of Beagle dogs for 6 months at target doses of 500, 1,000, and 2,000 mg per kg bw per day and reported no adverse effects. The NOAEL was determined to be >2,000 mg per kg bw per day.
- The oral administration of fermentative Reb A to Sprague-Dawley rats for 91 days did not lead to any adverse effects at consumption levels up to 2,057 mg per kg bw per day for males and 2,023 mg per kg bw per day for females, which were concluded to be the NOAELs (Rumelhard et al., 2016).

**3. Mutagenicity & Genotoxicity Studies**

- *In vitro* and *in vivo* genotoxicity assays covering mutation, chromosome damage, and deoxyribonucleic acid (DNA) strand breakage consistently and uniformly revealed negative results for Rebaudioside A.
- Evaluation of fermentation-derived Rebaudioside A demonstrated a similar safety profile to plant-derived Rebaudioside A (Rumelhard et al., 2016).

The following key mutagenicity studies have been conducted on Rebaudioside A and are negative for mutagenic responses:

- Bacterial mutagenicity studies negative for mutagenic response:
  - Wagner and Van Dyke (2006)
  - Williams and Burdock (2009)
  - Rumelhard et al. (2016)
- Mouse lymphoma (L5178Y/TK+/) studies negative for mutagenic response:
  - Clarke (2006)
  - Williams and Burdock (2009)
  - Human lymphocyte study negative for mutagenic response: Rumelhard et al. (2016)
- Chromosome aberration studies negative for mutagenic response:

  - Human lymphocytes – Williams and Burdock (2009)
  - Chinese hamster lung fibroblasts – Nakajima (2000a)
- Mouse micronucleus studies negative for mutagenic response:
  - Krsmanovic and Huston (2006)
  - Williams and Burdock (2009)
  - Nakajima (2000b) (BDF1 mouse bone marrow)
  - Unscheduled DNA synthesis (UDS) study negative for mutagenic response - Williams and Burdock (2009)
- Bacterial forward mutation study negative for mutagenic response – Pezzuto et al. (1985)

**4. Reproductive & Developmental Studies**

- Curry et al. (2008) conducted a 2-generation reproductive toxicity study on Rebaudioside A administered in the diet at 7,500, 12,500 and 25,000 ppm in Han Wistar rats. There were no signs of toxicity or adverse effects on body weights, body weight gain, or food consumption. Rebaudioside A did not affect reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology in either the $F_0$ or $F_1$ generations. The NOAEL for reproductive effects was 25,000 ppm, and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm, or 2,048 to 2,273 mg per kg bw per day (the highest dose tested).
- An unpublished study on Rebaudioside A was conducted on four groups of male and female Crl:CD(SD) rats (30 per sex per group) that were fed either a basal diet or the diet containing Rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter, 2008a). The test diet was offered to the offspring selected to become the $F_1$ generation following weaning (beginning on postnatal day 21). The $F_0$ and $F_1$ males continued to receive Rebaudioside A throughout mating, gestation, and lactation until the day of euthanasia. Both for parental systemic and reproductive toxicity, the NOAEL was ≥2,000 mg per kg bw per day (highest dose administered).
- In another unpublished study, the embryo/fetal developmental toxicity effects of Rebaudioside A when administered via gavage were studied in rats (Sloter, 2008b). The NOAEL for maternal and embryo/fetal development was determined to be >2,000 mg per kg bw per day.

**5. Clinical Studies on Rebaudioside A**

A summary of the clinical studies conducted on Rebaudioside A is presented in Table 10.3.

**Table 10.3. Human Studies with Rebaudioside A Preparations**

| Author/ Year | Substance Tested | Total Daily Dose | Population Characteristics | Study Design and Duration | Noted Effects Safety parameter Results |
|---|---|---|---|---|---|
| Maki et al. (2008a) | Rebaudioside A (97%) | Reb A: 1,000 mg Placebo: 0 | 100 patients with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) | Randomized, double-blind, placebo-controlled trial for 4 weeks | The extract of *Stevia rebaudiana* increased glucose tolerance. The extract decreased plasma glucose levels during the test and after overnight fasting in all volunteers. |
| Maki et al. (2008b) | Rebaudioside A (97%) | Reb A: 1,000 mg (n=60) Placebo: 0 (n=62) Age: 33-75 | Men and women with Type 2 diabetes | Randomized, double-blind, placebo-controlled trial for 16 weeks | No treatment related changes in blood pressure, body weight, and fasting lipids were noted. Rebaudioside A was well-tolerated, and records of hypoglycemic episodes showed no excess versus placebo |

## 6. Safety of Rebaudioside A

There have been a significant number of studies regarding the safety and toxicity of Rebaudioside A:

- GRAS notices submitted to FDA:
  - GRN 252: Merisant (2008) conducted studies that augmented genotoxicity data in three systems recognized by FDA as good predictors of carcinogenic potential. Two of these assays were conducted in mouse systems.
  - GRN 253: Cargill (2008) conducted studies that provided significant insight into the pharmacokinetics of Rebaudioside A, while demonstrating clinical safety of Rebaudioside A regarding lack of effects on blood pressure and glucose metabolism that could result from doses expected from use in food.
- JECFA concluded that all naturally occurring steviol glycosides are deemed to be safe as long as there is a combined purity of 95% and determined the ADI of the steviol glycosides applied to Rebaudioside A because the pharmacokinetics are virtually the same (FAO, 2017).
  - Carakostas et al. (2008) summarized the Cargill research program findings on rebaudioside A:
    - Steviol glycosides, Rebaudioside A, and Stevioside are not genotoxic *in vitro.*
    - In well-conducted *in vivo* assays, steviol glycosides, Rebaudioside A, and Stevioside have not been found to be genotoxic.
  - A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes et al., 2007a) and was improperly interpreted as a positive response.
  - Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.

  - The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
  - Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
  - While studies with Rebaudioside A indicated slight gastrointestinal (GI) absorption of the glycoside per se, the predominant metabolic pathway is comparable to that of Stevioside. The use of the ADI established by JECFA, which was determined on studies employing Stevioside as the main component, can be used as the ADI for Rebaudioside A.
  - The dietary levels expected from consumption of Rebaudioside A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.
- JECFA has evaluated the use of steviol glycosides in foods and agrees that, at the present time, the ADI for steviol glycosides of adequate purity, as defined by JECFA specifications, has been properly determined to be 4 mg per kg bw per person as steviol equivalents, which corresponds to 12 mg per kg bw per day for Rebaudioside A, on a dry weight basis. Therefore, the JECFA-derived ADI was adopted as a safe exposure for Rebaudioside A and the corresponding food uses meeting the specifications within the limits determined by this esteemed international body of food safety experts can be considered to be GRAS.
- Williams and Burdock (2009) reviewed three *in vitro* and two *in vivo* genotoxicity and mutagenicity studies on Rebaudioside A conducted according to Organisation for Economic Co-operation and Development (OECD) guidelines and found the studies revealed that rebaudioside A is:
  - non-mutagenic in an Ames test using *Salmonella typhimurium* and *Escherichia coli*
  - non-mutagenic in a chromosomal aberration test using Chinese hamster V79 cells
  - non-mutagenic in a mouse lymphoma assay using L5178Y+/- cells
  - non-mutagenic a bone marrow micronucleus test in mice at doses up 750 mg per kg bw
  - non-mutagenic in an unscheduled DNA synthesis test in rats at 2,000 mg per kg bw.
  - The authors note that these studies provide additional evidence that Rebaudioside A is not genotoxic at the doses tested and further support the GRAS determination of Rebaudioside A.

## PART 3. Studies on Principal Metabolite: Steviol

### A. Acute Toxicity Studies

- Toskulkac et al. (1997) administered single doses of steviol (90%) to various animals as follows:
  - Rat, oral $LD_{50}$ >15 g per kg
  - Hamster, oral $LD_{50}$ 5.2 g per kg bw in males and 6.1 g per kg bw in females

- Histopathological examination of the kidneys revealed severe degeneration of the proximal tubular cells, and these structural alterations were correlated with increased serum blood urea nitrogen and creatinine. The authors concluded that the cause of death was acute renal failure.

## B. Developmental Toxicity Studies

- Wasuntarawat et al. (1998) dosed groups of pregnant golden hamsters steviol (90%) at 0 mg (n not reported), 250 mg (n=20), 500 mg (n=20), or 1,000 mg (n=12) per kg bw per day by gavage in corn oil on days 6 -10 of gestation.
  - A significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12) were observed at the three highest doses.
  - The number of live fetuses per litter and mean fetal weight decreased in parallel.
  - No dose-dependent teratogenic effects were seen.
  - The NOEL for both maternal and developmental toxicity was 250 mg per kg bw per day.

## C. Mutagenicity & Genotoxicity Studies

The following key mutagenicity studies have been conducted on steviol and are negative for mutagenic responses:

- Bacterial mutagenicity studies negative for mutagenic response:
  - Klongpanichpak et al. (1997)
  - Procinska et al. (1991)
  - Compadre et al. (1988)
- Chromosome aberration studies negative for mutagenic response:
  - Chinese hamster lung fibroblasts – Matsui et al. (1996)
- DNA damage (Comet assay)
  - Sekihashi et al. (2002)
- Mouse bone marrow/liver micronucleus studies negative for mutagenic response:
  - Oh et al. (1999)
- Micronucleus studies negative for mutagenic response:
  - Temcharoen et al. (2000) (rat)
  - Temcharoen et al. (2000) (mouse)
  - Matsui et al. (1996) (mouse)
  - Temcharoen et al. (2000) (hamster)

The following key mutagenicity studies have been conducted on steviol and are positive or equivocal for mutagenic responses:

- Bacterial mutagenicity studies positive for mutagenic response:

  - Matsui et al. (1996) – Steviol was equivocal for mutagenicity. Steviol was weakly positive in Umu chromotest, either with or without metabolic activation. Steviol was negative in the reverse mutation and other bacterial assays even in presence of S9 activation
  - Terai et al. (2002) – Steviol was found to be mutagenic in Aroclor-induced rat liver S9 fraction.
  - Temcharoen et al. (2000) – Mutagenic effects of steviol and/or metabolites found in *S. typhimurium* TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene.
  - Pezzuto et al. (1985) – Mutagenicity was dependent on pretreatment of rats with Aroclor and NADPH addition, as unmetabolized steviol was inactive. None of the other metabolites tested was mutagenic.
  - Compadre et al. (1988) – Mass spectral analysis of steviol and analogues under conditions known to produce a mutagenic response. 15-oxo-steviol, a product of the metabolite, 15-alpha-hydroxysteviol was found to be a direct-acting mutagen.
- Chinese hamster lung fibroblast study positive for mutagenic response:
  - Matsui et al. (1996) – Gene mutations found in Chinese hamster lung fibroblasts after metabolic activation of steviol. In hamsters, several metabolites of Stevioside found that have not been found in rats or humans. Therefore, experimental relevance should be questioned when hamsters are used.

Each of the positive mutagenicity studies noted above had special circumstances or slightly different procedures. The positive mutagenicity studies were collectively not believed to present sufficient toxicological concern as determined by JECFA (WHO, 2006).

**D. Endocrine Disruption Studies**

- Shannon et al. (2016) investigated the endocrine disrupting potential of Stevioside, Rebaudioside A, and steviol in a series of *in vitro* bioassays and found that steviol:
  - antagonizes progesterone nuclear receptor transcriptional activity
  - increases progesterone production
  - induces an agonistic response on the progesterone receptor of sperm cells (Catsper)

The authors conclude that steviol might not qualify as a safer alternative to sugar or synthetic sweeteners. However, one must consider the fact that it is difficult to translate *in vitro* concentrations to local concentrations *in vivo* at the receptor level and no adverse effects have been noted in any reproductive studies.

# Appendix 11 Summary of the Regulatory History of Steviol Glycosides

## A. History of Traditional Medicinal and Human Food Use

- Stevia use as a sweetener and in traditional medicine by the Guarani tribes can be traced back for centuries (Esen, 2016; Gerwig et al., 2016; Brusick, 2008; Brandle et al., 1998).
- Stevia is commonly used to treat Type 2 diabetes in South America (Hawke, 2003). Doses in the range of 1 gram per person per day or more were reported to be necessary for therapeutic effects (Gregersen et al., 2004).
- Japan and Brazil approved stevia as a food additive in the 1980s (Raintree, 2012). Lester (1999) reported that 40% of the artificial sweetener market in Japan was stevia based.
- Use of steviol glycosides as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand, and as a natural health product in Canada.
- In 2005, it was estimated that sales of stevia in the US reached $45 million (Newsday, 2006).
- In 2010, Zenith International estimated stevia sales of 3,500 metric tons, which represents a 27% increase over 2009 figures. The market value is estimated to have increased to $285 million (Zenith, 2011).
- In 2013, worldwide sales of stevia was reported at 4,100 tons – representing a 6.5% increase over 2011 figures with an overall market value of $304 million (Zenith, 2013).
- In October 2014, it was reported that worldwide stevia sales increased 14% to 4,670 tons, with a market value of $336 million. It has been projected that the total market for stevia in 2017 would be 7,150 tons with an associated market value of $578 million (Zenith, 2014).
- NewHope360 reported that the global market for stevia in 2014 was $347 million, and that is expected to increase to $565.2 million by 2020. In addition, consumption is expected to increase from 2014 levels of 5,100.6 tons to 8,506.9 tons by 2020 (NewHope360, 2015).
- Nutritional Outlook reported that Mintel data indicated a 48% increase in stevia-containing products over the last five years (Decker and Prince, 2018).

## B. Summary of Regulatory History of Steviol Glycosides

### 1. U.S. Regulatory History

As of January 14, 2020, FDA has issued 64 “no questions” letters on GRAS notices on Rebaudioside A, Rebaudioside D, Rebaudioside M, Rebaudioside E, or steviol glycosides, including those undergoing enzyme treatment. There are currently two GRAS notices—GRN 878 for Glucosylated Steviol Glycosides and GRN 882 for Rebaudioside M—pending FDA review. At the notifier’s request, FDA ceased to evaluate GRN 867 for Rebaudioside M (FDA, 2020).

In addition, the Flavor and Extract Manufacturers Association (FEMA) has included twenty steviol glycosides preparations that are used to formulate flavors on their GRAS lists as shown in Table 10.1.

**Table 11.1. FEMA GRAS Status for Steviol Glycoside Preparations**

| Steviol Glycosides Preparation | FEMA Number | Reference |
|---|---|---|
| Rebaudioside A | 4601 | Smith et al. (2009) |
| Rebaudioside C; dulcoside B | 4720 | Leffingwell (2011) |
| Glucosyl steviol glycosides; enzymatically modified stevia extract | 4728 | Leffingwell and Leffingwell (2014); Marnett et al. (2013) |
| Stevioside | 4763 | Leffingwell and Leffingwell (2014); Marnett et al. (2013) |
| Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside A 60% | 4771 | Marnett et al. (2013) |
| Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside A 80% | 4772 | Marnett et al. (2013) |
| Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside C 30% | 4796 | Cohen et al. (2015a); Cohen et al. (2015b) |
| Steviol glycoside extract, *Stevia rebaudiana,* Rebaudioside A 22% | 4805 | Cohen et al. (2015a); Cohen et al. (2015b) |
| Steviol glycoside extract, *Stevia rebaudiana* Rebaudioside C 22% | 4806 | Cohen et al. (2015a); Cohen et al. (2015b) |
| Glucosylated stevia extract Steviol glycosides 80% | 4845 | Cohen et al. (2017) |
| Enzyme modified stevia, stevioside 20% | 4876 | Cohen et al. (2017) |
| Rebaudioside M; Rebaudioside X | 4895 | Cohen et al. (2019) |
| Glucosylated steviol glycosides, 70-80% | 4909 | Cohen et al. (2019) |
| Glucosylated steviol glycosides, 40% | 4910 | Cohen et al. (2019) |
| Stevia extract stevioside, 70% | 4911 | Cohen et al. (2019) |
| Rebaudioside D 95% | 4921 | Cohen et al. (2019) |
| Rebaudioside M 95%; Rebaudioside X 95% | 4922 | Cohen et al. (2019) |
| Glucosylated steviol glycosides, 90% | 4931 | Cohen et al. (2019) |
| Rebaudioside E ≥85% | 4936 | Cohen et al. (2019) |
| Rebaudioside I 95% | 4937 | Cohen et al. (2019) |

## 2. Canadian Regulatory History

- On September 18, 2009, the Natural Health Products Directorate, Health Canada (Health Canada, 2009) adopted and revised the maximum limit for steviol glycosides in Natural Health products (NHPs) to be in accordance with the full ADI of 4 mg steviol per kg bw established by JECFA (WHO, 2008).
    - As a Medicinal Ingredient: The maximum daily limit without cautionary labelling and additional safety evidence was set at 4 mg per kg bw per day expressed as steviol content. This limit is equivalent to 10 mg per kg bw per day (i.e. ~ 710 mg per day for an adult) for Stevioside or mixed steviol glycosides, 12 mg per kg bw per day (i.e. ~ 850 mg per day for an adult) for Rebaudioside A, or 50 mg per kg bw per day (i.e. ~ 3,550 mg per day for an adult) of stevia leaf.
    - As a Non-Medicinal Ingredient: As a sweetener or flavor enhancer, the quantity used should be according to conditions of CGMP and should not exceed the amount required to accomplish the purpose for which that non-medicinal ingredient is permitted to be added. As a non-medicinal ingredient, it should not exceed 4 mg per kg bw per day expressed as steviol content.
- On November 30, 2012, Health Canada published its final clearance for use of steviol glycosides as a sweetener in foods (Health Canada, 2012).
- In March 2014, Health Canada updated the List of Permitted Sweeteners (Lists of Permitted Food Additives) to include steviol glycosides in applications as a table-top sweetener and as an ingredient in a variety of foods, beverages, baked goods, meal replacement bars, condiments, and confectionary and gums (Health Canada, 2014).
- On January 15, 2016, Health Canada approved the use of Rebaudioside M for use as a high-intensity sweetener under the same conditions as the previously approved steviol glycosides (Health Canada, 2016).
- Health Canada (2017b) also modified the List of Permitted Sweeteners to include “all the steviol glycosides in the *Stevia rebaudiana* Bertoni plant (stevia plant).”
- On August 30, 2017, Health Canada’s Food Directorate updated its List of Permitted Sweeteners to allow for the use of steviol glycosides as a sweetener in ‘unstandardized snack bars,’ including granola bars, cereal bars, fiber bars, and protein isolate-based bars (Health Canada, 2017a).
- On August 27, 2018, Health Canada’s Food Directorate updated its List of Permitted Sweeteners to provide stakeholders with further information on the Lists of Permitted Food Additives as well as guidance on how to interpret and use these lists (Health Canada, 2018).
- On April 3, 2019, Health Canada’s Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides from *Stevia rebaudiana* Bertoni in canned fruit products (Health Canada, 2019).

- Most recently, on May 14, 2019, Health Canada's Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides derived from *Saccharomyces cerevisiae* strains CD15380 and CD15407 at the same maximum levels of use as steviol glycosides derived from *Stevia rebaudiana* Bertoni (Health Canada, 2019).

## 3. European Regulatory History

- The Joint Expert Committee on Food Additives (JECFA) reviewed steviol glycosides at its 51st, 63rd, 68th and 73rd meetings and published its original review in 2000 (WHO, 2000).
- In 2006, JECFA established a temporary ADI (acceptable daily intake) of 0 - 2 mg per kg (on a steviol basis) at its 63rd meeting (WHO, 2006).
- In 2007, JECFA finalized food grade specifications (FAO, 2007b), although they were subsequently updated in 2008 (FAO, 2008) and 2010 (FAO, 2010).
- In 2009, at the 69th meeting, the temporary status of the ADI was removed, and the ADI was raised to 0 - 4 mg per kg bw per day (on a steviol basis) as a result of the JECFA review of more recently completed clinical studies with steviol glycosides (WHO, 2008). In 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009).
- In 2009, several countries and the Calorie Control Council submitted a request to the Codex Committee on Food Additives to modify the JECFA specifications for steviol glycosides to include Rebaudioside D and Rebaudioside F as specifically named acceptable glycosides that would be considered as part of the minimum 95% steviol glycosides composition (CCFA, 2009). The proposal was discussed at the June, 2010 JECFA Meeting (FAO/WHO, 2009), and JECFA subsequently took final action in approving the modified steviol glycosides specifications to include Rebaudioside D and Rebaudioside F (FAO, 2010).
- In 2008, Switzerland's Federal Office for Public Health approved the use of stevia as a sweetener citing the favorable actions of JECFA (Switzerland Federal Office of Public Health, 2008).
- In 2009, France published its approval for the food uses of Rebaudioside A with a purity of 97% (AFSSA, 2009a; AFSSA, 2009b).
- In June 2008, the European Commission requested for EFSA to deliver a scientific opinion on the safety of steviol glycosides as a sweetener for use in the food categories specified in the dossiers from three petitioners.
    - EFSA reexamined the safety of steviol glycosides (EFSA, 2010), the EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg per bw per day, which is similar to JECFA's determination.
    - On May 25, 2011, EFSA published the daily dietary intake for use of Rebaudioside A as a flavoring substance in a variety of foods would be less than the ADI for steviol glycosides (EFSA, 2011a).
    - In 2014, EFSA evaluated extending the use of steviol glycosides as ingredients in food categories to include coffee, tea, and herbal and fruit infusions (assessed at 10 mg per L steviol glycosides) (EFSA, 2014).

  - In 2015, EFSA revised exposure estimates based on the EFSA Comprehensive European Food Consumption Database and the proposed extension of use for tea beverages and instant coffee and cappuccino products up to 29 mg per L of steviol equivalents, rather than 10 mg per L, as assessed in the previous 2014 EFSA opinion. EFSA noted that the mean exposure estimates remain below the ADI of 4 mg per kg bw per day for all population groups, with the exception of toddlers (in one country) at the upper range of the high-level exposure estimates (95$^{th}$ percentile: 4.3 mg per kg bw per day), which remains above the ADI. EFSA concluded that dietary exposure to steviol glycosides (E 960) is similar to the exposure estimated in 2014 and therefore does not change the outcome of the safety assessment (EFSA, 2015).
- On December 2, 2011, the EU approved steviol glycosides use as food additives (EU, 2011) based upon agreement between the JECFA and EFSA that steviol glycosides are safe for all populations to consume and are a suitable sweetening option for diabetics.
- On November 3, 2016, the EU food additives regulation 231/2012 was amended to remove the previous requirement for stevia blends to contain at least 75% Reb A or Stevioside.
- On October 13, 2016, the EU updated regulation EU 2016/1814 to permit the following steviol glycosides in stevia blends: Stevioside, Rebaudiosides A, B, C, D, E, F and M, Steviolbioside, Rubusoside, and Dulcoside (Searby, 2016).
- On January 31, 2018, the EFSA Panel of Food Additives and Nutrient Sources reviewed an application for glucosylated steviol glycoside preparations for use as a new food additive. The Panel concluded that the data supplied by the applicant were “insufficient to assess the safety” of the preparation. No safety concerns were raised by the EFSA Panel; however, their decision was based on the “limited” data provided in the dossier submitted by the applicant (EFSA, 2018).

## 4. Asian Regulatory History

- In May 2010, Hong Kong amended its food regulations to allow the use of steviol glycosides as a permitted sweetener in foods based upon the detailed safety evaluation and favorable findings as reported by JECFA (Hong Kong Centre for Food Safety, 2010).
- In July 2011, the Codex Alimentarius Commission adopted proposed maximum use levels for steviol glycosides in all major food and beverage categories which resulted in steviol glycoside approvals in Vietnam, the Philippines, Malaysia, Singapore and Thailand (Whitehead, 2013).
- The International Alliance of Dietary/Food Supplement Associations (IADSA) reported that the Codex Alimentarius Commission agreed to adopt the use of steviol glycosides for addition to chewable food supplements (Food Ingredients First, 2011).
- On September 20, 2012, the Food Safety and Standards Authority of India (FSSAI) approved the use of steviol glycosides as a non-nutritive sweetener in a variety of foods using specifications and purity established by JECFA (FSSAI, 2012).

- Since December 10, 2012, over thirty registrations have been granted by FDA Philippines to stand-alone steviol glycosides sweeteners or foods containing steviol glycosides as ingredients (Philippines, 2014).
- Steviol glycosides are also listed under International Numbering System (INS) number 960 in the Food Additives Permitted Under the Singapore Food Regulations document prepared by the Agri-Food & Veterinary Authority (AVA) of Singapore (AVA, 2014).

## 5. Australia and New Zealand Regulation History

- In 2008, the Food Standards Australia New Zealand (FSANZ) completed its evaluation of an application for use of steviol glycosides in foods and recommended that the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) amend the Australia New Zealand Food Standards Code to allow the use of steviol glycosides in food (FSANZ, 2008).
- On May 13, 2011, FSANZ approved an increase in the maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages, and flavored soy beverages up to 200 mg per kg, and in plain soy beverages up to 100 mg per kg (FSANZ, 2011).
- In 2015, FSANZ concluded that the use of Reb M does not pose any “public health and safety issues” (FSANZ, 2015).
- On January 14, 2016, Reb M was approved for use “as a food additive in accordance with the current permissions for steviol glycosides” (FSANZ, 2016a).
- In 2016, FSANZ called for submissions on permitting all minor steviol glycosides extracted from stevia leaf to be included in the definition of steviol glycosides in the Food Standards Code, noting that “[no] evidence was found to suggest that the proposed changes pose any public health and safety concerns” (FSANZ, 2016b).
- On February 8, 2017 FSANZ approved a draft variation of the definition of steviol glycosides to include all steviol glycosides present in the *Stevia rebaudiana* leaf (FSANZ, 2017).
- Most recently, FSANZ called for comments on the production of steviol glycosides produced with enzymes derived from genetically modified strains of *E. coli* (FSANZ, 2019a). The comment period closed on October 8, 2019, with 5 comments that supported the draft document. The approval report was issued on December 20, 2019 (FSANZ, 2019b).

## 6. South Africa

- On September 10, 2012, the South African Department of Health promulgated a new sweetener regulation: Regulation R733 (Regulations Relating to the Use of Sweeteners in Foodstuffs), allowed for the use of extracts of stevia rebaudiana, in composition and quantities in line with Codex standards, in food and beverages. Steviol glycosides can be used to a maximum level of 330 mg per kg (Food Stuff South Africa, 2012).

# Appendix 12 Summary of Published Safety Reviews

## A. Summary of JECFA Reviews

- 51st Meeting (WHO, 2000) – Stevioside evaluation determined that there was insufficient and inconsistent information on the Stevioside or steviol. No human metabolism data or mutagenicity data were available. JECFA determined that the ADI could not be determined without further data.
- 63rd Meeting (WHO, 2006) – More data were submitted; however, the data were inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g., those with hypotension or diabetes). The Committee allocated a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg per kg bw was established for steviol glycosides, expressed as steviol, based on a NOEL for Stevioside of 970 mg per kg bw per day (or 383 mg per kg bw per day, expressed as steviol) in the two-year study in rats and a safety factor of 200.
- 68th Meeting (WHO, 2007) – Further data were submitted showing the purity at 95% and that all steviol glycosides hydrolyze to steviol upon ingestion. JECFA determined that it was unnecessary to maintain a limit for the sum of Stevioside and Rebaudioside content that could include product that was at least 95% Stevioside or at least 95% Rebaudioside A. The Chemical and Technical Assessment report, written after the 2007 meeting, explained the Committee's thinking, which resulted in flexibility in the identity specifications (FAO, 2007a; FAO, 2007b).
- 69th Meeting (WHO, 2008) – Based on additional clinical studies, JECFA finalized the evaluation of steviol glycosides and raised the ADI to 0 - 4 mg per kg bw per day and removed the "temporary" designation. A summary of the Committee's key conclusions was published in the final toxicology monograph addendum (WHO, 2009).

## B. Summary of FSANZ Review of Steviol Glycosides

- In 2008, FSANZ reviewed the safety of steviol glycosides and concluded that they are well-tolerated and unlikely to have adverse effects on blood pressure, blood glucose, or other parameters in normal, hypotensive, or diabetic subjects at doses up to 11 mg per kg bw per day. FSANZ agreed with JECFA in setting an ADI of 4 mg steviol equivalents per kg bw per day (FSANZ, 2008).
- On May 13, 2011, FSANZ approved an increase in the maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages and flavored soy beverages up to 200 mg per kg and in plain soy beverages up to 100 mg per kg (FSANZ, 2011).
- On January 16, 2016, FSANZ approved the addition of Rebaudioside M as a steviol glycoside intense sweetener (FSANZ, 2016a).

- On February 20, 2017, FSANZ broadened the definition and, hence, specification for steviol glycosides preparations to include any mixture of individual steviol glycosides extracted from the stevia leaf.

## C. Summary of EFSA Review of Steviol Glycosides

- On March 10, 2010, EFSA adopted a scientific opinion on the safety of steviol glycosides (mixtures that comprise not less than 95% of Stevioside and/or Rebaudioside A) as a food additive based upon JECFA's 2008 findings and in response to the European Commission's request to reevaluate the safety of steviol glycosides as a sweetener (EFSA, 2010).
    - EFSA agreed that the results of toxicology studies on either Stevioside or Rebaudioside A are applicable for the safety assessment of steviol glycosides.
    - EFSA established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg per kg bw per day primarily based on the application of a 100-fold uncertainty factor to the NOAEL in the two-year carcinogenicity study in the rat when administering 2.5% Stevioside in the diet (Toyoda et al., 1997).
- On January 11, 2011, EFSA revised the exposure assessment of steviol glycosides from its use as a food additive, for children and adults, based on the revised proposed uses presented.
    - EFSA reduced usage levels in 16 foods by a factor of 1.5 to 3, with no changes for 12 food groups.
    - The mean estimated exposure to steviol glycosides (equivalents) in European children (aged 1-14 years) ranged from 0.4 to 6.4 mg per kg bw per day and from 1.7 to 16.3 mg per kg bw per day at the 95$^{th}$ percentile.
    - A correction was considered to be necessary for the consumption of non-alcoholic flavored drinks (soft drinks) by children, and the corrected exposure estimate at the 95$^{th}$ percentile for children ranged from 1.0 to 12.7 mg per kg bw per day.
    - For adults, the mean and 97.5$^{th}$ percentile intakes were estimated to range from 1.9 to 2.3 and 5.6 to 6.8 mg per kg bw per day, respectively.
    - These revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg per kg bw (steviol equivalent).

## D. Other Published Reviews

- Stevia and steviol glycosides have been extensively investigated for their biological, toxicological, and clinical effects (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002).
- Four additional reviews have appeared on the toxicology and biological activity of stevia extracts and steviol glycosides (Yadav and Guleria, 2012; Brown and Rother, 2012; Brahmachari et al., 2011; Chatsudthipong and Muanprasat, 2009). The studies are not always closely comparable because:
    - These reviews do not clearly differentiate between studies on crude stevia extract and purified steviol glycosides.

  - Studies on biological activity used routes of administration other than oral.
  - Some studies may have used doses that are much higher than anticipated human use levels.
  - Roberts and Munro (2009) criticized the Chatsudthipong and Muanprasat (2009) review with points that are applicable – in general – to all the reviews:
    - Lack of purity of the material,
    - Route of exposure in relation to metabolism and safety assessment - *in vitro* and intravenous, intraperitoneal, or subcutaneous dosing studies are not relevant to the safety of steviol glycosides consumed orally.
    - Paucity of discussion of worldwide regulatory authorities affirming the safety of purified forms of Stevioside and Rebaudioside A as a food ingredient.
- Urban et al. (2015) reviewed the potential allergenicity of steviol glycosides. The authors noted that: “hypersensitivity reactions to stevia in any form are rare” and concluded that current data do not support claims that steviol glycosides are allergenic. In addition, the authors stated that there is “little substantiated scientific evidence” to warrant consumer warning statements to consumers about allergy to highly purified stevia extracts.

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# Appendix 13 GRAS Associates Expert Panel Report

**The Generally Recognized as Safe (GRAS) Status of the Proposed Uses of BESTEVIA® Rebaudioside I**

January 17, 2020

## Foreword

An independent panel of experts ("Expert Panel") was convened by GRAS Associates, LLC on behalf of their client, Blue California, to evaluate the safety and Generally Recognized as Safe (GRAS) status of Blue California's proposed uses of BESTEVIA® Rebaudioside I in conventional foods. The members of this Expert Panel† are qualified to serve in this capacity by qualification of scientific training and experience in the safety of food and food ingredients.

## Discussion

A significant amount of safety information related to the consumption of steviol glycosides is generally available, and has been discussed in Part 6, as well as Appendices 9-12, of Blue California's dossier. First, there is a history of safe consumption of steviol glycosides when used as an ingredient in food products in the U.S., Canada, South America, Europe, Asia, and Australia and New Zealand. Second, a number of experimental studies have investigated the safety of steviol glycosides. The composite evidence from historical safe consumption and experimental studies collectively demonstrate the safety of BESTEVIA® Rebaudioside I for human food consumption.

The majority of the studies reviewed on steviol glycosides and steviol have been discussed in detail in previous GRAS Notices (GRNs), including GRN 278, GRN 667, GRN 715, and GRN 823, which were submitted by Blue California.

With regard to the safety documentation, the key pharmacokinetic data establish that steviol glycosides are not absorbed through the GI tract, per se; they are converted to steviol by bacteria normally present in the large intestine, and the steviol is absorbed but rapidly metabolized to steviol glucuronide and excreted. It has been well-established experimentally from various published studies that the steviol glycoside molecules are not absorbed from the GI tract (Gardana et al., 2003; Koyama et al., 2003b). The action of bacteria in the large intestine is directly supported by the published study that showed that steviol glycosides can be converted to steviol in

† Dr. Emmel, Chair of the Expert Panel, is a chemist with substantial food safety experience in addressing steviol glycosides and other food ingredients. Dr. Kapp is a toxicologist with over 35 years of experience. He is a Fellow of the Academy of Toxicological Sciences, a Fellow of the Royal Society of Biology, and a European Registered Toxicologist. Dr. Lewis is a biologist with more than 10 years of experience preparing GRAS dossiers. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety and in participating in deliberations of GRAS Expert Panels.

the large intestine by normal anaerobic GI flora as demonstrated by an *in vitro* study in fecal homogenates (Koyama et al., 2003a; Renwick and Tarka, 2008).

A review of published literature indicates that Rebaudioside I has not been observed in *Stevia rebaudiana* Bertoni, though it was first isolated from the closely related cultivar, *Stevia rebaudiana* Morita (Ohta et al., 2010). It should be noted that Rebaudioside I is listed as a recognized steviol glycosides by JECFA in the "Steviol Glycosides from *Stevia rebaudiana* Bertoni" monograph published in 2017 (FAO, 2017). There is substantial structural similarity between Rebaudioside I and the other steviol glycosides, therefore Rebaudioside I is expected to be metabolized to steviol in a parallel manner. Furthermore, FEMA recently determined that Rebaudioside I (95%) is GRAS in certain flavoring applications with an average maximum use level of 30 parts per million (ppm).

The Expert Panel reviewed the recent publications on steviol glycosides by Sánchez-Delgado et al. (2019), Wang and Wu (2019), and Zhou et al. (2019), and did not identify any that raise safety concerns with regard to the use of steviol glycosides in conventional foods.

The ADI for steviol glycosides has been set largely based on a published chronic study in rats (Toyoda et al., 1997) and several published clinical studies that show there are no pharmacological effects in humans at doses several fold higher than the ADI (Barriocanal et al., 2006; Barriocanal et al., 2008; Wheeler et al., 2008). Recently, Roberts et al. (2016) noted in a persuasive argument using a chemical-specific adjustment factor (CSAF) that the ADI could be higher. The toxicity of the metabolite, steviol, has been well reviewed in the published literature (Geuns, 2003; WHO, 2006; Urban et al., 2013). In addition, FDA has issued "no questions" letters in response to 64 GRN submissions for steviol glycosides preparations.

The Expert Panel notes that Blue California's manufacturing process for BESTEVIA® Rebaudioside I is similar to the processes described for other GRAS steviol glycosides materials synthesized from *Stevia rebaudiana* extracts by genetically modified yeast, as described in GRN 667, GRN 715, and GRN 823.

The GRAS Associates Expert Panel convened on behalf of Blue California has reviewed the proposed uses for BESTEVIA® Rebaudioside I. The highest 90$^{th}$ percentile consumption by any population subgroup of BESTEVIA® Rebaudioside I was calculated to be approximately 5.93 mg per kg bw per day, which is equivalent to 1.67 mg per kg bw per day steviol equivalents (calculated by a weighted sum estimate) for any population group, on a worst-case scenario basis. This estimated intake value is well below the JECFA ADI of 4 mg per kg bw per day expressed as steviol equivalents. Therefore, BESTEVIA® Rebaudioside I is expected to be safe within established allowable limits.

A compelling case can be made that scientific consensus exists regarding the safety of steviol glycosides when of sufficiently high purity. The central role of conversion to steviol and subsequent elimination with these naturally occurring steviol glycosides extends to the manner in which the

various steviol glycosides molecules are metabolized and eliminated from the body. While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Panel believes that a wide consensus does exist in the scientific community to support a GRAS conclusion as evidenced by several publications (Carakostas, 2012; Geuns, 2007; Urban et al., 2013; Waddell, 2011; Williams, 2007; Brusick, 2008) that refute safety concerns expressed by a minority of scientists. In addition, Roberts et al. (2016) suggest that the ADI for steviol glycosides could be as high as 6 to 16 mg per kg bw per day, which is higher than has been previously accepted by the scientific community, providing the potential for an even more robust safety profile.

In summary, sufficient qualitative and quantitative scientific evidence in the composite is available to support the safety-in-use of Blue California's BESTEVIA® Rebaudioside I (≥95% Rebaudioside I) preparation given the following conditions:

- Blue California's BESTEVIA® Rebaudioside I continues to meet the designated specifications;
- The minimum sweetness intensities for BESTEVIA® Rebaudioside I remains unchanged; and
- BESTEVIA® Rebaudioside I is produced in accordance with Current Good Manufacturing Practices (CGMPs).

**Conclusion**

The Expert Panel critically reviewed the data provided by Blue California for their BESTEVIA® Rebaudioside I preparation, as well as publicly available published information obtained from peer-reviewed journals and other safety assessments prepared by other Expert Panels and well-respected international regulatory bodies.

The ingestion of Blue California's BESTEVIA® Rebaudioside I from the intended uses results in intakes that are safe within the limits of established historical use and published safety studies and the widely accepted ADI of 4 mg per kg bw per day steviol equivalents.

The Expert Panel unanimously concluded that the proposed uses of Blue California's BESTEVIA® Rebaudioside I preparation, manufactured as described in Part 2.B. of their dossier, and declared within the subject notification meets the FDA definition of safety in that there is "reasonable certainty of no harm under the intended conditions of use" as described herein, and Blue California's BESTEVIA® Rebaudioside I preparation is generally recognized as safe (GRAS).

Robert W. Kapp, Jr., Ph.D.
Fellow ATS, FRSB, & ERT(UK)

Kara Lewis, Ph.D.

Katrina V. Emmel, Ph.D.
Panel Chair

**END**