ORIGINAL SUBMISSION

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March 7, 2016

GRN 000638

Dr. Antonia Mattia
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Subject: GRAS Notice for High Purity Rebaudioside A

Dear Dr. Antonia Mattia:

On behalf of Hunan Huacheng Biotech, Inc., we are submitting for FDA review a GRAS notification for High Purity Rebaudioside A. The attached documents contain the specific information that addresses the safe human food uses for the notified substance. We believe that this determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

We enclose an original and two copies of this notification for your review. Please feel free to contact me if additional information or clarification is needed as you proceed with the review. We would appreciate your kind attention to this matter.

Sincerely,

(b) (6)

3/7/2016

Susan Cho, Ph.D.
Susanscho1@yahoo.com
Agent for Huacheng Biotech, Inc.

enclosure

GRAS EXEMPTION CLAIM FOR HIGH PURITY REBAUDIOSIDE A MANUFACTURED BY HUNAN HUACHECNG BIOTECH, INC.

GRN 000638

Prepared by: NutraSource, Inc.
6309 Morning Dew Court
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Tel: 410-531-3336;
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GRAS EXEMPTION CLAIM

A. Rebaudioside A: Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36

Hunan Huacheng Biotech, Inc. (hereinafter referred to as Hunan HBI) has determined that its High Purity Rebaudioside A (Reb A; powder form) is Generally Recognized As Safe (GRAS). Consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act,* this determination is based on scientific procedures described in the following sections. Since these procedures specify the conditions of its intended use in food, the use of Hunan HBI's Reb A is exempt from the requirement of premarket approval.

Signed

(b) (6)

3/5/2016

Susan Cho Date:
Agent for Hunan Huacheng Biotech, Inc (Hunan HBI).

B. Notifier's Name and Address

Fiona Chu
Hunan Huacheng Biotech Inc.
188#, Tongzi'po West. Rd., Changsha, Hunan, China
Tel: 0086-731-8898-8198
Fax: 0086-731-8898-8118
Email: fionachu@huachengbio.com

C. Name of GRAS Substance

The common name of the notified substance is High Purity Rebaudioside A (Reb A).

Trade name is Bestevia™

D. Product Description

D.1. Identity

GRAS EXEMPTION CLAIM FOR HIGH PURITY REBAUDIOSIDE A MANUFACTURED BY HUNAN HUACHECNG BIOTECH, INC.

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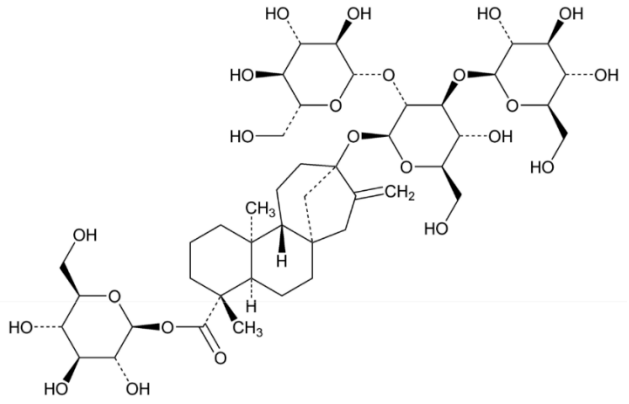
Rebaudioside A (Reb A) is a sweet tasting steviol glycoside extracted and purified from *Stevia rebaudiana* (Bertoni). *Stevia rebaudiana* Bertoni is a small perennial shrub of the Asteraceae (Compositae) family that is native to South America, particularly Brazil and Paraguay, where it is known as "stevia" or "honey leaf" for its powerful sweetness (Carakostas, 2012). Stevia leaves were used by indigenous peoples in Paraguay and Brazil since before recorded history (Carakostas, 2012). Stevia became more widely known outside central South America following the 1887 ''discovery" of stevia by botanist, Antonio Bertoni. Stevia is the generic term used for food ingredients derived from the herb *Stevia rebaudiana* (Bertoni) (Carakostas et al., 2008). Reb A is non-nutritive and tastes approximately 200-300 times sweeter than sucrose.

This GRAS document discusses Reb A products containing more than 97% Reb A. These products are similar to the products described in GRNs 253, 278, 282, 354, and 461 for which FDA has issued "no question" letters. Figure 1 presents chemical structures of Reb A. A typical compositional analysis shows that HBI's High Purity Reb A is composed of ≥97% Reb A, 2.2% moisture, and 0.1% ash.

CAS number: 58543-16-1
Chemical name: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D- glucopyranosyl ester
Chemical formula: $C_{44}H_{70}O_{23}$
Formula weight: 967.03 daltons

Fig. 1. Chemical Structures of Reb A.



D.2. Manufacturing process
The following outlines the procedures by which Hunan HBI processes its Reb A:

1. *S. rebaudiana* leaves are dried, crushed, and subjected to extraction with hot water.
2. The extract is then centrifuged to precipitate plant substances such as pectin and pigment, this is followed by filtration steps.
3. The filtrate is passed through adsorption resin to trap the steviol glycoside components. Subsequently, the glycosides adsorbed on the resin are eluted with ethanol.
4. The ethanol extract is decolored using active carbon.

5. The decolored concentrated solution is cooled to crystallize steviol glycosides at 2-5℃ for 24-30 h.
6. The concentrated solution containing Reb A is filterd, then ethanol/water solution or methanol/water solution is added. After dissolving adequately, this solution is filtered through plate-frame filter press. The filtrate is cooled to crystallize Reb A at 2-5℃ for 24-30 h. This is filtered via suction filtration and crude Reb A crystals are washed with 2-5℃ of pure water.
7. The crude Reb A crystals are dissolved in ethanol/water solution or methanol/water solution. The dissolved liquor is cooled to re-crystallize Reb A at 2-5℃ for 24 h. This is then filtered through suction filtration to get the recrystallized Reb A crystals.
8. The recrystallized Reb A crystals are then placed in 2-5℃ of pure water and stirred evenly. This is then filtered through suction filtration and washed with pure water at 2-5℃. The Reb A content of recrystallized crystals is more than 97.0%.
9. The Reb A product is dried under vacuum, ground, and passed through an 80 mesh screen before packaging.

All chemical reagents used in the process are food grade. Processing aids used in the purification process comply with FCC 5th Edition specifications, and the filters and adsorption resins used in the manufacturing comply with 21 CFR 173.25. The Hunan HBI's High Purity Reb A is prepared in accordance with current Good Manufacturing Practices (cGMP). Hunan HBI uses a Hazard Analysis and Critical Control Points (HACCP)-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications.

D.3. Specifications

Table 1 presents specifications of Hunan HBI's Reb A. Hunan HBI's specifications generally meet or exceed those established by FCC or the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

Table 1. Specifications for Steviol glycosides

Physical and chemical parameters	Hunan HBI's Reb A	JECFA[a] specifications for steviol glycosides	FCC[b] specifications for Reb A	Method
Appearance Form	powder or crystal	Powder	Crystal, granule, or powder	Visual
Appearance Color	White to light yellow	White to light yellow	White to off-white	Visual
Solubility[d]	Soluble in water and ethanol	Freely soluble in water	Freely soluble in water:ethanol (50:50)	USP
Purity (by HPLC)	≥97%	NS	≥ 95%	JECFA HPLC
Residual Ethanol[c]	≤5,000 ppm	≤5,000 ppm	≤5,000 ppm	USP 37
Residual Methanol	≤200 ppm			USP 37
Loss on Drying	≤6.0%	≤6.0%	≤6.0%	USP

pH, 1% Solution	4.5-7.0	4.5-7.0	4.5-7.0	USP
Total Ash	≤1.0%	≤1.0%	≤1.0%	USP
Arsenic	≤1 ppm	≤1 ppm	≤1 ppm	AOAC 2006.03
Lead	≤1 ppm	≤1 ppm	≤1 ppm	AOAC 2006.03
Total Plate Count	≤1000 cfu/g	NA	NA	CP
Yeast & Mold	≤100 cfu/g	NA	NA	CP
Total Coliform	≤100 mpn/g	NA	NA	CP
Salmonella spp	Negative	NA	NA	CP
Staphylococcus aureus	Negative	NA	NA	CP
E. coli	Negative	NA	NA	CP

[a] From the 73rd JECFA (2010).
[b] Food Chemicals Codex (FCC, 7th Ed.). 2010. Rebaudioside A monograph.
[c] No specification is necessary for residual methanol because this solvent is not used in the process.
NS = not specified; NA = not applicable; cfu=Colony Forming Units; CP=Chinese Pharmacopia; mpn=most probable number; Hunan HBI = Hunan Huacheng Biotech, Inc.®; Reb A= Rebaudioside A; AOAC=Association of Analytical Communities; HPLC= high-performance liquid chromatography; JECFA= Joint FAO/WHO Expert Committee on Food Additives; mpn=most probable number.

E. Applicable Conditions for Use of the Notified Substance

E.1. Current Regulatory Status

USA

The FDA has issued 'no question' letters on thirty-nine GRAS notifications related to food uses of Reb A and related compounds: 18 notices for Reb A, 11 notices of steviol glycosides - Reb A and stevioside as principal components; 6 notices related to other steviol glycosides, and 4 notices for enzyme-modified steviol glycosides (FDA, 2008a-b, 2009 a-d, 2010 a-e, 2011 a-j, 2012a-e; 2013a-f, 2014a-c, 2015 a-d). In these GRAS notices, toxicity-related studies on Reb A and related compounds from the literature were presented to support the safety in use of Reb A and other steviol glycosides. The FDA did not question the acceptability and suitability of these studies to establish the safety of Reb A for the proposed food uses. The FDA did not have questions regarding the summary of safety, concluding that daily intake of Reb A up to 5.0 mg /person/day is safe. The safety and related information in the above- mentioned GRAS notices are hereby incorporated by reference into this independent GRAS determination.

Joint FAO/WHO Food Standards Programme

The JECFA reviewed the safety of steviol glycosides multiple times (JECFA, 2006, 2007, 2008, 2009, 2010).

In a 2009 report titled "Safety evaluation of certain food additives" (Food additives series 60), JECFA noted that the results of the new studies presented to the Committee have shown no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw/day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. The Committee assigned a permanent Acceptable Daily Intake (ADI) for steviol glycosides of 0–4 mg/kg/bw expressed as steviol content.

In March 2015, the 47th session of the Committee on Food Additives (JECFA, 2015) noted that the current specifications for steviol glycosides would remain valid.

Food Standards Australia New Zealand (FSANZ)

The FSANZ (2008, 2011) also established an ADI for steviol glycosides of 4 mg/kg bw/day (expressed as steviol) based on the NOAEL value found from the 2-year rat carcinogenicity study by Toyoda et al. (1997).

European Union

The European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources Added to Food issued an opinion concluding that steviol glycosides are neither carcinogenic, genotoxic, or associated with any reproductive or developmental toxicity. Like the JECFA and FSANZ before them, the EFSA Panel considered the 2-year carcinogenicity study published by Toyoda et al. (1997) to be the pivotal study, and established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on application of a 100-fold uncertainty factor to the NOAEL for stevioside of 967 mg/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day) (EFSA, 2011).

Health Canada

In 2012, Health Canada approved 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 (Health Canada, 2014).

E.2. Intended Use Levels and Food Categories

The subject of the present GRAS determination contains ≥97% Reb A. Hunan HBI's Reb A product, containing Reb A as the principal sweetening component, is intended to be used as a table-top sweetener and general purpose non-nutritive sweetener and as a flavor modifier in various foods other than in infant formulas and meat and poultry products. The intended use will be as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19). The intended use levels will vary by food category, but the actual levels are self-limiting due to organoleptic characteristics. The amounts of purified Reb A to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation (21 CFR 182.1(b)(1)).

E.3. Estimated Dietary Intakes (EDIs) of Reb A Based upon Intended Food Uses

Using the methodology presented in Renwick (2008), the EDI of the Reb A have been calculated. To predict EDIs for Reb A, Renwick (2008) used a substitution method that takes

into account actual intake data of high consumption artificial sweeteners (expressed as sucrose equivalents). The estimates were calculated from published intake data of existing artificial sweeteners which had varying age ranges for children. In order to be conservative, Renwick (2008) used data from the age group of each study that showed the highest intake.

Using this method, the estimated dietary exposures for Reb A in average and high consumers are predicted to be 1.3 and 3.4 mg/kg body weight (bw)/day for the general population, 2.1 and 5.0 mg/kg bw/day for non-diabetic children and 3.4 and 4.5 mg/kg bw/day for children with diabetes. The highest predicted intake of Reb A would be in diabetic and non-diabetic children and diabetic adults, but predicted that dietary exposure would always be less than 5 mg/kg bw/day (Table 2).

The molecular weight of Reb A (967) is about three times that of steviol (318) and therefore, the overall intakes by average and high non-diabetic adult consumers given in Table 2 are equivalent to approximately 0.43 and 1.12 mg/kg bw, respectively, when expressed as steviol. The highest predicted dietary exposure, which is in high non-diabetic children consumers, would be equivalent to about 1.64 mg steviol/kg bw/day. These intakes are all below the ADI levels defined by the JECFA for steviol glycosides (JECFA, 2008) of 0-4 mg/kg bw expressed as steviol.

Table 2. The EDIs of Reb A Based on Sweetener Substitution

Population	Intakes of intense sweeteners expressed as sucrose equivalents, mg/kg bw/day		Predicted intakes of Reb A, mg/kg bw/day		Predicted intakes of Reb A as steviol equivalent, mg/kg bw/day	
	Average consumer	High consumer	Average consumer	High consumer	Average consumer	High consumer
Non-diabetic adults	255	675	1.3	3.4	0.43	1.12
Diabetic adults	280	897	1.4	4.5	0.46	1.48
Non-diabetic children	425	990	2.1	5.0	0.69	1.64
Diabetic children	672	908	3.4	4.5	1.12	1.48

Adopted from Renwick (2008). Reb A= Rebaudioside A.

As described in Renwick (2008), the EDIs for Reb A products represent very optimistic estimates of the potential intake.

E.4. Basis for the GRAS Determination

The intended use of Reb A (Bestevia™; powder form) has been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the Generally Recognized as Safe (GRAS) determination. In addition, because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

Technical Element (Safety) of the GRAS Determination

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of Reb A, as well as appropriate corroborative data.

1. Hunan HBI's Reb A is manufactured under cGMP using common food industry materials and processes. Hunan HBI uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to specifications.
2. Hunan HBI intends to market its Reb A (commercially known as Bestevia^{TM}) as a table-top and general-purpose sweetener and as a flavor modifier that will consist of not less than 97% Reb A. Analytical data from multiple lots indicate that Reb A complies reliably with the established food-grade product specifications and meet all applicable purity standards. Physical and chemical specifications of Hunan HBI's Reb A generally meet or exceed FCC and/or JECFA specifications. Additionally, results of the analysis show the absence of any pesticide residues in this Reb A preparation.
3. It is anticipated that the estimated consumption of Reb A for the defined food uses (1.64 mg/kg bw/day, as steviol equivalents, for high consumers) is expected to be much lower than the ADI values (0 to 4 mg/kg bw/day, as steviol equivalents) established by JECFA, EFSA, and FSANZ.
4. The EDI estimates are based on the assumption that Hunan HBI's Reb A will replace currently marketed high intensity sweeteners including all steviol glycosides. Thus, cumulative exposures are not expected to change. In addition, the EDIs presented in this notice are highly optimistic estimates.
5. In the previous GRAS notices to the FDA, the safety of Reb A has been established in toxicological studies in animals and in mutagenicity studies, and is further supported by clinical studies in human. The FDA responses to GRAS notifications on Reb A indicate that the FDA is satisfied with the safety-in-use of the Reb A, as long as consumption is 5.0 mg/kg bw/day or less.
6. Subchronic studies reported that NOAELs for Reb A were over 2,000 mg/kg bw/day in rats (Curry and Roberts, 2008; Nikiforov et al., 2008). In addition, a study found that LD_{50} values for stevioside were over 15 g/kg bw in rats, mice, and hamsters, indicating that steviol glycosides, including Reb A, are relatively harmless (Toskulkao et al., 1997).
7. The results of the human clinical trials demonstrate that steviol glycosides are safe and are well-tolerated in normotensive or hypotensive individuals and in subjects with type 2 diabetes following long-term consumption at doses of up to 1-1.5 g/day of Reb A or stevioside, or about 16-25 mg/kg bw/day (Chan et al., 2000; Hsieh et al., 2003; Maki et al., 2008a, 2008b).
8. Historical consumption of stevia and steviol glycosides supports the safety of Reb A. Intakes of the Reb A preparation have been estimated based on the extensive history of dietary intake assessments by numerous international governments and agencies and the intake assessments contained in 18 GRAS Notifications to the U.S. FDA.
9. Additional animal studies (Holvoet et al., 2015; Nikiforov et al., 2013) and a meta-analysis of human clinical trials (Onakpoya and Heneghan, 2015) published subsequent to the most recent FDA GRAS notice continued to support the safety of using Reb A as a food ingredient.

<u>Common Knowledge Element of a GRAS Determination</u>

FDA notes that general recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or

indirectly added to food. FDA discusses what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community. The two following components meet a common knowledge element of a GRAS determination:

1. 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
2. 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. 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.

Because this safety evaluation was based on generally available and widely accepted data and information, as well there was consensus among qualified scientists about the safety of the Reb A for its intended use, it also satisfies the "common knowledge" element of a GRAS determination.

F. Availability of Information

The detailed data and information that serve as a basis for this GRAS determination will be provided to the U. S. FDA upon request, or are available for the FDA's review and copying during reasonable business hours at the offices of NutraSource, Inc. located at 6309 Morning Dew Ct., Clarksville, MD 21029, USA.

G. Basis of GRAS determination: Through scientific procedures.

References

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EXPERT PANEL STATEMENT

OF THE
GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF
HIGH PURITY REBAUDIOSIDE A
AS A FOOD INGREDIENT

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1. INTRODUCTION

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by NutraSource, Inc., at the request of Hunan Huacheng Biotech, Inc.® (Hunan HBI), to determine the Generally Recognized As Safe (GRAS) status of its Rebaudioside A (hereinafter referred to as 'Reb A') as a table top sweetener and general purpose non-nutritive sweetener as defined in 21 CFR 170.3(o)(19) and as a flavor modifier in foods. A comprehensive search of the scientific literature for safety and toxicity information on Reb A was conducted and made available to the Expert Panel members. The Expert Panel members independently and critically evaluated materials submitted by Hunan HBI and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel unanimously agreed to the decision described herein.

The purpose of this dossier is to (1) Outline the identity and composition of Reb A, (2) Estimate exposure under the intended conditions of use, (3) Document the literature pertaining to the safety of Reb A, and (4) Assemble an independent panel of recognized experts to review the literature and assess the GRAS status of Reb A. The data and information summarized in this dossier demonstrate that the intended use of Reb A, produced using current Good Manufacturing Practices (cGMP) and meeting food-grade specifications, is GRAS, based on scientific procedures, as described herein.

II. INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

II.A. Background

Rebaudioside A (Reb A) is a sweet tasting steviol glycoside extracted and purified from *Stevia rebaudiana* (Bertoni). *Stevia rebaudiana* Bertoni is a small perennial shrub of the Asteraceae (Compositae) family that is native to South America, particularly Brazil and Paraguay, where it is known as "stevia" or "honey leaf" for its powerful sweetness (Ferrazzano et al., 2015). Stevia leaves were used by indigenous peoples in Paraguay and Brazil since before recorded history (Carakostas, 2012). Stevia became more widely known outside central South America following the 1887 ''discovery" of stevia by botanist, Antonio Bertoni. Stevia is the generic term used for food ingredients derived from the herb *Stevia rebaudiana* (Bertoni) (Carakostas et al., 2008).

Stevia rebaudiana leaves contain sweetening diterpene glycosides, known as steviol glycosides, which constitute 4–20% of the dry leaf weight (Ferrazzano et al., 2015). The leaf extracts are complex mixtures comprised of steviol glycosides (stevioside, Reb A, Reb B, Reb C, Reb D, Reb E, Reb F, dulcoside A, rubusoside, steviolbioside), labdane-type diterpenes, triterpenoids, steroids, volatile oils, and flavonoids. Stevioside and Reb A are the predominant steviol glycosides found in leaves of *S. rebaudiana*. Compared to stevioside, Reb A is considered more suitable for use to sweeten foods and beverages due to its less bitter aftertaste and greater solubility in water. Reb A is non-nutritive and tastes approximately 200-300 times sweeter than sucrose.

Several studies have suggested that in addition to their sweetness, steviosides and their related compounds, including rebaudioside A and isosteviol, may offer additional therapeutic benefits. These benefits include anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, anti-diarrheal, diuretic, and immunomodulatory actions (Ferrazzano et al., 2015). In addition, steviol glycosides are known to have antioxidant activities (Bender et al., 2015).

III. CLAIM OF GRAS STATUS

III.A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

High purity Reb A derived from *S. rebaudiana* for use as a table top sweetener and non-nutritive sweetener and as a flavor modifier has been determined to be Generally Recognized As Safe (GRAS), and therefore, is exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

III.B. Common or Trade Name:

The specific substances that are the subjects of this safety evaluation are identified as high purity rebaudioside A (Reb A) as produced and sold by Hunan Huacheng Biotech, Inc. (Hunan HBI) under the trade names of Bestevia™.

This GRAS document discusses High Purity Reb A products containing more than 97% Reb A. These products are similar to the products described in GRNs 253, 278, 282, 354, and 461 for which FDA has issued "no question" letters.

III.C. Name and Address of the Responsible Individual:

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Hunan Huacheng Biotech Inc (Hunan HBI)
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Fax: 0086-731-8898-8118
Email: fionachu@huachengbio.com

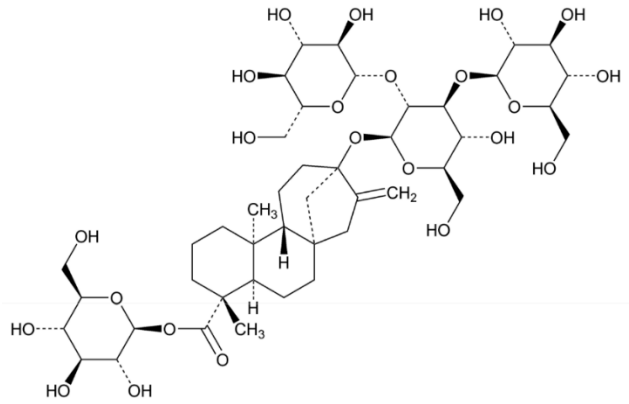
III.D. Chemistry, Physicochemical Properties, and Structure

Figure 1 presents chemical structures of Reb A and its related compounds. Table 1 summarizes the chemical characteristics of Reb A. Stevioside and Reb A are structurally very similar, with Reb A having one more glucose moiety when compared to stevioside. A typical compositional analysis shows that Huan HBI's Reb A is composed of 97.3% Reb A, 2.2% moisture, and 0.1% ash. The specifications meet or exceed those specified by the Food Chemical Codex (FCC, 2010).

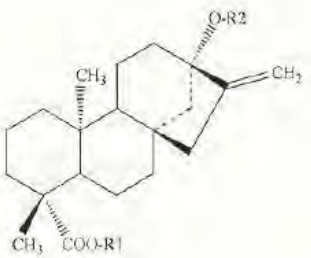
Table 1. Chemical Characterization of Reb A

Common Name	Rebaudioside A
Chemical name	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
Chemical formula	$C_{44}H_{70}O_{23}$
Formula weight	967.03 daltons
CAS Number	58543-16-1

Fig. 1. Chemical Structures of Reb A and Related Compounds.



Reb A



	Compound name	C.A.S. No.	R1	R2
1	Steviol	471-80-7	H	H
2	Steviolbioside	41093-60-1	H	β-Glc-β-Glc(2→1)
3	Stevioside	57817-89-7	β-Glc	β-Glc-β-Glc(2→1)
4	Rebaudioside A	58543-16-1	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
5	Rebaudioside B	58543-17-2	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
6	Rebaudioside C (dulcoside B)	63550-99-2	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
7	Rebaudioside D	63279-13-0	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
8	Rebaudioside E	63279-14-1	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
9	Rebaudioside F	438045-89-7	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
10	Rubusoside	63849-39-4	β-Glc	β-Glc
11	dulcoside A	64432-06-0	β-Glc	β-Glc-α-Rha(2→1)

[a] From FAO, 2007 and GRN 555.

III.E. Manufacturing Process of Hunan HBI's High Purity Reb A

The flow diagram in Figure 2 describes an overview of HBI's Reb A manufacturing process. In brief,

1. *S. rebaudiana* leaves are dried, crushed, and subjected to extraction with hot water.
2. The extract is then centrifuged to precipitate plant substances such as pectin and pigment, this is followed by filtration steps.
3. The filtrate is passed through adsorption resin to trap the steviol glycoside components. Subsequently, the glycosides adsorbed on the resin are eluted with ethanol.
4. The ethanol extract is decolored using active carbon.
5. The decolored concentrated solution is cooled to crystallize steviol glycosides at 2-5℃ for 24-30 h.
6. The concentrated solution containing Reb A is filterd, then ethanol/water solution or methanol/water solution is added. After dissolving adequately, this solution is filtered through plate-frame filter press. The filtrate is cooled to crystallize Reb A at 2-5℃ for 24-

30 h. This is filtered via suction filtration and crude Reb A crystals are washed with 2-5℃ of pure water.

7. The crude Reb A crystals are dissolved in ethanol/water solution or methanol/water solution. The dissolved liquor is cooled to re-crystallize Reb A at 2-5℃ for 24 h. This is then filtered through suction filtration to get the recrystallized Reb A crystals.
8. The recrystallized Reb A crystals are then placed in 2-5℃ of pure water and stirred evenly. This is then filtered through suction filtration and washed with pure water at 2-5℃. The Reb A content of recrystallized crystals is more than 97.0%.
9. The Reb A product is dried under vacuum, ground, and passed through an 80 mesh screen before packaging.

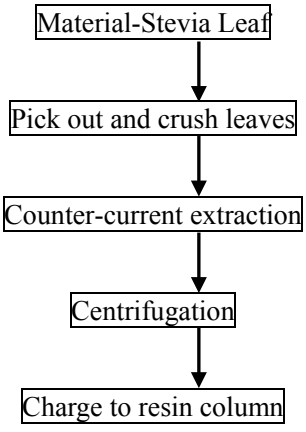
All chemical reagents used in the process, the adsorption resin, and the ion exchange resins are food grade. The processing aids used in the purification process comply with FCC 5th Edition specifications, and ion exchange resins used in the manufacturing comply with 21 CFR 173.25. The Hunan HBI's Reb A is prepared in accordance with current Good Manufacturing Practices (cGMP). Hunan HBI uses a Hazard Analysis and Critical Control Points (HACCP)-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications.

Certificates of analysis of five representative lots of Bestevia™, the market name for Hunan HBI's Reb A, are detailed in Appendix A. Results from pesticide analyses of representative Stevia Pure samples are also provided in Appendix B. Results of the analysis showed the absence of any pesticide residues in this Reb A preparation.

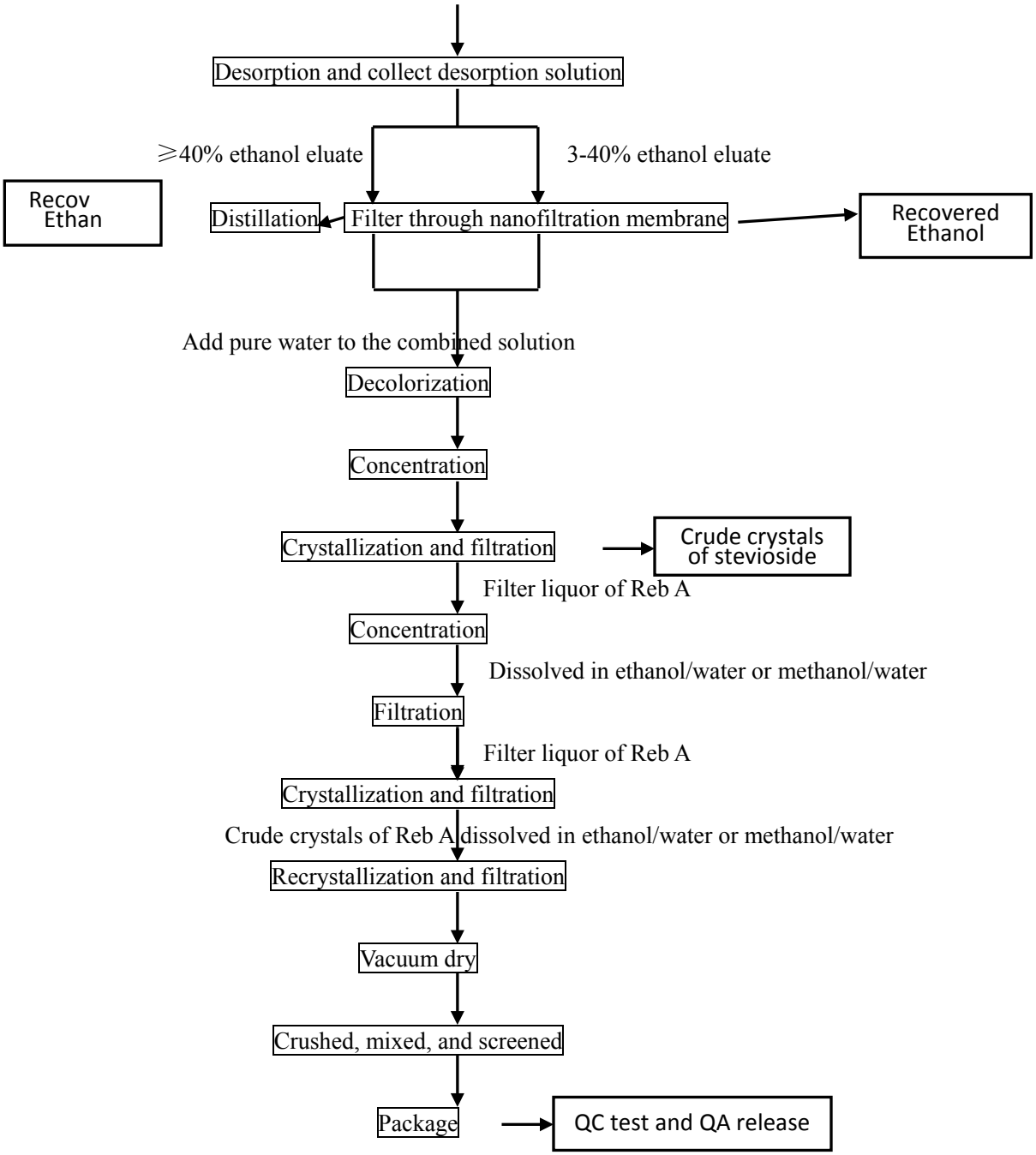
Stability

Reb A is very stable when stored as a powder. Storage for 24 months in polyethylene bags resulted in loss of only 1–2% of Reb A (Prakash et al., 2008).

Figure 2. Flow Diagram for Manufacturing Process of Reb A



Rebaudioside A



III. F. Specifications of Hunan HBI's High Purity Reb A

Table 2 presents specifications of Hunan HBI's high purity Reb A. Hunan HBI's specifications generally meet or exceed those established by FCC or the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

Table 2. Specifications for Steviol glycosides

Physical and chemical parameters	HunanHBI's Reb A	JECFA[a] specifications for steviol glycosides	FCC[b] specifications for Reb A	Method
Appearance Form	Powder or crystal	Powder	Crystal, granule, or powder	Visual
Appearance Color	White to light yellow	White to light yellow	White to off-white	Visual
Solubility[d]	Soluble in water and ethanol	Freely soluble in water	Freely soluble in water:ethanol (50:50)	USP
Purity (by HPLC)	≥97%	NS	≥ 95%	JECFA HPLC
Residual Ethanol[c]	≤5,000 ppm	≤5,000 ppm	≤5,000 ppm	USP 37
Residual Methanol	≤200 ppm			USP 37
Loss on Drying	≤6.0%	≤6.0%	≤6.0%	USP
pH, 1% Solution	4.5-7.0	4.5-7.0	4.5-7.0	USP
Total Ash	≤1.0%	≤1.0%	≤1.0%	USP
Arsenic	≤1 ppm	≤1 ppm	≤1 ppm	AOAC 2006.03
Lead	≤1 ppm	≤1 ppm	≤1 ppm	AOAC 2006.03
Total Plate Count	≤1000 cfu/g	NA	NA	CP
Yeast & Mold	≤100 cfu/g	NA	NA	CP
Total Coliform	≤100 mpn/g	NA	NA	CP
Salmonella spp	Negative	NA	NA	CP
Staphylococcus aureus	Negative	NA	NA	CP
E. coli	Negative	NA	NA	CP

[a] From JECFA (2010).
[b] Food Chemicals Codex (FCC, 7th Ed.). 2010. Rebaudioside A monograph.
[c] No specification is necessary for residual methanol because this solvent is not used in the process.
NS = not specified; NA = not applicable; cfu=Colony Forming Units; CP=Chinese Pharmacopia; mpn=most probable number; Hunan HBI = Hunan Huacheng Biotech, Inc.®; Reb A= Rebaudioside A; AOAC=Association of Analytical Communities; HPLC= high-performance liquid chromatography; JECFA= Joint FAO/WHO Expert Committee on Food Additives; mpn=most probable number.

IV. INTENDED USES AND EXPOSURE ESTIMATES

IV.A. Food Sources of Reb A
Leaves of stevia or *S. rebaudiana* are the source of Reb A.

IV.B. Intended Technical Effects
Reb A can be used as a naturally occurring high intensity, non-nutritive sweetener.

IV.C. Intended Use
The subject of the present GRAS determination contains ≥97% Reb A. Hunan HBI's Reb A product, containing Reb A as the principal sweetening component, is intended to be used as a table-top sweetener and general purpose non-nutritive sweetener and as a flavor modifier in various foods other than in infant formulas and meat and poultry products. The intended use will be as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19). The intended use levels will vary by food category, but the actual levels are self-limiting due to organoleptic characteristics. The amounts of purified Reb A to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation (21 CFR 182.1(b)(1)).

IV.D. Estimated Dietary Intakes (EDIs) Under the Intended Use
The EDIs were adopted from calculations of Renwick (2008). To predict EDIs for Reb A, Renwick (2008) used a substitution method that takes into account actual intake data of high consumption artificial sweeteners (expressed as sucrose equivalents). The estimates were calculated from published intake data of existing artificial sweeteners that had varying age ranges for children. In order to be conservative, Renwick (2008) used data from the age group of each study that showed the highest intake.

Using this method, the estimated dietary exposures for Reb A in average and high consumers are predicted to be 1.3 and 3.4 mg/kg body weight (bw)/day for the general population, 2.1 and 5.0 mg/kg bw/day for non-diabetic children and 3.4 and 4.5 mg/kg bw/day for children with diabetes. The highest predicted intake of Reb A would be in children and diabetic adults, but predicted dietary exposure would always be less than 5 mg/kg bw/day (Table 3).

Table 3. The EDIs of Reb A Based on Sweetener Substitution

Population	Intakes of intense sweeteners expressed as sucrose equivalents, mg/kg bw/day		Predicted intakes of Reb A, mg/kg bw/day		Predicted intakes of Reb A as steviol equivalent, mg/kg bw/day	
	Average consumer	High consumer	Average consumer	High consumer	Average consumer	High consumer
Non-diabetic adults	255	675	1.3	3.4	0.43	1.12
Diabetic adults	280	897	1.4	4.5	0.46	1.48
Non-diabetic children	425	990	2.1	5.0	0.69	1.64
Diabetic children	672	908	3.4	4.5	1.12	1.48

Adopted from Renwick (2008). Reb A= Rebaudioside A.

The molecular weight of Reb A (967) is about three times that of steviol (318) and, therefore, the overall intakes by average and high non-diabetic adult consumers given in Table 3 are equivalent to approximately 0.43 and 1.12 mg/kg bw, respectively, when expressed as steviol. The highest predicted dietary exposure, which is in high non-diabetic child consumers, would be equivalent to about 1.64 mg steviol/kg bw/day. These intakes are all below the acceptable daily intake (ADI) levels defined by JECFA for steviol glycosides (JECFA, 2008) of 0-4 mg/kg bw expressed as steviol.

As described in Renwick (2008), the EDIs for Reb A products represent very optimistic estimates of the potential intake for the following reasons: 1) the calculations are based on the assumption that Reb A products will capture the entire intense sweetener market and the intense sweetener market is unlikely to be dominated by a single sweetener. The presence of multiple sweeteners is likely to significantly reduce the intake of individual compounds compared with the data presented in the present GRAS assessment. Indeed, a market survey indicated that stevia-based sweeteners have only 8% of the intense sweetener market share (Food Navigator, 2013); 2) the analysis focuses on those studies and sweeteners that show the highest sucrose replacement, and studies giving low intakes were not used to calculate the values; 3) the analysis of the dietary exposures of children in Table 3 used data for the age group with the highest intakes; and 4) the assumptions, such as the use of food groups rather than individual food items and brands, and the use of maximum permitted use levels, have been made in many of the studies.

V. BASIS FOR GRAS DETERMINATION

V.A. Current Regulatory Status

USA

The FDA has issued ‘no question’ letters on thirty-nine GRAS notifications related to food uses of Reb A and related compounds: 18 notices for Reb A, 11 notices of steviol glycosides - Reb A and stevioside as principal components; 6 notices related to other steviol glycosides, and 4 notices for enzyme-modified steviol glycosides (Table 5; FDA, 2008a-b, 2009 a-d, 2010 a-e, 2011 a-j, 2012a-e; 2013a-f, 2014a-c, 2015 a-d). In these GRAS notices, toxicity-related studies on Reb A and related compounds from the literature were presented to support the safety in use of Reb A and other steviol glycosides. The FDA did not question the acceptability and suitability of these studies to establish the safety of Reb A for the proposed food uses. The FDA did not have questions regarding the summary of safety, concluding that Reb A intake up to 5.0 mg Reb A/person/day is safe. The safety and related information in the above- mentioned GRAS notices are hereby incorporated by reference into this independent GRAS determination. The pertinent information is summarized in Table 4.

Table 4. Summary of GRAS Notices of Steviol Glycosides

Company	FDA GRN number (Year)	Purity	Intended food uses
Reb A			
1. Merisant	GRN 252, (FDA, 2008a)	Reb A ≥95%	Variety of food categories and table-top sweetener
2. Cargill Inc.	GRN 253, (FDA, 2008b)	Reb A ≥97%	General-purpose sweetener, excluding meat and poultry products
3. McNeil Nutritionals LLC	GRN 275 (FDA, 2009a)	Steviol Glycosides – Reb A Principal Component	Table-top sweetener
4. Blue California	GRN 278 (FDA, 2009b)	Reb A ≥97%	General-purpose and table-top sweetener
5. Sweet Green Fields LLC	GRN 282 (FDA, 2009c)	Reb A ≥97%	General-purpose sweetener, excluding meat and poultry products
6. Sunwin USA LLC & WILD Flavors	GRN 303 (FDA, 2010a)	Reb A ≥95%/ ≥98%	General-purpose sweetener, excluding meat and poultry products and infant formulas
7. Pyure Brands, LLC	GRN 318 (FDA, 2010b)	Reb A 95%/ 98%	General-purpose and table-top sweetener, excluding meat and poultry products and infant formulas
8. PureCircle USA Inc.	GRN 323 (FDA, 2010c)	Steviol Glycosides – Reb A Principal Component	General-purpose and table-top sweetener, excluding meat and poultry products and infant formulas
9. GLG Life Tech Ltd.	GRN 329 (FDA, 2010d)	Reb A ≥97%	General-purpose sweetener, excluding meat and poultry products
10. Guilin Layn Natural Ingredients, Corp.	GRN 354 (FDA, 2011a)	Reb A ≥97%	General-purpose and table-top sweetener, excluding meat and poultry products and infant formulas
11. BrazTek International Inc.	GRN 365 (FDA, 2011b)	Reb A	General-purpose sweetener, excluding meat and poultry products
12. Shanghai Freemen Americas LLC.	GRN 369 (FDA, 2011c)	Reb A	General-purpose sweetener, excluding meat and poultry products
13. GLG Life Tech Ltd.	GRN 380 (FDA, 2011d)	Reb A	General purpose and table-top sweetener, excluding meat and poultry products

14. Chengdu Wagott Pharmaceutical	GRN 388 (FDA, 2012a)	Reb A	General purpose and table-top sweetener, excluding meat and poultry products
15. Daepyung Co., Ltd.	GRN 393 (FDA, 2012b)	Reb A	General purpose and table-top sweetener, excluding meat and poultry products
16. MiniStar International, Inc.	GRN 418 (FDA, 2012c)	Reb A	General-purpose sweetener, excluding meat and poultry products and infant formulas
17. Almendra, Ltd.	GRN 461 (FDA, 2013a)	Reb A ≥97%	General-purpose sweetener, excluding meat and poultry products and infant formulas
18. Qufu Xiang-zhou Stevia Products Co., Ltd.	GRN 467 (FDA, 2013b)	Reb A ≥98%	General-purpose sweetener, excluding meat and poultry products and infant formulas
Purified Steviol Glycosides - Reb A and Stevioside as principal component			
1. Wisdom Natural Brands	GRN 287 (FDA, 2009d)	Steviol Glycosides ≥95%	General-purpose sweetener, excluding meat and poultry products and infant formulas
2. Sunwin USA LLC & WILD Flavors	GRN 304 (FDA, 2010e)	Steviol Glycosides ≥95%	General-purpose sweetener, excluding meat and poultry products and infant formulas
3. GLG Life Tech Ltd.	GRN 348 (FDA, 2011e)	Steviosides	General-purpose and table-top sweetener, excluding meat and poultry products and infant formulas
4. GLG Life Tech Ltd.	GRN 349 (FDA, 2011f)	Steviol Glycosides ≥97%	General-purpose and table-top sweetener, excluding meat and poultry products and infant formulas
5. GLG Life Tech Ltd.	GRN 493 (FDA, 2014a)	Steviol Glycosides ≥95%	General purpose and table top sweetener, excluding meat and poultry products
6. Sinochem Qingdao Co. Ltd.	GRN 367 (FDA, 2011g)	Steviol Glycosides ≥95%	General-purpose and table top sweetener, excluding meat and poultry products and infant formulas
7. Daepyung Co., Ltd.	GRN 395 (FDA, 2012d)	Steviol Glycosides	General purpose and table-top sweetener, excluding meat and poultry products
8. Chengdu Wagott Pharmaceutical	GRN 389 (FDA, 2012e)	Steviol Glycosides	General purpose and table-top sweetener, excluding meat and poultry products
9. NOW Foods	GRN 337 (FDA, 2011h)	Steviosides	General-purpose sweetener in foods, excluding meat and poultry products, at levels determined by good manufacturing practices

10. Almendra (Thailand) Ltd.	GRN 516 (FDA, 2014b)	Steviol Glycosides	A table top and general purpose sweetener, excluding infant formulas and meat and poultry products
11. Productora Alysa SpA	GRN 555 (FDA, 2015a)	Steviol Glycosides ≥95%	A table top and general purpose non-nutritive sweetener, excluding infant formulas and meat and poultry products
Other Steviol Glycosides			
1. PureCircle USA, Inc.	GRN 456 (FDA, 2013c)	Reb D ≥95%	General-purpose sweetener, excluding meat and poultry products and infant formulas
2. PureCircle USA, Inc.	GRN 473 (FDA, 2013d)	Purified Steviol Glycosides – Reb M (Reb X) Principal	General-purpose sweetener, excluding meat and poultry products and infant formulas
3. GLG Life Tech Corp.	GRN 512 (FDA, 2014c)	Reb M	A table top and general purpose sweetener, excluding infant formulas and meat and poultry products
4. GLG Life Tech Corp	GRN 536 (FDA, 2015d)	Reb C	A table top and general purpose sweetener, excluding infant formulas and meat and poultry products
5. GLG Life Tech Corp	GRN 538 (FDA, 2015e)	Reb C	A table top and general purpose sweetener, excluding infant formulas and meat and poultry products
6. GLG Life Tech Corp	GRN 548 (FDA, 2015f)	Reb D	A table top and general purpose sweetener, excluding infant formulas and meat and poultry products
Enzyme Modified Steviol Glycosides			
1. NOW Foods	GRN 336 (FDA, 2011i)	Enzyme-modified Steviol Glycosides Preparation	General-purpose sweetener in foods, excluding meat and poultry products, at levels determined by good manufacturing practices
2. Toyo Sugar Refining Co., Ltd. & Nippon Paper Chemicals Co., Ltd.	GRN 375 (FDA, 2011j)	Enzyme-modified Steviol Glycosides	General-purpose sweetener in foods, excluding meat and poultry products, at levels determined by good manufacturing practices
3. Daepyung Co., Ltd.	GRN 448 (FDA, 2013e)	Enzyme-modified Steviol Glycosides	General-purpose sweetener, excluding meat and poultry products and infant formulas
4. Daepyung Co., Ltd.	GRN 452 (FDA, 2013f)	Enzyme-modified Steviol Glycosides	General-purpose sweetener, excluding meat and poultry products and infant formulas

[a] This table was updated and modified from GRN 516 and 555. Reb = Rebaudioside.

Joint FAO/WHO Food Standards Programme
The JECFA reviewed the safety of steviol glycosides multiple times (JECFA, 2006, 2007, 2008, 2009, 2010).

In its 69th meeting report titled "Evaluation of certain food additives", the JECFA Committee concluded that the new data were sufficient to allow the safety factor of 100, instead of 200, and the temporary designation to be removed, and so they established an ADI for steviol glycosides of 0-4 mg/kg bw/day expressed as steviol content (JECFA, 2008). For the purposes of comparing intake and safety limits, all steviol glycosides are converted to their steviol equivalents (Roberts and Renwick, 2008). Based on their relative molecular weights, stevioside quantities are multiplied by 0.40 and Reb A quantities by 0.33 to convert both to steviol equivalents. The ADI of 0-4 mg/kg bw/day on a steviol equivalent basis corresponds to 0-12 mg Reb A/kg bw/day using this molecular weight conversion.

In a 2009 report titled "Safety evaluation of certain food additives" (Food additives series 60), JECFA noted that the results of the new studies presented to the Committee have shown no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw/day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. The Committee assigned a permanent ADI for steviol glycosides of 0-4 mg/kg/bw expressed as steviol content.

In March 2015, the 47th session of the Committee on Food Additives (JECFA, 2015) noted that the current specifications for steviol glycosides would remain valid.

Food Standards Australia New Zealand (FSANZ)
The FSANZ (2008, 2011) also established an ADI for steviol glycosides of 4 mg/kg bw/day (expressed as steviol) based on the NOAEL value found from the 2-year rat carcinogenicity study by Toyoda et al. (1997).

European Union
The European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources Added to Food issued an opinion concluding that steviol glycosides are neither carcinogenic, genotoxic, or associated with any reproductive or developmental toxicity. Like the JECFA and FSANZ before them, the EFSA Panel considered the 2-year carcinogenicity study published by Toyoda et al. (1997) to be the pivotal study, and established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day. This determination was based on application of a 100-fold uncertainty factor to the NOAEL for stevioside of 967 mg/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day) (EFSA, 2011).

Health Canada
In 2012, Health Canada approved 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 (Health Canada, 2014).

V.B. Review of Safety Data

As noted above, the FDA has issued 'no question' letters on thirty-nine GRAS notices related to food uses of Reb A and related compounds. As Hunan HBI's Reb A product in this GRAS determination is similar in specifications compared to the other Reb A in the previous FDA GRAS notices, it is recognized that the information and data in the other GRAS notices are pertinent to the safety of the Reb A in this GRAS determination. This information is hereby incorporated by reference in this document and will not be discussed in detail. Additionally, this notice discusses an additional animal study and a meta-analysis of human clinical trials that have been published since the FDA's last reviews of 2014-2015. The subject of the present GRAS assessment is Reb A.

V.B.1. Metabolism of Steviol Glycosides (modified from GRN 473)

With the exception of having different numbers and types of sugar moieties, steviol glycosides share the same structural backbone, steviol. As such, all steviol glycosides are expected to follow the same metabolic pathway as demonstrated for Reb A and stevioside. Therefore, the results of toxicology studies on either stevioside or Reb A are applicable to the safety of all steviol glycosides.

Toxicokinetic studies confirmed that intact stevioside and Reb A are poorly absorbed, but they are hydrolyzed by the intestinal microflora to the aglycone, steviol, which is well absorbed (JECFA, 2008). All steviol glycosides are hydrolyzed to steviol prior to absorption (Koyama et al., 2003). After absorption, steviol is metabolized mainly to steviol glucuronide, which is excreted in the urine of humans.

Studies comparing the metabolic fate of Reb A and stevioside demonstrate that both glycosides have similar pharmacokinetics in the rat; they are both metabolized in the gut to steviol prior to absorption followed by glucuronidation to steviol glucuronide in the liver and excretion in the feces via the bile (Roberts and Renwick, 2008). However, in humans, elimination of steviol glycosides, primarily as steviol glucuronide with very small amounts of the unchanged glycoside or steviol, occurs via the urine (Wheeler et al., 2008). The difference in excretion pathways between humans and rats is explained by the different molecular weight thresholds for human and rat biliary excretion of organic anions such as steviol glucuronide (Renwick and Tarka, 2008).

Overall, the data demonstrate that Reb A and stevioside have similar metabolism and pharmacokinetics in the rat. Similarity of metabolism in humans and rats allows for risk assessment based on the results of available rodent toxicity studies.

V.B.2. Mutagenicity and Genotoxicity Studies

Since the FDA's last completed review of 2015, no new mutagenicity or genotoxicity studies of steviol glycosides have been published. It should be noted that national and international food safety agencies and numerous expert panels have recently concluded that steviol glycosides, including the widely used sweeteners, stevioside and Reb A, are not genotoxic (Urban et al., 2013, 2015). The 2013 review by Urban et al. (2013) included an evaluation of the adequacy of the database including more recent genotoxicity data not mentioned in those publications. The authors of this 2013 review concluded that the current database of *in vitro* and *in vivo* studies

for steviol glycosides is robust and does not indicate that either stevioside or Reb A are genotoxic.

V.B.3. Animal Toxicity Studies

Since the FDA's last completed review of 2014-2015, no new animal toxicity studies have been published. Major toxicity studies were published in or before 2008, and no new toxicity studies were found from the literature after the GRAS notice by Cargill (GRN 253) in 2008-2009 (FDA, 2008b). The exception is the subacute study by Nikiforov et al. which was published in 2013. Thus, this GRAS notice summarizes the studies already reviewed in previous GRAS notices, in particular, GRN 253. The notified substance in this notice is Reb A.

A Recent Animal Toxicity Study Published Since FDA's Last Review

Nikiforov et al. (2013) compared the subacute toxicity (28 days) of Reb A and Reb D using Sprague-Dawley rats. Dose levels tested were 500, 1000, and 2,000 mg/kg bw/d for Reb D and 2,000 mg/kg bw/day for Reb A. There were no treatment-related abnormalities in the general condition or behavior of the animals, hematology, serum chemistry, urinalysis or histopathological examinations. Results were comparable between the group administered 2,000 mg/kg bw/day Reb D and the group administered 2,000 mg/kg bw/day Reb A. The results indicate that the NOAELs were 2,000 mg/kg bw/day for both Reb A and Reb D. The results confirmed the previous findings by Nikiforov et al. (2008) which reported the NOAEL of 2,000 mg/kg bw/day for Reb A.

Toxicity Studies Referenced in Previous GRNs

No overt signs of toxicity were noted after a single oral dose (2,000 mg/kg bw) of Reb A (purity, not specified) and stevioside (purity 96%) in mice (Medon et al., 1982), indicating that the oral lethal dose, 50% (LD_{50}) is far greater than 2 g/kg bw, the highest dose tested, and that both Reb A and stevioside have comparable toxicity profiles. Toskulkao et al. (1997) reported that stevioside at a dose as high as 15 g/kg bw was not lethal to mice, rats, or hamsters.

Curry and Roberts (2008) conducted a 4-week study in which Wistar rats were administered Reb A at dietary concentrations of 0, 2.5, 5.0, 7.5, or 10.0%. The NOAEL was determined to be 10% of the diet.

The NOAEL in the 13-week subchronic toxicity study was 5.0% of the diet or approximately 4,161 mg/kg bw/day in male and 4,645 mg/kg bw/day in female rats, the highest concentrations tested (Curry and Roberts, 2008). A two-generation reproductive/developmental toxicity study demonstrated lack of reproductive toxicity of Reb A in rats (Curry et al., 2008).

No carcinogenicity or chronic toxicity studies of Reb A were identified in the literature. However, chronic toxicity studies with rats involving 22-24 month administration of stevioside show that stevioside is not carcinogenic (Table 9; Toyoda et al., 1997; Xili et al., 1992; Yamada et al., 1985). In a study by Toyoda et al. (1997), stevioside was added to a powdered diet at concentrations of 0 (control), 2.5%, and 5% for 24 months to examine the carcinogenic potential of stevioside in F344 rats of both sexes. It was concluded that stevioside was not carcinogenic in F344 rats under the experimental conditions described. Authors did not assign a NOAEL. However, the NOAEL was calculated to be 970 mg/kg bw/day in males (mid-dose, JECFA,

2006). Two othr studies using lower doses (Xili et al., 1992; Yamada et al., 1985) also did not find carcinogenicity of stevioside. Xili et al. (1992) concluded that the NOAEL was 794 mg/kg bw/day in rats and suggested that the ADI for humans was 7.9 mg/kg bw/day. Table 5 summarizes a recent animal toxicity study and the studies referenced in previous GRNs.

Table 5. Summary of Animal Toxicity Studies

Species	Dose	Duration	NOAEL	Reference
A Recent Animal Toxicity Study Published Since FDA's Last Review				
Subacute Toxicity Studies of Reb A				
Rat, SD	2,000 mg/kg bw/d	4 wk	2,000 mg/kg bw/d	Nikiforov et al., 2013
Toxicity Studies Referenced in Previous GRNs				
Subacute Toxicity Studies of Reb A				
Rat (HsdBR1 Han:Wist; 20/sex/ group)	0, 2.5, 5.0, 7.5, and 10.0% Reb A (97% purity) in diet	4 wk	10% in diet	Curry and Roberts, 2008
Subchronic Toxicity Studies of Reb A				
Rat (HsdBR1 Han:Wistar; 20/sex/group)	0, 1.25, 2.5, or 5.0% Reb A (97% purity in diet [M-wk 1:0, 1,506, 3,040, and 5,828 mg/kg bw/d; wk 13: 0, 698, 1,473, and 3,147 mg/kg bw/d; F-wk1: 0, 1,410, 2,841, and 5,512 mg/kg bw/d; wk 13: 0, 980, 1,914, and 3,704 mg/kg bw/d	13 wk	5.0% of diet or approximately 4,161 mg/kg bw/day in males and 4,645 mg/kg bw/day in female rats	Curry and Roberts, 2008
Rat (Crl:CD(SD); 20/sex/group)	0, 517, 1,035, or 2,055 mg/kg bw/d for males and 0, 511, 1,019, or 2,050 mg/kg bw/d for females (diet)	13 wk	2,000 mg/kg bw/day	Nikiforov and Eapen, 2008
Developmental and Reproductive Toxicity Studies on Reb A				
Rat (female HsdRcc: Han Wistar; F_0=6/group, F_1=10/sex/ group); preliminary study	0, 2.5, 3.75, or 5.0% (97% purity) of diet (0, 4, 711, 8,021, or 9,484 mg/kg bw/day during first 4 days of treatment; and 0, 6,291, 10,045, or 11,386 mg/kg bw/d during day 17 to 20 of lactation)	F_0 females: Day 14 to 21 of lactation	5.0% of diet	Curry et al., 2008
	0, 2.5, 3.75, or 5.0% (97% purity) in diet	F_1: Day 0 to 14 (nursing), day 14 to	2.5% of diet	

	(0, 5,814, 9,849, or 14,076 mg/kg bw/day, males and 0, 5,679, 9,338, or 13,088 mg/kg bw/d, females)	35 post-partum (diet)		
Rat (HsdRcc: Han Wistar; 30/sex/group)	0, 0.75, 1.25, or 2.5% of diet (97% purity) (0, 586, 975, or 2,048 mg/kg bw/d, males; 0, 669, 1,115, or 2,273 mg/kg bw/day, pre-mating females; 0, 648-713, 1,119-1,169, or 2,263-2,381 mg/kg bw/day, gestation; and 0, 715-1,379, 1,204-2,388, or 2,602-5,019, lactation)	F_0 males: 17 weeks F_0 females: Pre-mating, 10 weeks; Mating, up to 3 weeks; gestation, days 1 to 20 after conception; Lactation, days 1 to 21 after parturition (total ~20 weeks) F_1 offspring (all offspring): Post-partum day 1 to 21 (nursing). Unselected F_1 offspring: killed at 30 days of age	2.5% of diet or 2,048-2,273 mg/ kg bw/day, the highest level tested	Curry et al., 2008
Carcinogenicity and Chronic Toxicity Studies of Stevioside				
F344 rat/ 50 per sex per group	Ad libitum 0, 2.5, or 5% of diet (95.6% stevioside)	24 mo	Author did not assign a NOAEL. (mid-dose calculates to 970 mg/ kg bw/day in males; JECFA, 2006)	Toyoda et al., 1997
Wistar rat/ 45 per sex per group	0, 0.2, 0.6, 1.2% of diet (85% stevioside)	24 mo	794 mg/ kg bw/day (high dose)	Xili et al., 1992
F344 rat/ 70 per sex per group, 30 per sex per group in low-dose	0.1, 0.3, 1% of diet/95.2% Steviol glycosides (75% stevioside; 16% Reb A)	M-22 mo; F-24 mo	550 mg/ kg bw/day (high dose)	Yamada et al., 1985

bw= body weight; Reb A= Rebaudioside A; d=day; mo=months; wk= week.

V.B.4. Animal Efficacy Studies

Our review is limited to one animal efficacy study published since the FDA's last review of 2014-2015 (Holvoet et al., 2015). This study reported that stevia-derived compounds attenuated the toxic effects of ectopic lipid accumulation in the liver of obese mice. Twenty-four ob/ob and

LDLR-double deficient mice were orally administered with stevioside (10 mg/kg bw/day; n = 8), Reb A (12 mg/kg bw/day; n = 8), or steviol (5 mg/kg bw/day; n = 8). All stevia-derived compounds reduced hepatic steatosis to a similar extent, indicating that liver toxicity can be reduced through several pathophysiological changes. No adverse effects of steviol glycosides were observed.

V.B.5. Human Clinical Studies

Recent Meta-Analysis of Human Clinical Studies

Since the FDA's last review of 2015, one systematic review/meta-analysis evaluating the effect of steviol glycosides on cardiovascular risk factors has been published (Onakpoya and Heneghan, 2015). In this systematic review, 9 studies with a total of 756 participants were evaluated (Barriocarnal et al., 2008; Chan et al., 2000, Ferri et al., 2006; Gisleine et al., 2006; Gregersen et al., 2004; Hsieh et al., 2003; Maki et al., 2008a, 2008b, 2009). Meta-analysis revealed a non-significant difference in systolic blood pressure between steviol glycoside and placebo. Significant reductions in diastolic blood pressure (DBP; mean difference: -2.98 mm Hg, P=0.03) and fasting blood glucose (mean difference: 0.66 mmol/l, P<0.00001) were observed. There was no significant effect on blood lipid profile. This meta-analysis also suggested that stevioside may reduce blood pressure and fasting blood glucose although the size of the effects is small and the substantial heterogeneity limits the robustness of any conclusions. Reb A does not appear to have any significant effects on blood pressure or cardiovascular risk factors.

This meta-analysis reported adverse events that included abdominal fullness, epigastric pain, and dizziness. In general, there were no significant differences in the frequencies of adverse events between steviol glycosides and placebo; however, nausea, abdominal fullness, and vomiting were severe enough to stop treatment with stevioside in one study (Chan et al., 2000). In total, there were 38 dropouts across seven studies; there were no significant differences in the rates of study withdrawals between steviol glycosides and placebo groups.

Studies Referenced in Previous GRNs

The results of the human clinical trials demonstrate that Reb A and other steviol glycosides such as stevioside are safe (Barriocanal et al., 2008; Chan et al., 2000; Ferri et al., 2006; Hsieh et al., 2003; Maki et al., 2008a, 2008b). Long-term consumption of Reb A at doses of up to 1,000 mg/day, or about 16 mg/kg bw/day, was well-tolerated in normotensive or hypotensive individuals and subjects with type 2 diabetes (Maki et al., 2008a, 2008b). Daily consumption of 1,000 mg is about 3 times the EDIs for high-intake adult consumers. Also, this dose is more than 10-fold greater than the EDI of Reb A in children with diabetes (1.5-1.6 mg/kg bw/day).

In addition, long-term administration of stevioside was also well tolerated at daily doses of up to 1,500 mg for 1-2 years (or about 25 mg/kg bw/day) in Chinese patients with mild to moderate hypertension (Chan et al., 2000; Hsieh et al. 2003).

VI. GENERAL RECOGNITION OF THE SAFETY OF HIGH PURITY REB A

The safety assessment to ascertain GRAS status for Reb A with the defined food uses has met FDA criteria for reasonable certainty of no harm by considering both technical and common knowledge elements. Numerous human and animal studies have reported benefits of Reb A with no major adverse effects. Hunan HBI conducts a HACCP-controlled manufacturing process and

rigorously tests its final production batches to verify adherence to quality control specifications. There is broad-based and widely disseminated knowledge concerning Reb A and other steviol glycosides. This GRAS determination is based on the data and information generally available and consented opinion about the safety of Reb A. The literature indicates that Reb A offers consumers benefits without adverse effects.

VI.A. Technical Element (Safety) of the GRAS Determination

The intended use of these high purity Reb A has been determined to be safe through scientific procedures as set forth in 21 CFR§170.3(b), thus satisfying the so called "technical" element of the GRAS determination.

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of Reb A, as well as appropriate corroborative data. Hunan HBI's Reb A is manufactured under cGMP using common food industry materials and processes. Hunan HBI uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications.

1. Hunan HBI intends to market its Reb A preparation (commercially known as Bestevia^{TM}) as a table-top and general-purpose sweetener and as a flavor modifier that will consist of not less than 97% Reb A. Analytical data from multiple lots indicate that Reb A comply reliably with the established food-grade product specifications and meet all applicable purity standards. Physical and chemical specifications of Hunan HBI's Reb A generally meet or exceed FCC and/or JECFA specifications. Additionally, results of the analysis showed the absence of any pesticide residues in this steviol glycoside preparation.

2. It is anticipated that the estimated consumption of Reb A for the defined food uses (1.64 mg/kg bw/day, as steviol equivalents, for high consumers) is expected to be much lower than the ADI values (0 to 4 mg/kg bw/day, as steviol equivalents) established by JECFA, EFSA, FSANZ, and Health Canada.

3. The EDI estimates are based on the assumption that Hunan HBI's Reb A will replace currently marketed high intensity sweeteners including all steviol glycosides. Thus, cumulative exposures are not expected. In addition, the EDIs presented in this notice are highly optimistic estimates.
4. In the previous GRAS notices to the FDA, the safety of Reb A has been established in toxicological studies in animals and in mutagenicity studies, and is further supported by clinical studies in human. The FDA responses to GRAS notifications on Reb A indicate that the FDA is satisfied with the safety-in-use of the Reb A, as long as consumption is 5.0 mg/kg bw/day.

5. Subchronic studies reported that NOAELs for Reb A were over 2,000 mg/kg bw/day in rats. In addition, studies found that LD50 values for stevioside were over 15 g/kg bw in rats, mice, and hamsters, indicating that steviol glycosides, including Reb A, are relatively harmless.

6. The results of the human clinical trials demonstrate that steviol glycosides are safe and are well-tolerated in groups of normotensive or hypotensive individuals and type 2 diabetics following long-term consumption at doses of up to 1.5 g/day or about 25 mg/kg bw/day.

7. Historical consumption of stevia and steviol glycosides supports the safety of Reb A. Intakes of the Reb A preparation have been estimated based on the extensive history of dietary intake assessments by numerous international governments and agencies and the intake assessments contained in numerous GRAS Notifications to the U.S. FDA.

8. An additional animal study and a meta-analysis of human clinical trials published subsequent to the most recent FDA GRAS notice continued to support the safety of using Reb A as a food ingredient.

VI.B. Common Knowledge Element of a GRAS Determination

FDA notes that general recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food. FDA discusses what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community. The two following components meet a common knowledge element of a GRAS determination:

1. Data and information relied upon to establish safety must be generally available, and this has been established by utilizing published, peer-reviewed scientific journals, and

2. There is 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.

Because this safety evaluation was based on generally available and widely accepted data and information, as well there was consensus among qualified scientists about the safety of the Reb A for its intended use, it also satisfies the "common knowledge" element of a GRAS determination.

VII. CONCLUSIONS: DETERMINATION OF GRAS STATUS OF HIGH PURITY REBAUDIOSIDE A

The intended use of Reb A has been determined to be safe through scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the technical element of the Generally Recognized as Safe (GRAS) determination. In addition, because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the common knowledge element of a GRAS determination.

On behalf of Hunan Huacheng Biotech, Inc. (Hunan HBI), we, the undersigned expert panel members, Susan S. Cho, Ph.D., George C. Fahey, Jr, Ph.D., and Joanne Slavin, Ph.D., R.D. have independently evaluated the materials summarized in the Reb A GRAS report. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that Reb A, produced consistent with current Good Manufacturing Practice (cGMP) and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concludes that these uses of Reb A are GRAS based on scientific procedures, and other experts qualified to assess the safety of food and food ingredients would concur with these conclusions.

Therefore, the Panel has concluded that this Reb A preparation is GRAS under the intended conditions of use on the basis of scientific procedures and, therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR. The Panel is not aware of any information that would be inconsistent with a finding that the proposed use of high purity Reb A as a table-top sweetener and general-purpose non-nutritive sweetener and as a flavor modifier, meeting appropriate specifications and used according to current Good Manufacturing Practice, is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

(b) (6)

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George C. Fahey, Jr, Ph.D.
Professor Emeritus, University of Illinois, Urbana, IL

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Date

VII. CONCLUSIONS: DETERMINATION OF GRAS STATUS OF HIGH PURITY REBAUDIOSIDE A

The intended use of Reb A has been determined to be safe through scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the technical element of the Generally Recognized as Safe (GRAS) determination. In addition, because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the common knowledge element of a GRAS determination.

On behalf of Hunan Huacheng Biotech, Inc. (Hunan HBI), we, the undersigned expert panel members, Susan S. Cho, Ph.D., George C. Fahey, Jr, Ph.D., and Joanne Slavin, Ph.D., R.D. have independently evaluated the materials summarized in the Reb A GRAS report. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that Reb A, produced consistent with current Good Manufacturing Practice (cGMP) and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concludes that these uses of Reb A are GRAS based on scientific procedures, and other experts qualified to assess the safety of food and food ingredients would concur with these conclusions.

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_____ _____

Susan Cho, Ph.D. Date
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_____ _____

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_____ _____

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Appendix A.

Certification of Analysis

Product Name: Stevia Extract 97% Rebaudioside A Lot No.: (b) (6)
Manufacture Date: Oct 21, 2015 Expire Date: Oct 20,2017
Analysis Date: Oct 28, 2015 Shelf Life:2 Years

Analysis Item	Specification	Acutal value	TEST METHOD
Identification	Positive	Conform	TLC
Appearance	White to Light yellow powder or crystal	Conform	Visual
Solubility	Freely soluble in water	Conform	USP
Rebaudioside A	⩾ 97%	98.2	JECFA HPLC
PH (1%solution)	4.5-7.0	4.81	USP
Loss on drying	⩽ 6.0%	2.11	USP
Total Ash	⩽ 1.0%	0.15	USP
Residual of Methanol	⩽ 200 ppm	⩽ 200 ppm	USP
Residual of Ethanol	⩽ 5000 ppm	⩽ 5000 ppm	USP
Arsenic (As)	⩽ 1 ppm	⩽ 1 ppm	AOAC
Lead (Pb)	⩽ 1 ppm	⩽ 1 ppm	AOAC
Total Plate Count	⩽ 1000cfu/g	270	CP
Yeast & Mold	⩽ 100cfu/g Max	30	CP
Total Coliform	Negative	Conform	CP
S. aureus	Negative	Conform	CP
Salmonella	Negative	Conform	CP
E.Coli	Negative	Conform	CP

Hunan Huacheng Biotech, Inc. QC TEST

Analyst: (b) (6) **QC:** (b) (6) **QA:** (b) (6)

Certification of Analysis

Product Name: Stevia Extract 97% Rebaudioside A Lot No.: (b) (6)
Manufacture Date: Nov 9, 2015 Expire Date: Nov 8,2017
Analysis Date: Nov 16, 2015 Shelf Life:2 Years

Analysis Item	Specification	Acutal value	TEST METHOD
Identification	Positive	Conform	TLC
Appearance	White to Light yellow powder or crystal	Conform	Visual
Solubility	Freely soluble in water	Conform	USP
Rebaudioside A	⩾ 97%	98.9	JECFA HPLC
PH (1%solution)	4.5-7.0	4.88	USP
Loss on drying	⩽ 6.0%	2.00	USP
Total Ash	⩽ 1.0%	0.11	USP
Residual of Methanol	⩽ 200 ppm	⩽ 200 ppm	USP
Residual of Ethanol	⩽ 5000 ppm	⩽ 5000 ppm	USP
Arsenic (As)	⩽ 1 ppm	⩽ 1 ppm	AOAC
Lead (Pb)	⩽ 1 ppm	⩽ 1 ppm	AOAC
Total Plate Count	⩽ 1000cfu/g	240	CP
Yeast & Mold	⩽ 100cfu/g Max	20	CP
Total Coliform	Negative	Conform	CP
S. aureus	Negative	Conform	CP
Salmonella	Negative	Conform	CP
E.Coli	Negative	Conform	CP

Hunan Huacheng Biotech, Inc. QC TEST

Analyst: (b) (6) **QC:** (b) (6) **QA:** (b) (6)

Certification of Analysis

Product Name: Stevia Extract 97% Rebaudioside A Lot No.: (b) (6)
Manufacture Date: Nov 25, 2015 Expire Date: Nov24,2017
Analysis Date: Dec 01, 2015 Shelf Life:2 Years

Analysis Item	Specification	Acutal value	TEST METHOD
Identification	Positive	Conform	TLC
Appearance	White to Light yellow powder or crystal	Conform	Visual
Solubility	Freely soluble in water	Conform	USP
Rebaudioside A	⩾ 97%	98.4	JECFA HPLC
PH (1%solution)	4.5-7.0	4.90	USP
Loss on drying	⩽ 6.0%	2.23	USP
Total Ash	⩽ 1.0%	0.14	USP
Residual of Methanol	⩽ 200 ppm	⩽ 200 ppm	USP
Residual of Ethanol	⩽ 5000 ppm	⩽ 5000 ppm	USP
Arsenic (As)	⩽ 1 ppm	⩽ 1 ppm	AOAC
Lead (Pb)	⩽ 1 ppm	⩽ 1 ppm	AOAC
Total Plate Count	⩽ 1000cfu/g	200	CP
Yeast & Mold	⩽ 100cfu/g Max	40	CP
Total Coliform	Negative	Conform	CP
S. aureus	Negative	Conform	CP
Salmonella	Negative	Conform	CP
E.Coli	Negative	Conform	CP

Hunan Huacheng Biotech, Inc. QC TEST

Analyst: (b) (6) **QC:** (b) (6) **QA:** (b) (6)

Certification of Analysis

Product Name: Stevia Extract 97% Rebaudioside A Lot No.: (b) (6)
Manufacture Date: Dec 05, 2015 Expire Date: Dec04,2017
Analysis Date: Dec 12, 2015 Shelf Life:2 Years

Analysis Item	Specification	Acutal value	TEST METHOD
Identification	Positive	Conform	TLC
Appearance	White to Light yellow powder or crystal	Conform	Visual
Solubility	Freely soluble in water	Conform	USP
Rebaudioside A	⩾ 97%	97.9	JECFA HPLC
PH (1%solution)	4.5-7.0	4.91	USP
Loss on drying	⩽ 6.0%	2.25	USP
Total Ash	⩽ 1.0%	0.13	USP
Residual of Methanol	⩽ 200 ppm	⩽ 200 ppm	USP
Residual of Ethanol	⩽ 5000 ppm	⩽ 5000 ppm	USP
Arsenic (As)	⩽ 1 ppm	⩽ 1 ppm	AOAC
Lead (Pb)	⩽ 1 ppm	⩽ 1 ppm	AOAC
Total Plate Count	⩽ 1000cfu/g	230	CP
Yeast & Mold	⩽ 100cfu/g Max	30	CP
Total Coliform	Negative	Conform	CP
S. aureus	Negative	Conform	CP
Salmonella	Negative	Conform	CP
E.Coli	Negative	Conform	CP

Hubei Huacheng Biotech,Inc. QC TEST

Analyst: (b) (6) **QC:** (b) (6) **QA:** (b) (6)

Certification of Analysis

Product Name: Stevia Extract 97% Rebaudioside A Lot No.: (b) (6)
Manufacture Date: Dec 15, 2015 Expire Date: Dec14,2017
Analysis Date: Dec 21, 2015 Shelf Life:2 Years

Analysis Item	Specification	Acutal value	TEST METHOD
Identification	Positive	Conform	TLC
Appearance	White to Light yellow powder or crystal	Conform	Visual
Solubility	Freely soluble in water	Conform	USP
Rebaudioside A	⩾ 97%	99.0	JECFA HPLC
PH (1%solution)	4.5-7.0	4.83	USP
Loss on drying	⩽ 6.0%	2.04	USP
Total Ash	⩽ 1.0%	0.11	USP
Residual of Methanol	⩽ 200 ppm	⩽ 200 ppm	USP
Residual of Ethanol	⩽ 5000 ppm	⩽ 5000 ppm	USP
Arsenic (As)	⩽ 1 ppm	⩽ 1 ppm	AOAC
Lead (Pb)	⩽ 1 ppm	⩽ 1 ppm	AOAC
Total Plate Count	⩽ 1000cfu/g	220	CP
Yeast & Mold	⩽ 100cfu/g Max	40	CP
Total Coliform	Negative	Conform	CP
S. aureus	Negative	Conform	CP
Salmonella	Negative	Conform	CP
E.Coli	Negative	Conform	CP

Hunan Huacheng Biotech Inc. QC TEST

Analyst: (b) (6) **QC:** (b) (6) **QA:** (b) (6)

Appendix B. Pesticide Analysis

NSF International

NSF (Shanghai) Testing Technology Co., Ltd.
Unit D/F, 1 Floor, Building 10, 1188 Lianhang Road,
Minhang District, Shanghai, China
Tel: +86.21.2428.6300 | Fax: +21.2428.6299
www.nsfchinalab.org

TEST REPORT

Send To: C0194893

Hunan Huacheng Biotech, Inc
188#, Tongzi'po West Road, Hi-Tech Zone,
Changsha China ,410205

Facility: C0194894

Hunan Huacheng Biotech, Inc
188#, Tongzi'po West Road, Hi-Tech Zone,
Changsha China ,410205

Result **Complete** Report Date 03-Feb-2016

Customer Name	Hunan Huacheng Biotech, Inc
Description	Stevia Extract(high purity Rebaudioside A)
Lot No.:	2015102101+2015110901+2015112501+2015120501+2015121501
Test Type	Test Only
Job Number	J-00207972
Sample Reception Date	28-Jan-2016
Testing Completion Date	03-Feb-2016

Summary of Results

Testing Parameters and Standards	Result
Determination of Pesticides Residues in the Sample Refer to USP 38<561>	See Page 2-6

(b) (6)

Report Authorization

Dongjing Liu – Laboratory Site Manager
NSF (Shanghai) Testing Technology Co., Ltd.

Date 03-Feb-2016

1. **Determination of Pesticides Residues in the Sample Refer to USP 38<561>.**

1.1 Pesticides residues testing procedure:

Accurately weight testing samples, add acetonitrile to extract. After a purification step, inject the extract and analyze by GC-MS/MS and LC-MS/MS

1.1.1 Pesticides residues testing results by GC-MS/MS and LC-MS/MS:

Testing Parameter	CAS. No.	Unit	Result
Aldrin and Dieldrin (sum of)	309-00-2	µg/kg	ND(20)
	60-57-1		
Bromophos-methyl	2104-96-3	µg/kg	ND(20)
Bromopropylate	18181-80-1	µg/kg	ND(20)
cis-Chlordane trans-Chlordane and Oxychlordane(sum of)	5103-71-9	µg/kg	ND(20)
	5103-74-2	µg/kg	
	27304-13-8	µg/kg	
Cyfluthrin (sum of)	68359-37-5	µg/kg	ND(20)
λ-Cyhalothrin	91465-08-6	µg/kg	ND(20)
DDT (sum of p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-TDE and o,p'-TDE)	50-29-3	µg/kg	ND(30)
	789-02-6		
	72-55-9		
	3424-82-6		
	72-54-8		
	53-19-0		
Dichlorvos	62-73-7	µg/kg	ND(20)
Endosulfan (sum of isomers and endosulfan sulphate	959-98-8	µg/kg	ND(20)
	33213-65-9	µg/kg	
	1031-07-8	µg/kg	
Heptachlor (Sum of heptachlor , cis-Heptachlorepoxide,and tran-Heptachlorepoxide(sum of)	76-44-8	µg/kg	ND(20)
	28044-83-9	µg/kg	
	1024-57-3	µg/kg	

Testing Parameter	CAS. No.	Unit	Result
Endrin	72-20-8	μg/kg	ND(20)
Dicofol	115-32-2	μg/kg	ND(20)
Hexachlorobenzene	118-74-1	μg/kg	ND(20)
Hexachlorocyclohexane(sum of isomers α-, β-, δ-, and ξ-)	319-84-6	μg/kg	ND(20)
	319-85-7	μg/kg	
	319-86-8	μg/kg	
	6108-10-7	μg/kg	
Lindane (γ-Hexachlorocyclohexane)	58-89-9	μg/kg	ND(20)
Chlorpyriphos-methyl	5598-13-0	μg/kg	ND(20)
Mecarbam	2595-54-2	μg/kg	ND(20)
Methoxychlor	72-43-5	μg/kg	ND(20)
Pendimethalin	40487-42-1	μg/kg	ND(20)
Pirimiphos-ethyl	23505-41-1	μg/kg	ND(20)
Permethrin and isomers (sum of)	54774-45-7	μg/kg	ND(20)
	51877-74-8	μg/kg	
Quintozene(sum of quintozene,pentachloraniline, and methylpentachlorphenyl sulfide)	82-68-8	μg/kg	ND(20)
	527-20-8	μg/kg	
	1825-19-0	μg/kg	
Tecnazene	117-18-0	μg/kg	ND(20)
Tetradifon	116-29-0	μg/kg	ND(20)
Vinclozolin	50471-44-8	μg/kg	ND(20)
Piperonyl butoxide	51-03-6	μg/kg	ND(20)
Pyrethrum (sum of pyrethrin I and pyrethrin II)	121-21-1	μg/kg	ND(20)
	121-29-9	μg/kg	
Deltamethrin	52918-63-5	μg/kg	ND(20)
Fenvalerate	51630-58-1	μg/kg	ND(20)
Flucytrinate	70124-77-5	μg/kg	ND(20)

Testing Parameter	CAS. No.	Unit	Result
Cypermethrin and isomers (sum of)	52315-07-8	μg/kg	ND(20)
Alachlor	15972-60-8	μg/kg	ND(20)
fenchlorphos (sum of fenchlorphos and Fenchlorphos-oxon)	299-84-3	μg/kg	ND(20)
	3983-45-7	μg/kg	
S-421	127-90-2	μg/kg	ND(10)
Mirex	2385-85-5	μg/kg	ND(5)
Pentachloroanisole	1825-21-4	μg/kg	ND(5)
Chlorthal-dimethyl	1861-32-1	μg/kg	ND(5)
Acephate	30560-19-1	μg/kg	ND(20)
Azinphos-ethyl	2642-71-9	μg/kg	ND(20)
Azinphos-methyl	86-50-0	μg/kg	ND(20)
Bromophos-ethyl	4824-78-6	μg/kg	ND(20)
Chlorfenvinphos	470-90-6	μg/kg	ND(20)
Chlorpyriphos-ethyl	2921-88-2	μg/kg	ND(20)
Diazinon	333-41-5	μg/kg	ND(20)
Dichlofluanid	1085-98-9	μg/kg	ND(20)
Dimethoate and omethoate(sum of)	60-51-5	μg/kg	ND(20)
	1113-02-6	μg/kg	
Ethion	563-12-2	μg/kg	ND(20)
Etrimfos	38260-54-7	μg/kg	ND(20)
Fenitrothion	122-14-5	μg/kg	ND(20)
Fenpropathrin	64257-84-7	μg/kg	ND(20)
Fensulfothion (sum of fensulfothion, fensulfothion-oxon, fensulfothion-oxonsulfon, fensulfothion-sulfon	115-90-2	μg/kg	ND(20)
	6552-21-2	μg/kg	
	6132-17-8	μg/kg	
	14255-72-2	μg/kg	

Testing Parameter	CAS. No.	Unit	Result
Fenthion(sum of fenthion, fenthion-oxon, fenthion-oxon-sulfon, fenthion-oxon-sulfoxid, fenthion-sulfone, fenthion-sulfoxid)	55-38-9	µg/kg	ND(30)
	6552-12-1	µg/kg	
	14086-35-2	µg/kg	
	6552-13-2	µg/kg	
	3761-42-0	µg/kg	
	3761-41-9	µg/kg	
Fonophos	994-22-9	µg/kg	ND(20)
Malathion and Malaoxon(sum of)	121-75-5	µg/kg	ND(20)
	1634-78-2	µg/kg	
Methacrifos	62610-77-9	µg/kg	ND(20)
Methamidophos	10265-92-6	µg/kg	ND(20)
Methidathion	950-37-8	µg/kg	ND(20)
Monocrotophos	6923-22-4	µg/kg	ND(20)
Parathion-ethyl and Paraoxon-ethyl(sum of)	56-38-2	µg/kg	ND(20)
	311-45-5	µg/kg	
Parathion-methyl and Paraoxon-methyl(sum of)	298-00-0	µg/kg	ND(20)
	950-35-6	µg/kg	
Phosalone	2310-17-0	µg/kg	ND(20)
Phosmet	732-11-6	µg/kg	ND(20)
Pirimiphos-methyl (sum of pirimiphos-methyl and N-desethyl-pirimiphos-methyl)	29232-93-7	µg/kg	ND(20)
	67018-59-1	µg/kg	
Procymidone	32809-16-8	µg/kg	ND(20)
Profenophos	41198-08-7	µg/kg	ND(20)
Prothiophos	34643-46-4	µg/kg	ND(20)
Quinalphos	13593-03-8	µg/kg	ND(20)
Tau-Fluvalinate	102851-06-9	µg/kg	ND(20)

Remark: ND = Not Detected, less than reporting limit.

1.2 Bromide inorganic testing results:

1.2.1 Bromide ion testing procedure:

Accurately weight testing samples, add water to extract, ultrasonic extract for 30 min, inject the extract and analyze by GC-ECD.

1.2.2 Bromide ion testing results:

Testing Parameter	Unit	Result
Bromide, inorganic (Calculated as bromide ion)	mg/kg	ND(5.0)

Remark: ND = Not Detected, less than reporting limit.

1.3 Dithiocarbamates(CS_2) testing

1.3.1 Dithiocarbamates(CS_2) testing procedure:

Accurately weight testing samples, add n-isooctane and $SnCl_2$ to extract, ultrasonic extract for 60 min, inject the extract and analyze by GC-MSD.

1.3.2 Dithiocarbamates (CS_2) testing results:

Testing Parameter	CAS.No	Unit	Result
Dithiocarbamates (expressed as CS_2)	75-15-0	mg/kg	ND(1.0)

Remark: ND = Not Detected, less than reporting limit.

Picture of sample

Sample in Bag

End of Report

Pages 000066-006719 have been removed in accordance with copyright laws. The list of the references that have been removed are on pages 000012-000019 and pages 000044-000052.

SUBMISSION END