

GRAS Notice (GRN) No. 983

<https://www.fda.gov/food/generally-recognized-safe-gras/gras-notice-inventory>



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October 14, 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 Notice – *High Purity Steviol Glycosides*

Dear Dr. Carlson:

GRAS Associates, LLC, acting as the Agent for Zhucheng Haotian Pharm Co., Ltd. ("ZCHT"), is submitting for FDA review Form 3667 and the enclosed CD, free of viruses, containing a GRAS Notice for *High Purity Steviol Glycosides*. Along with ZCHT'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,

A grey rectangular box redacting the signature of William J. Rowe.

William J. Rowe, President
Agent for Blue California
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Suite 500
North Bethesda, MD 20852
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Enclosure: GRAS Notice for Zhucheng Haotian Pharm. Co., Ltd. – *High Purity Steviol Glycosides*



GRAS Notification

of

High Purity Steviol Glycosides

Food Usage Conditions for General Recognition of Safety

on behalf of

Zhucheng Haotian Pharm Co., Ltd.

Shandong

People's Republic of China

10/14/20

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FOREWORD

Zhucheng Haotian Pharm Co., Ltd. (“ZCHT”) based our Generally Recognized as Safe (GRAS) assessment of SoPure Stevia™ high purity steviol glycosides preparations (≥95% total steviol glycosides), 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 August 17, 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 ZCHT’s request, GRAS Associates, LLC (“GA”) convened an Expert Panel to complete an independent safety evaluation of ZCHT’s high purity steviol glycosides preparations. The purpose of the evaluation is to ascertain whether ZCHT’s high purity steviol glycosides preparations as described in Part 3 are generally recognized as safe, i.e., GRAS, under the intended conditions of use. In addition, ZCHT 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 E¹

ZCHT has concluded that our high purity steviol glycosides preparations (≥95% total steviol glycosides), referred to collectively as “SoPure Stevia™”, and which meet the specifications described below, are 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 sections that follow. The evaluation accurately reflects the intended conditions of food use for the designated high purity steviol glycosides preparations.

Signed:
Agent for ZCHT

A rectangular area of the document is redacted with a grey box, obscuring the signature of the agent for ZCHT. There are some faint blue ink marks visible around the redacted area.

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 5/16/20).

William J. Rowe
President
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North Bethesda, MD
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Date: October 14, 2020

B. Name and Address of Responsible Party

Zhucheng Haotian Pharm Co., Ltd.
Xinxing, Zhucheng,
Shandong Province
262218
The People's Republic of China

As the Responsible Party, ZCHT accepts responsibility for the GRAS conclusion that has been made for our SoPure Stevia™ high purity steviol glycosides preparations 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 is “high purity steviol glycosides” and “stevia extract.” ZCHT also plans to market our high purity steviol glycosides preparations under the trade name “SoPure Stevia™.”

D. Conditions of Intended Use in Food

ZCHT's SoPure Stevia™ high purity steviol glycosides ($\geq 95\%$ total steviol glycosides) preparations are intended for use as general purpose sweeteners 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, ZCHT's SoPure Stevia™ high purity steviol glycosides ($\geq 95\%$ total steviol glycosides) preparations have been concluded to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

² <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRSearch.cfm?fr=170.30> (Accessed 8/17/20).

ZCHT's SoPure Stevia™ purified steviol glycosides preparations are not subject to premarket approval requirements of the FD&C Act based on ZCHT's conclusion that the substances are GRAS under the conditions of its intended food use.

ZCHT 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 unfavorable, available and pertinent to the evaluation of the safety and GRAS status of ZCHT's SoPure Stevia™ high purity steviol glycosides preparations.

F. Availability of Information

The data and information that serve as the bases for this GRAS Notice will be maintained at the offices of ZCHT and will be made available during customary business hours.

ZCHT 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

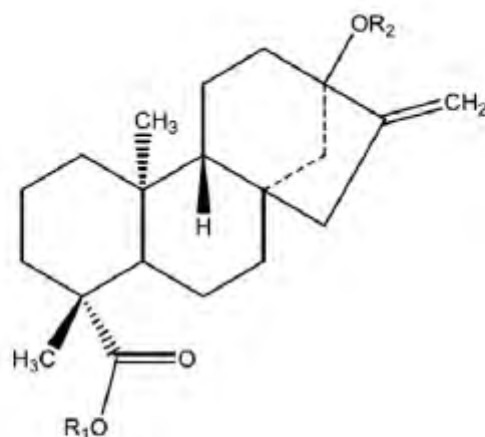
The Joint FAO/WHO Expert Committee on Food Additives (JECFA) adopted the term “steviol glycosides” for the family of steviol derivatives with sweetness properties derived from the stevia plant. In the scientific literature, steviol glycosides have been referred to as “stevia,” “stevioside,” “steviol glycosides,” and “stevia glycoside.” The term “stevia” is presently used more narrowly to describe the plant or crude extracts of the plant.

Herein, “high purity steviol glycosides” is the common or usual name of the non-nutritive sweetener derived from an extract of *Stevia rebaudiana* Bertoni. The compositional features of SoPure Stevia™ high purity steviol glycosides preparations are described in more detail in this section.

The general chemistry of high purity steviol glycosides has previously been reviewed in a number of GRAS Notices (GRNs) including GRN 275 (McNeil Nutritionals, 2009), GRN 287 (Wisdom Natural Brands, 2009), GRN 790 (GLG Life Tech, 2019), and GRN 795 (Steviana Bioscience, 2018). Representative chemical structures of some of the steviol glycosides that have been identified in *Stevia rebaudiana* Bertoni to date are presented in Figure 1.

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

Figure 1. Chemical Structures of Various Steviol Glycosides^a



Compound	R1	R2
Steviol	H-	H-
Stevioside	Glcβ1-	Glcβ(1-2)Glcβ1-
Rebaudioside A	Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
Rebaudioside B	H-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
Rebaudioside C	Glcβ1-	Rhaα(1-2)[Glcβ(1-3)]Glcβ-
Rebaudioside D	Glcβ(1-2)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
Rebaudioside E	Glcβ(1-2)Glcβ1-	Glcβ(1-2)Glcβ1-
Rebaudioside F	Glcβ1-	Xylβ(1-2)[Glcβ(1-3)]Glcβ1-
Rebaudioside M	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
Steviolbioside	H-	Glcβ(1-2)Glcβ1-
Dulcoside A	Glcβ1-	Rhaα(1-2)Glcβ1-
Rubusoside	Glcβ1-	Glcβ1-

Glc, Rha, and Xyl represent glucose, rhamnose, and xylose sugar moieties, respectively

^a From Perrier et al. (2018)

B. Manufacturing Processes

ZCHT's manufactures its SoPure Stevia™ high purity steviol glycosides (≥95% total steviol glycosides) preparations from extracts derived from *Stevia rebaudiana* leaves using a fairly typical process used in the industry for the production of stevia-derived sweeteners under CGMP practices. In short, steviol glycosides are extracted from dried stevia leaves with water and flocculant (ferrous sulfate and calcium oxide) is used to purify the resulting stevia extract solution. An alternative process is ethanol extraction of the steviol glycosides from dried stevia leaves. The steviol glycosides extract solution is then filtered through pressed filter equipment, and impurities are removed using cation and anion exchange columns. The steviol glycosides are removed from the filtrate as it passes through an adsorption resin and the column is subsequently washed with an ethanol/water solution to collect the desired steviol glycosides

eluent. The solvent is removed by evaporation and the remaining liquid is dried to obtain crude steviol glycosides extract.

There are several ways in which ZCHT further purifies the crude steviol glycosides extract to obtain the high purity steviol glycosides preparations. The crude extract is dissolved in food grade ethanol mixed with water and crystals are formed under temperature-adjusted conditions. A centrifuge is used to separate the high purity steviol glycosides from the mother liquor. The mother liquor from the crude extract is used for further crystallization to produce other high purity steviol glycosides and a second mother liquor. The second mother liquor can be further purified to produce other high purity steviol glycosides through an additional crystallization step.

The resulting high purity steviol glycosides crystals are then dried, milled, sieved, blended, and subjected to magnets and metal detection. The crystals are then packaged to achieve the final SoPure Stevia™ high purity steviol glycosides products.

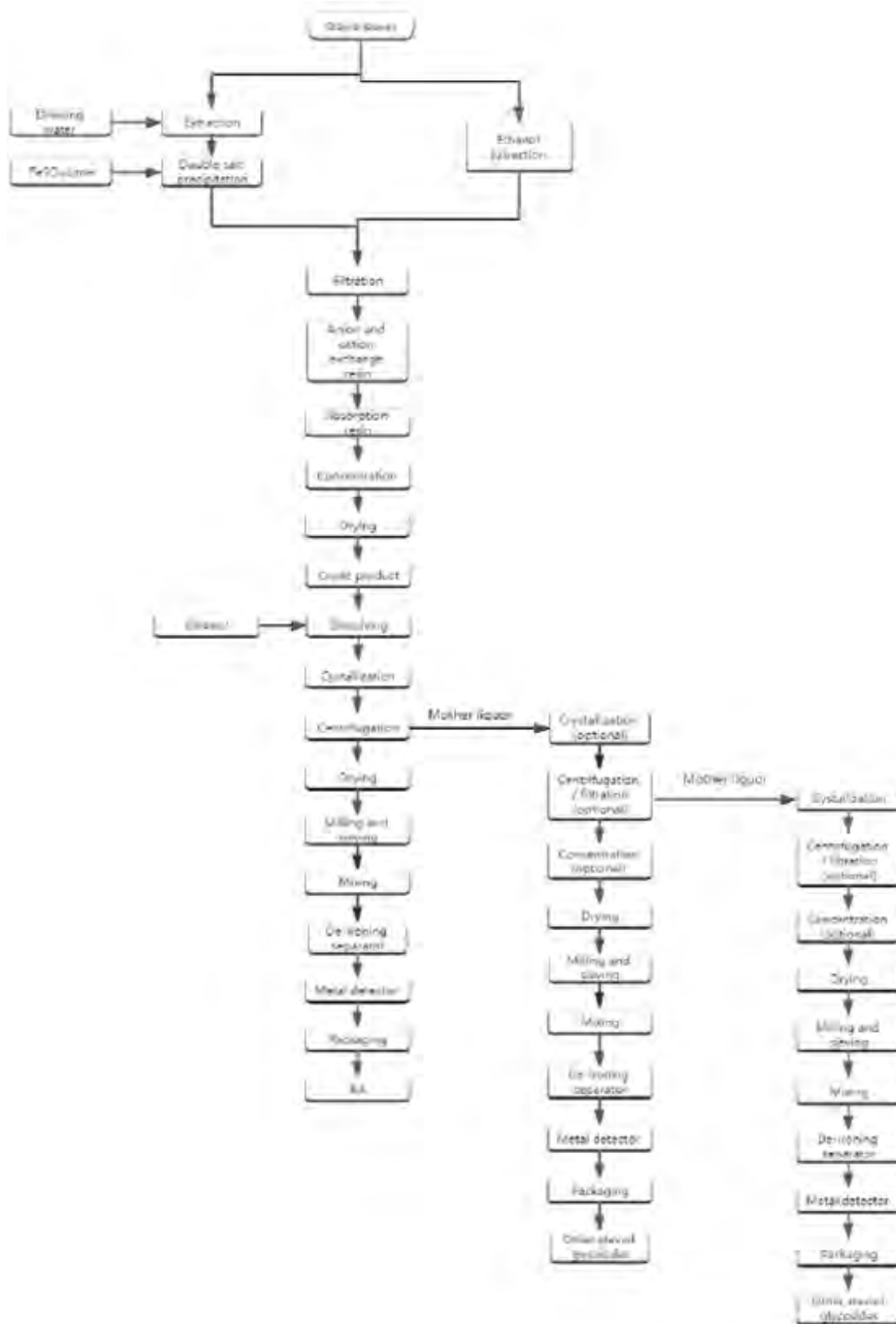
The ferrous sulfate, calcium oxide (lime), and ethanol used in the purification processes comply with Food Chemicals Codex (FCC) 12th Edition specifications. The resins comply with 21 CFR § 173.65³ and 21 CFR § 173.25⁴ specifications. Supporting documentation for the raw materials and processing aids are provided in Appendix 1. The manufacturing process for SoPure Stevia™ high purity steviol glycosides preparations is summarized in the flow charts provided in Figure 2.

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³ <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/cfrsearch.cfm?fr=173.65>

⁴ <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/cfrsearch.cfm?fr=173.25>

Figure 2. Flow Chart of Manufacturing Process for ZCHT’s SoPure Stevia™ High Purity Steviol Glycosides Preparations



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 have 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.

2. Specifications for ZCHT’s SoPure Stevia™ High Purity Steviol Glycosides Preparations and Supporting Methods

ZCHT has adopted product specifications for our SoPure Stevia™ high purity steviol glycosides preparations based upon current JECFA recommendations, while also complying with Food Chemicals Codex (FCC) specifications for steviol glycosides as a consumable human food substance. The compositions of five non-consecutive lots of ZCHT’s SoPure Stevia™ (≥95% total steviol glycosides) preparations are compared with the JECFA and FCC specifications in Table 1.

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Table 1. Specifications for ZCHT’s SoPure Stevia™ High Purity Steviol Glycosides Preparation

Physical & Chemical Parameters	JECFA ^a Specifications Steviol Glycosides	FCC ^b Specifications Steviol Glycosides	ZCHT’s Minimum Specifications for SoPure Stevia™ High Purity Steviol Glycosides	Results of SoPure Stevia™ High Purity Steviol Glycosides					
				20190106 TSG95	20191101 TSG95	20200101 TSG95	20200203 TSG95	20200301 TSG95	Method of Analysis
Appearance Form	Powder	Powder, flakes, or granules	Fine powder	Powder	Powder	Powder	Powder	Powder	Visual
Appearance Color	White to light yellow	White to light yellow	White to off-white	White	White	White	White	White	Visual
Solubility	Freely soluble in water: ethanol (50:50)	Freely soluble in water: ethanol (50:50)	NS	NS	NS	NS	NS	NS	NS
Purity (HPLC Area)	≥ 95% Steviol Glycosides	≥ 95% Steviol Glycosides	NLT 95% Total Steviol Glycosides	96.5%	96.6%	95.7%	95.4%	96.3%	JECFA 2017
Residual Ethanol	NMT 5,000 mg/kg	NMT 0.50%	NMT 5,000 ppm	<25 ppm	<25 ppm	51 ppm	25 ppm	178 ppm	JECFA 2017
Residual Methanol	NMT 200 mg/kg	NMT 0.020%	NMT 200 ppm	< 50 ppm	< 50 ppm	< 50 ppm	< 50 ppm	< 50 ppm	JECFA 2017
Loss on Drying	NMT 6.0%	NMT 6.0%	NMT 6.0%	3.9%	3.4%	4.0%	4.4%	3.6%	JECFA 2017
pH, 1% Solution	4.5 - 7.0	4.5 - 7.0	4.5-7.0	5.5	4.9	5.6	5.4	5.7	JECFA 2017
Total Ash	NMT 1%	NMT 1%	NMT 1.0%	0.04%	0.06%	0.07%	0.05%	0.05%	JECFA 2017
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	NMT 1.0 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)
Lead	NMT 1 mg/kg	NMT 1 mg/kg	NMT 0.5 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)
Cadmium	NS	NS	NMT 1.0 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)
Mercury	NS	NS	NMT 0.1 ppm	ND	ND	ND	ND	ND	AAS ChP2015 Part 4 (2321)
Total Plate Count (cfu/g)	NMT 1,000	NS	NMT 1,000	< 10	10	< 10	< 10	< 10	ChP 2015 Part 4 (1105)
Yeast & Mold (cfu/g)	NMT 200	NS	NMT 100	< 10	< 10	< 10	< 10	< 10	ChP 2015 Part 4 (1105)
<i>E. coli</i>	Negative in 1 g	NS	Negative in 1 g	Negative	Negative	Negative	Negative	Negative	ChP 2015 Part 4 (1105)
<i>Salmonella spp.</i>	Negative in 25 g	NS	Negative in 25 g	Negative	Negative	Negative	Negative	Negative	ChP 2015 Part 4 (1105)

^a Prepared at 84th JECFA (2017)

^b Steviol Glycosides monograph. Food Chemicals Codex (12th Ed.) (FCC, 2020)

AAS – atomic absorption spectrometry; cfu – colony forming units; ChP – Chinese Pharmacopeia; g – gram; HPLC – high-performance liquid chromatography; kg – kilogram; mg – milligram;

NA – not applicable; ND – not detected; NLT – not less than; NMT – not more than; NS – not specified; ppm – parts per million

It is important to note that individual steviol glycosides and steviol glycosides blends 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 these 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 or certain steviol glycosides blends, which exhibit lower solubility in water: ethanol solutions.

Based on the solubility definitions provided in Table 2, ZCHT’s SoPure Stevia™ high purity steviol glycosides preparations range from freely soluble to slightly soluble.

Table 2. 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)
g – gram; mL – milliliter

ZCHT analyzes our SoPure Stevia™ high purity steviol glycosides preparations following the JECFA (2017) monograph method. The certificates of analysis and representative chromatograms for five representative lots of material are presented in Appendix 2 and Appendix 3. Test reports for the analysis of pesticides residues in the stevia leaf raw material and in a representative SoPure Stevia™ high purity steviol glycosides finished product are provided in Appendix 4. The collection of these reports demonstrates that ZCHT’s high purity steviol glycosides preparations are well characterized and meet the established purity criteria.

D. Physical or Technical Effect

ZCHT conducted sweetness equivalence evaluations for SoPure Stevia™ high purity steviol glycosides preparations. Taste panels determined that SoPure Stevia™ high purity steviol glycosides preparations are 150 - 400 times sweeter than sucrose, depending on the use level and application.

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 275 (McNeil Nutritionals, 2009), GRN 287 (Wisdom Natural Brands, 2009), GRN 790 (GLG Life Tech, 2019), and GRN 795 (Steviana Bioscience, 2018).

Stevioside has been reported to be stable over the pH range 3-9 and can be heated at 100°C for 1 hour without decomposing, but, at pH 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 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 concentrations 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.

Buniowska et al. (2020) studied the stability of steviol glycosides (from an aqueous *Stevia rebaudiana* Bertoni leaf extract) in a fruit juice beverage after thermal treatment ranging from 60.0 to 99.0°C. The authors reported that decreases in rebaudioside A, rebaudioside C, and rebaudioside F concentrations were observed after thermal processing at all temperatures, independent of initial concentration. However, for stevioside, both temperature and concentration affected the concentration after thermal processing, where stevioside was stable in solution at temperatures up to 80°C.

In a shelf stability study conducted by Salar et al. (2020), a stevia sweetener of unknown composition that was purchased from Agriestevia S.L (Molina de Segura, Murcia, Spain) was used to sweeten a fruit juice prepared with maqui powder and lemon and other citrus juices at a concentration of 4 mg per 100 mL. The samples were pasteurized at 85°C for 15 seconds, after which aliquots were drawn and used in studies that investigated the effects of light and temperature on the stabilities of vitamin C and phenolic compounds. The stabilities of vitamin C

and phenolic compounds in the beverages prepared with sucrose and stevia were similar. Storage temperature had more of an effect on the analyzed bioactive compounds than light exposure, which was not deemed to be a “critical factor.” Compared with the sucrose-containing juice samples, there was a greater reduction in total flavonones content under light exposure at room temperature and a slightly higher loss of vitamin C during the first month was observed in juice containing the stevia sweetener. The authors noted that “stevia could be considered as an alternative sweetener by the industry,” even with these observations.

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 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 ZCHT’s SoPure Stevia™ High Purity Steviol Glycosides Preparations

ZCHT conducted long-term stability studies on five lots of our SoPure Stevia™ high purity steviol glycosides preparations. Samples were stored at 25°C ± 2°C at a relative humidity of 60% ± 5%. A summary of the stability results is provided in Table 3.

Table 3. ZCHT’s SoPure Stevia™ Stability Data

Lot Number: 20150922						
Duration	Appearance	Loss on Drying (%)	Total Steviol Glycosides (%)	Total Aerobic Bacteria (cfu/g)	Mold and Yeast (cfu/g)	<i>E. coli</i>
t=0	White Powder	3.0	99.7	< 10	< 10	Negative
3 months	White Powder	3.5	99.0	< 10	< 10	Negative
6 months	White Powder	3.8	99.3	< 10	< 10	Negative
9 months	White Powder	4.0	99.5	< 10	< 10	Negative
12 months	White Powder	4.1	99.4	< 10	< 10	Negative
18 months	White Powder	4.1	99.6	< 10	< 10	Negative
24 months	White Powder	4.2	99.0	< 10	< 10	Negative
36 months	White Powder	4.2	99.6	< 10	< 10	Negative

Lot Number: 20160801RA60						
Duration	Appearance	Loss on Drying (%)	Total Steviol Glycosides (%)	Total Aerobic Bacteria (cfu/g)	Mold and Yeast (cfu/g)	<i>E. coli</i>
t=0	White Powder	3.4	98.5	10	< 10	Negative
3 months	White Powder	3.5	98.4	< 10	< 10	Negative
6 months	White Powder	3.5	98.5	< 10	< 10	Negative
9 months	White Powder	3.6	98.6	< 10	< 10	Negative
12 months	White Powder	3.8	98.5	< 10	< 10	Negative
18 months	White Powder	3.7	98.4	< 10	< 10	Negative
24 months	White Powder	3.8	97.8	< 10	< 10	Negative
36 months	White Powder	3.7	98.3	< 10	< 10	Negative
Lot Number: 20160805RA80						
Duration	Appearance	Loss on Drying (%)	Total Steviol Glycosides (%)	Total Aerobic Bacteria (cfu/g)	Mold and Yeast (cfu/g)	<i>E. coli</i>
t=0	White Powder	3.8	98.2	< 10	< 10	Negative
3 months	White Powder	3.9	98.1	< 10	< 10	Negative
6 months	White Powder	3.9	98.2	< 10	< 10	Negative
9 months	White Powder	4.0	98.4	< 10	< 10	Negative
12 months	White Powder	4.1	98.3	< 10	< 10	Negative
18 months	White Powder	4.0	98.0	< 10	< 10	Negative
24 months	White Powder	4.1	98.5	10	< 10	Negative
36 months	White Powder	4.2	98.1	< 10	< 10	Negative
Lot Number: R1X20160501						
Duration	Appearance	Loss on Drying (%)	Total Steviol Glycosides (%)	Total Aerobic Bacteria (cfu/g)	Mold and Yeast (cfu/g)	<i>E. coli</i>
t=0	White Powder	4.5	99.7	< 10	< 10	Negative
3 months	White Powder	4.6	99.3	< 10	< 10	Negative
6 months	White Powder	4.6	99.3	< 10	< 10	Negative
9 months	White Powder	4.7	98.8	< 10	< 10	Negative
12 months	White Powder	4.7	99.1	< 10	< 10	Negative
18 months	White Powder	4.8	98.6	< 10	< 10	Negative
24 months	White Powder	4.8	98.5	< 10	< 10	Negative
36 months	White Powder	4.8	99.2	< 10	< 10	Negative

Lot Number: R1X20160502						
Duration	Appearance	Loss on Drying (%)	Total Steviol Glycosides (%)	Total Aerobic Bacteria (cfu/g)	Mold and Yeast (cfu/g)	<i>E. coli</i>
t=0	White Powder	4.1	98.2	< 10	< 10	Negative
3 months	White Powder	4.3	98.3	< 10	< 10	Negative
6 months	White Powder	4.2	97.9	10	< 10	Negative
9 months	White Powder	4.4	97.5	< 10	< 10	Negative
12 months	White Powder	4.3	98.0	10	< 10	Negative
18 months	White Powder	4.4	97.8	< 10	< 10	Negative
24 months	White Powder	4.5	97.7	< 10	< 10	Negative
36 months	White Powder	4.5	98.0	< 10	10	Negative

cfu – colony forming unit; g – gram

The stability data for steviol glycosides in the scientific literature, the JECFA report, and the extensive stability testing for structurally similar rebaudioside A as presented in GRN 252 (Merisant, 2008) and GRN 304 (Sunwin/WILD, 2010), along with ZCHT’s stability testing results, support the position that ZCHT’s SoPure Stevia™ high purity steviol glycosides preparations are well-suited for the intended food uses.

F. Calculation of Steviol Equivalents of SoPure Stevia™

For comparative purposes, the content of steviol glycosides is often expressed as steviol or steviol equivalents. Each component steviol glycoside has a steviol equivalence factor that is calculated based upon the ratio of the molecular weights (MW) of steviol and a particular steviol glycoside, as shown in Table 4.

Table 4. Steviol Equivalency Factors for Various Steviol Glycosides

Component Steviol Glycoside	Molecular Weight	Steviol Equivalency Factor ^a
Rubusoside	643	0.495
Steviolbioside	643	0.495
Dulcoside A	789	0.403
Rebaudioside B	805	0.395
Stevioside	805	0.395
Rebaudioside F	937	0.339
Rebaudioside C	951	0.334
Rebaudioside A	967	0.329

Component Steviol Glycoside	Molecular Weight	Steviol Equivalency Factor ^a
Rebaudioside E	967	0.329
Rebaudioside D	1129	0.282
Rebaudioside M	1291	0.246

^a Calculated by dividing the molecular weight of steviol (MW=318) by the molecular weight of each glycoside.

Using these steviol equivalency factors, along with the representative percent composition of a SoPure Stevia™ high purity steviol glycosides preparation, it is possible to determine the worst-case scenario steviol equivalency of SoPure Stevia™, as presented in Table 5.

Table 5. Steviol Equivalency of Steviol Glycosides Extract Raw Material

Component Steviol Glycoside	Typical Composition ^a (%)	Steviol Equivalents ^b (%)
Rubusoside	0.20	0.10
Steviolbioside	0.20	0.10
Dulcoside A	0.20	0.081
Rebaudioside B	0.60	0.24
Stevioside	34.00	13.43
Rebaudioside C	7.20	2.41
Rebaudioside F	1.10	0.37
Rebaudioside A	52.50	17.3
Rebaudioside D	0.50	0.14
Total Steviol Equivalence	--	34.2

^a Based on the typical levels of steviol glycosides in a representative SoPure Stevia™ high purity steviol glycosides preparation.

^b Calculated by multiplying the % of the steviol glycoside by the steviol equivalency factor.

PART 3. DIETARY EXPOSURE

The subject SoPure Stevia™ high purity steviol glycosides preparations are 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, as defined in 21 CFR 170.3(o)(19).⁵ 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 SoPure Stevia™ high purity steviol

⁵ Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.

glycosides 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 21 CFR 182.1(b)(1).⁶

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 5. 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 intake of a sweetener that is 100 times as sweet as sucrose when used as a total sugar replacer for a 90th percentile would be a maximum of 9.9 mg per kg body weight (bw) per day for any population subgroup.

The relative sweetness intensity of SoPure Stevia™ high purity steviol glycosides preparations ranges between 150X and 400X times that of sucrose. A weighted sum estimate was used to determine the worst-case scenario steviol equivalency factor for SoPure Stevia™, which was determined to be 34.2 g steviol per 100 g SoPure Stevia™ (as described in Part 2.F).

The highest 90th percentile consumption by any population subgroup of SoPure Stevia™ using the lowest relative sweetness intensity and worst-case scenario steviol equivalence would be approximately 6.60 mg per kg steviol glycosides bw per day. Based on a weighted sum estimate for steviol equivalents provided in Table 5, the consumption would be less than 2.26 mg per kg bw per day on a steviol equivalents basis for any population group for ZCHT’s SoPure Stevia™ high purity steviol glycosides preparations described herein. These calculations are summarized in Table 6.

Table 6. Daily Intake of Sweeteners (in Sucrose Equivalents) & Estimated Daily Intakes of SoPure Stevia™

Population Group	Intakes of Sweeteners (mg sucrose/kg bw/day) ^a		Calculated Intake of SoPure Stevia™ (mg/kg bw/day) ^b		Calculated Intake of SoPure Stevia™ as Steviol Equivalents (mg/kg bw/day)	
	Low	High	Low	High	Low	High
Healthy Population	255	675	1.70	4.50	0.58	1.54
Diabetic Adults	280	897	1.87	5.98	0.64	2.05
Healthy Children	425	990	2.83	6.60	0.97	2.26
Diabetic Children	672	908	4.48	6.05	1.53	2.07

^a From Renwick (2008)

^b Calculated by dividing the sucrose intake by the minimum average relative sweetness value of 150 for SoPure Stevia™.
 bw – body weight; kg – kilogram; mg – milligram

⁶ <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=182.1>

The values in Table 6 are based on the assumption that ZCHT's SoPure Stevia™ high purity steviol glycosides preparations constitute 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 worst-case scenario estimated maximum intake of purified steviol glycosides is 3.38 mg per kg bw per day (1.13 mg per kg steviol equivalents) for SoPure Stevia™ high purity steviol glycosides. For healthy children, the worst-case scenario estimated maximal intake is 6.60 mg per kg bw per day (2.26 mg per kg as steviol equivalents) for SoPure Stevia™ high purity steviol glycosides. In all population groups, the worst-case scenario 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 ZCHT's SoPure Stevia™ high purity steviol glycosides preparations, which would be chemically stable under 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 $\geq 95\%$ 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 6.

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).

In a global market projection prepared by Maximize Market Research Pvt. Ltd., the global stevia market was \$488.8 million in 2019 and is expected to reach \$980.5 million by 2027.⁷

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 7.

⁷ <https://www.maximizemarketresearch.com/market-report/global-stevia-market/27578/>

1. U.S. Regulatory History

Based on available information from FDA's GRAS Notice Inventory website (FDA, 2020) as of August 26, 2020, FDA has issued 64 “no questions” letters on GRAS notices on rebaudioside A, rebaudioside D, rebaudioside M, or high purity 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 7, six of which are for enzymatically modified stevia extracts, 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, 2017b). (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, 2019c). In May 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, 2019b). Most recently, on June 27, 2019, Health Canada's Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides from various sources in “standardized flavoured milks” (Health Canada, 2019a).

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% rebaudioside 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) 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).

On September 24, 2019, the EFSA Panel on Food Additives and Flavourings concluded that there is no safety concern for rebaudioside M produced via enzymatic bioconversion and recommended that the European Commission consider establishing specifications for the preparation (EFSA, 2019).

On March 24, 2020, EFSA published a scientific opinion in response to a proposed amendment of the specifications for steviol glycosides, stating that all steviol glycosides share the same metabolic fate, and therefore the safety of 60 steviol glycosides identified in the leaves of *Stevia rebaudiana* Bertoni can be based on “read-across” from previously evaluated toxicological data. EFSA maintained that the ADI of 4 mg per kg bw applies to all 60 steviol glycosides. The EFSA Panel noted that the inclusion of more steviol glycosides, “whilst maintaining the assay value of not less than 95%, would allow less pure preparations” onto the market. The Panel stated that they “cannot conclude on the safety of the proposed amendment to the specifications of steviol glycosides (E 960) as [a] food additive if the purity assay value of not less than 95% for the total content of steviol glycosides is maintained.” Furthermore, the Panel noted that it is possible to manufacture steviol glycosides with a purity higher than 95% total steviol glycosides (EFSA, 2020).

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 7.

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, 2016). 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).

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).

On May 14, 2020, FSANZ published an approval report for a draft variation to amend the specification for steviol glycosides from *Stevia rebaudiana* Bertoni in section S3—35 of the Australia New Zealand Food Standards Code to include rebaudioside E produced by enzymatic conversion from stevia leaf extract. The approved draft variation allows for the use of high purity rebaudioside E ($\geq 85\%$ rebaudioside E; $\geq 95\%$ total steviol glycosides) within the existing permissions and limits for steviol glycosides (FSANZ, 2020a). Subsequently, on July 28, 2020, Amendment No. 193 was published to include rebaudioside E produced by enzymatic conversion from stevia leaf extract (FSANZ, 2020b).

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--- 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 8.

Since the JECFA evaluation (WHO, 2008), FDA has received and not objected to over fifty-five 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 publication by Roberts et al. (2016) indicates that the ADI could be higher, as discussed further in Appendix 8. 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 6.

Because of their sweetness characteristics, steviol glycosides have viable uses as a non-nutritive sweetener in foods.⁸ 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 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 8 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., 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).

More recently, 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 69th meeting.

The Committee noted that the most recent short-term toxicity studies were consistent with those reviewed at or prior to the 69th meeting, and that the new toxicokinetic study in humans did not

⁸ It has also been reported that steviol glycosides may have pharmacological properties, which 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.

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* Bertonii” while retaining the requirement that the total percentage of steviol glycosides is $\geq 95\%$ (WHO, 2017).

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

Several published and unpublished studies (summarized in Appendix 6) 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; Slotter, 2008a) and developmental effects (Slotter, 2008b) in rats. Most notably, pharmacokinetic studies in rats (Roberts and Renwick, 2008) and humans (Wheeler et al., 2008) on 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.

Purkayastha et al. (2014) compared the anaerobic *in vitro* metabolism of rebaudiosides A, B, D, and M with human fecal homogenates. In all cases, the rebaudiosides were hydrolyzed to steviol within 24 hours, with the majority of metabolism occurring within the first 8 hours. Metabolism of rebaudiosides took longer at higher concentrations (2.0 mg per mL vs. 0.2 mg per mL). There were no marked differences in rate or extent of hydrolysis observed between male and female fecal homogenates or individual rebaudiosides.

In a follow up study, Purkayastha et al. (2016) investigated the metabolic fate of two concentrations of steviolbioside, dulcoside A, and rebaudiosides A, B, C, D, E, F, and M in an *in vitro* study using pooled human fecal homogenates over the course of 24 to 48 hours. It was reported that the glycosidic side chains ---containing glucose, rhamnose, xylose, fructose, and those with deoxy-glucose including combinations of $\alpha(1-2)$, $\beta-1$, $\beta(1-2)$, $\beta(1-3)$, and $\beta(1-6)$ linkages ---were mostly degraded to steviol within 24 hours. This observation supports the extrapolation of safety data for specific steviol glycosides and steviol to other steviol glycosides found in *Stevia rebaudiana* leaf extract. As previously observed, the rate of metabolism was slower at higher concentrations (2.0 mg per mL vs. 0.2 mg per mL). In addition, Purkayastha et al. (2016) reported that no appreciable differences in metabolism were observed between fecal homogenates obtained from males and females or those obtained from different ethnicities.

Most recently, Purkayastha and Kwok (2020) investigated the metabolic fate of steviol glycosides in fecal homogenates collected from adults and children. Steviol glycosides obtained from stevia leaf extract (composed of more than 20 steviol glycosides, with Reb D and Reb M as the principal components), bioconversion reaction product (composed of Reb D and Reb M), minor steviol glycosides extracted from a stevia leaf extract (composed of Reb AM, Reb W2, Reb U2, Reb V,

Reb N, and Reb O), enzyme modified steviol glycosides, and rebaudioside A standard were used as test samples for *in vitro* incubation in pooled human fecal homogenate samples obtained from adult and pediatric donors. Purkayastha and Kwok (2020) reported that all steviol glycosides preparations tested “shared qualitatively similar *in vitro* metabolic fates.” In addition, the authors concluded that “safety data for individual steviol glycosides can be used to support safety of all steviol glycosides produced by extraction and enzymatic conversion of stevia leaf extract.”

ZCHT concludes that these studies by Purkayastha et al. (2020; 2016; 2014) and the studies on rebaudioside A strengthen the argument that all steviol glycosides that follow the same metabolic pathway are safe at the JECFA established ADI.

ZCHT has also reviewed the findings from human clinical studies. ZCHT noted that, with regard to the clinical effects reported in humans, in order to corroborate the observations in these studies, these effects of steviol glycosides only occur in patients with either elevated blood glucose or blood pressure (or both). JECFA called for studies in individuals that 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. ZCHT 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 6 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). ZCHT 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. ZCHT concurs with Carakostas (2012) and Waddell (2011) evaluations of the Awney et al. (2011) study and have concluded that it does not impact the safety discussion of ZCHT's SoPure™ Stevia.

Urban et al. (2013) examined the extensive genotoxicity database on steviol glycosides because some concern has been expressed in two 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 do 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. ZCHT agrees with the Urban et al. (2013) conclusions.

In addition, a paper by Shannon et al. (2016) raises a possible concern of endocrine disruption by steviol. ZCHT 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. ZCHT 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 ZCHT's opinion that steviol glycosides preparations are generally recognized as safe. A summary of this study is provided in Appendix 6.

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.

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 increases in CYP3A29 mRNA expression (< 2.0-fold), “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*.

A recently published study addressed the genotoxic activity of stevia (Svetia™, purity not reported⁹). 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 consistent with the weight of the evidence supporting negative genotoxicity and at odds with data reported by Nunes et al. (2007a). As discussed above, the validity and importance of the Nunes et al. study has been discounted given the questions surrounding the methodology.

In a seven-week study, 13 subjects (ages 18 to 30 years) without a history of hypertension or hyperglycemia were supplemented with four commercial 1 g packets per day of steviol glycosides (equivalent to 0.1 g steviol glycosides per day, brand and composition not reported) for six weeks. Decreases in triglyceride, cholesterol, and serum TNF- α concentrations were observed. No adverse effects were reported (Sánchez-Delgado et al., 2019).

Wang and Wu (2019) investigated the angiotensin-converting enzyme (ACE) inhibiting activity of a 95% pure steviol glycosides extract (composition not reported) obtained from an ethanol extract of stevia leaves. Steviol glycosides were reported to have doubled the ACE inhibitory activity of an ethanolic extract of steviol leaves, were well-accepted in a sensory test in decaffeinated coffee, decaffeinated tea, and peanut protein beverages, and had a significant antihypertensive effect in spontaneously hypertensive rats. No adverse events in humans or rats were reported.

The interaction between select prescription drugs and steviol acyl glucuronide, the major metabolite of rebaudioside A, was investigated by Zhou et al. (2019). Organic anion transporter 3 (OAT3) – mediated uptake of steviol acyl glucuronide was examined *in vitro* using human embryonic kidney 293 (HEK293) cells. HEK293 cells were transfected with human organic anion transporter 3 (hOAT3) and rat organic anion transporter 3 (rOAT3). Both probenecid and glimpiride were potent inhibitors of hOAT3 and rOAT3 with no apparent species differences observed. Pharmacokinetic studies in male Sprague-Dawley rats revealed that both probenecid

⁹ 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) indicates that the trademarked material is a blend of cane sugar and 97% pure rebaudioside A.

and glimepiride significantly elevated plasma steviol acyl glucuronide concentrations, particularly between 6 and 8 hours after oral administration of rebaudioside A. The inhibition of OAT3 is a potential mechanism for the interaction between steviol acyl glucuronide and probenecid and glimepiride, which could be clinically relevant. The authors concluded that “care should be given to populations with concomitant use of stevia leaf extracts and probenecid or glimepiride.”

ZCHT has reviewed the Zhou et al. (2019) publication in detail and notes that the pharmacokinetic oral dose used in the study was 15 mg per kg rebaudioside A. Plasma concentrations of steviol acyl glucuronide were observed to be dose-dependent after oral administration of rebaudioside A, with an average concentration maximum (C_{max}) of approximately 39 ng per mL at 5 mg per kg rebaudioside A and 170 ng per mL at 15 mg per kg rebaudioside A, respectively, observed at 6 hours post-dosing. Given the observed dose-dependency, it is possible that a reduced interaction would be observed between steviol acyl glucuronide and probenecid and glimepiride at lower doses of rebaudioside A. Based on a steviol equivalence factor of 0.329 for rebaudioside A as listed in Table 4, the 15 mg per kg dose corresponds to 4.9 mg per kg steviol equivalents, which is higher than the JECFA-established ADI of 4.0 mg per kg bw per day. Given that the investigational dose is higher than the accepted ADI for steviol glycosides and the history of safe use of steviol glycosides, including the paucity of reported case studies regarding the concomitant ingestion of steviol glycosides and probenecid and glimepiride, ZCHT concludes that the use of steviol glycosides as proposed herein remains safe for the general population and agrees with Zhou et al. (2019) in an abundance of caution that care should be exercised in the small subset of the population for which probenecid and glimepiride are prescribed; however, more data are necessary to draw any specific conclusions about their findings.

Halasa et al. (2020) published a case study vignette on the investigation of the presence of steviol glycosides metabolites in plasma, cerebrospinal fluid, amniotic fluid, and cord blood in samples collected as early as 2004. The end date was not provided. Steviol glucuronide, the primary steviol metabolite, was detected in all types of samples, but was observed primarily in the plasma. Of the samples, seven of the 38 adults (18%) had detectable steviol glucuronide concentrations, while two of 13 (15%) amniotic fluid samples and one of 15 (7%) cord blood samples contained steviol glucuronide. The authors noted that steviol glucuronide was detected only in samples obtained in and after 2008, which corresponds to the dates of the first GRAS Notices submitted to FDA for steviol glycosides.

It should be noted that Halasa et al. (2020) did not discuss their findings in relation to the time of consumption and intake levels of steviol glycosides. As steviol glucuronide is a known metabolite of steviol glycosides and is expected to be present in plasma following steviol glycosides ingestion, this study serves to support previous published findings.

Recent studies in rats have been identified in the published literature. Assi et al. (2020) investigated the use of an ethanolic extract of dried *S. rebaudiana* leaves (chemical composition not reported) to treat diabetic rats. No adverse effects or unplanned animal deaths were reported.

Cho et al. (2018) investigated the impact of stevia and obesity on fertility and reproductive outcomes in Sprague Dawley rats. Rats were administered 2-3 mg per kg bw per day rebaudioside A in drinking water starting two weeks prior to mating and throughout lactation. The authors reported that obese rats supplemented with rebaudioside A displayed a lower fertility index than untreated obese rats (53.3% vs. 85.7%, respectively); however, the rate of successful pregnancies was higher in obese rats supplemented with rebaudioside A than untreated obese rats (100% vs. 60.7%). No animal deaths were reported.

A follow-up study examined the impact of maternal low-dose rebaudioside A consumption on adiposity, glucose tolerance, gut microbiota, and the mesolimbic pathway in obese dams and their offspring (Nettleton et al., 2020). Pregnant obese rats and their offspring were fed high fat/sucrose diet plus 3 mg per kg bw per day rebaudioside A (Sigma-Aldrich) through 18 weeks postpartum. The authors noted that rebaudioside A consumption reduced the fertility of dams, as previously reported (Cho et al., 2018). The study supports findings that low-calorie sweeteners may not be metabolically inert. No animal deaths were reported.

ZCHT notes that the effect of steviol glycosides on fertility and reproductive outcomes has been the subject of a number of investigations as discussed further in Appendix 6, and that these recent publications by Cho et al. (2018) and Nettleton et al. (2020) corroborate previous findings by Planas and Kuć (1968), where 5% crude stevia leaf extract was observed to reduce fertility to 21% in female rats.

The effects of non-nutritive low-calorie sweeteners on gut microbiota were reviewed by Plaza-Diaz et al. (2020). It was noted that there have been no reports of negative interactions between steviol glycosides and colonic microbiota; however, it is possible that steviol glycosides modify the gut microbiota. The authors note further studies are necessary to “clarify its specific effects.”

A recent review by Ray et al. (2020) focused on the effects of *Stevia rebaudiana* on glucose homeostasis, blood pressure, and inflammation. The authors reported that no hypersensitivities or allergies were reported since 2008, and that the few prior reports were for “improperly filtered stevia extracts.” Furthermore, Ray et al. noted that additional randomized controlled trials are needed to confirm the beneficial effects of stevia. No significant adverse effects were noted from any study included in the review.

Zhao et al. (2020) reported that stevioside improved hyperglycemia-induced cardiac dysfunction in male C57BL/6 mice. Stevioside supplementation reduced the expression levels of cardiac fibrosis producing lysyl oxidase family and weakened the collagen cross-linking lysyl oxidase-like 2 caused by hyperglycemia, as well as promoted the elimination of existing fibrosis via the regulation of matrix metalloproteinase and tissue inhibitors of metalloproteinase. No adverse events or unplanned deaths were reported.

The effect of steviol on cytotoxicity, adipogenesis, ROS concentration, and gene expression were studied in the murine 3T3-L1 cell line. Kurek et al. (2020) reported that there was no observed

effect on the proliferation of cells, lipid accumulation, or intracellular ROS generation at steviol concentrations up to 100 μ M. Furthermore, it was reported that steviol reduced the expression of genes regulating the adipogenesis and lipogenesis process. Results of this study further support the safety of steviol—and by extension—steviol glycosides.

Abolhasani et al. (2020) evaluated the *in vitro* cytotoxicity of stevioside on cancerous liver (HepG2), colon (HT29), and breast (MCF7) cells, as well as normal kidney cells (Hek293), compared with cisplatin. Stevioside was reported to display higher cell growth inhibition on the HepG2 cell line and was not observed to have high toxicity on the Hek293 normal cell line. The authors concluded that stevioside “showed less cytotoxic effects compared to cisplatin” (abstract only).

ZCHT agrees with the safety conclusions of the 64 GRAS Expert Panels in the notices 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.

ZCHT 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 SoPure Stevia™ high purity steviol glycosides. Therefore, with the steviol equivalence values shown in Table 6, ZCHT concludes that, for the general population, the estimated maximum daily intake of the SoPure Stevia™ preparation described herein is 6.60 mg per kg bw or 2.26 mg per kg expressed as steviol equivalents. Based upon these calculations, the intake of ZCHT’s SoPure Stevia™ high purity steviol glycosides preparation described herein safely aligns with the 4 mg per kg bw per day ADI expressed as steviol equivalents as determined by JECFA.

ZCHT’s SoPure Stevia™ high purity steviol glycosides preparations contain not less than 95% total steviol glycosides. 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 SoPure Stevia™. 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 rebaudioside A, other steviol glycosides and steviol has been summarized, with key studies summarized in Appendix 6.

Furthermore, ZCHT has reviewed this safety information and has concluded that SoPure Stevia™ high purity steviol glycosides 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.”¹⁰

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.”¹¹

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:¹²

- 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

¹⁰ 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 6/22/20).

¹¹ See 81 FR 54959 Available at: <https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe> (Accessed on 10/12/2020).

¹² See Footnote 1.

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 SoPure Stevia™ High Purity Steviol Glycosides

An evaluation of the safety and GRAS status of the intended use of ZCHT’s SoPure Stevia™ high purity steviol glycosides ($\geq 95\%$ total steviol glycosides) preparation 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 ZCHT’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 9.

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 exist 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 6. 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 above, 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).

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, over fifty-five GRAS notifications 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, ZCHT 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, ZCHT concludes that SoPure Stevia™ ($\geq 95\%$ total steviol glycosides) defined in the subject notification are 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 ZCHT's SoPure Stevia™ 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 ZCHT's SoPure Stevia™ preparation for several population groups summarized in Part 3.A. are no greater than 2.26 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 high purity steviol glycosides composed of at least 95% total steviol glycosides are used as a general non-nutritive sweetener.

Accordingly, SoPure Stevia™ high purity steviol glycosides ($\geq 95\%$ total steviol glycosides) as produced by ZCHT and declared within the subject notification meet 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, are generally recognized as safe (GRAS).

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

A. References

1. List of Acronyms

AAS	Atomic Absorption Spectrometry
ACE	Angiotensin-converting enzyme
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
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
ChP	Chinese Pharmacopeia
C _{max}	Maximum serum concentration
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
GRNs	GRAS Notices
HbA1c	Glycated hemoglobin
HEK293	Human embryonic kidney 293
hOAT3	Human organic anion transporter 3
HPLC	High performance liquid chromatography
IADSA	International Alliance of Dietary/Food Supplement Associations

INS	International Numbering System
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg	Kilogram
LD ₅₀	Median lethal dose
mg	Milligram
mL	Milliliter
MPL	Maximum permitted level
mRNA	Messenger ribonucleic acid
MW	Molecular Weight
NA	Not applicable
ND	Not detected
ng	Nanogram
NHANES	National Health and Nutrition Examination Surveys
NHPs	Natural Health Products
NLT	Not less than
NMT	Not more than
NOAEL	No observed adverse effect level
NS	Not specified
OAT3	Organic anion transporter 3
OECD	Organisation for Economic Co-operation and Development
ppm	Parts per million
Reb A	Rebaudioside A
Reb M	Rebaudioside M
rOAT3	Rat organic anion transporter 3
SBP	Systolic blood pressure
TAC	Total antioxidant capacity
TFC	Total flavonoid content
TPC	Total phenolic content
TRAP	Tartrate-resistant alkaline phosphatase
UDS	Unscheduled DNA synthesis
ug	Microgram
WHO	World Health Organization
WHO/JECFA	World Health Organization/Joint FAO/WHO Expert Committee on Food Additives

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B. Appendices

Appendix 1 Specifications and Certificates of Analyses for Raw Materials and Production Processing Aids

Appendix 1.1 Ferrous Sulfate

Appendix 1.2 Calcium Oxide

Appendix 1.3 Ethanol

Appendix 1.4 Sunresin Statement

Appendix 1.5 Anion Resin

Appendix 1.6 Adsorption Resin

Appendix 1.2 Calcium Oxide



新乡市源丰钙业有限公司

XIN XIANG YUAN FENG CALCIUM CO., LTD.

河南省新乡市凤泉区宝山路东段 电话: 13523239150 传真: 0373-3919228

产品质量检验报告单

Product Inspection Report

产品名称: 食品级氧化钙
Product Name: Food Grade Calcium Oxide
 执行标准 **Standard:** FCC
 批号: 200530
Batch No.: 200530
 数量: 25kg
Quantity: 25kg

用户名称: 诸城市浩天药业有限公司
User Name: Zhucheng Haotian
 Pharmaceutical Co., Ltd
 生产日期: 2020.05.30
Production Time: 200530
 报告日期: 2020.08.20
Report Time: 2020.08.20

序号 No.	检测项目 Test Items	技术要求 Requirements	检测结果 Result	单项判定 Judge	备注 Remarks
1	色泽 Colour	白色或灰白色 White or grayish white	白色 White	符合 Qualified	/
2	状态 Status	颗粒或粉末 Particles or powder	粉末 Powder	符合 Qualified	/
3	氧化钙 (CaO) 含量 (以干基计, 质量分数) Content (on dry basis, w/w)	% 95-100.5	96.8	符合 Qualified	/
4	镁和碱金属 (质量分数) Alkalis or Magnesium	% ≤3.6	2.56	符合 Qualified	/
5	无机砷 (以 As 计) Arsenic	mg/kg ≤3	0.8	符合 Qualified	/
6	氟 (F) Fluorine	mg/kg ≤150	36	符合 Qualified	/
7	铅 (Pb) Lead	mg/kg ≤2	0.48	符合 Qualified	/
8	酸不溶物 (质量分数) Acid-Insoluble Substances	% ≤1	0.2	符合 Qualified	/
9	灼烧减量 (质量分数) Loss on Ignition	% ≤10	1.6	符合 Qualified	/
10	鉴别试验 Identification	通过实验 Pass the test	通过实验 Pass the test	符合 Qualified	/
11	检测结果 Test Results		合格 Qualified		/

包装: 25kg/牛皮纸袋
 Packing: 25Kg/ Kraft bag

Inspector

Approver

Appendix 1.3 Ethanol



食用酒精检验报告 Certificate of Analysis of Edible Ethanol

报告编号 Report Number: YXPG-2020-0731001		YXSJ-ZG-03-R-047
批号 Batch Number: 200728-N3	规格 Grade: 优级 Special grade	数量 Qty: / MT
检验日期 Test Date: 2020.07.31	报告日期: 2020.07.31	
检验项目 Test Parameter	要求 Requirements	测试结果 Test Result
外观 Appearance	透明, 无色易挥发液体 Clear, colorless and volatile liquid	符合 Comply
气味 Odor	典型中性气味 Typical neutral odor with no abnormal odor	符合 Comply
口味与气味 Taste and Odor	没有异常的口味与气味 No abnormal taste and odor when	符合 Comply
含量 (%Vol) Assay	≥95.0	96.1
酸度 (以乙酸计) (%) Acidity (as acetic acid)	≤0.003	<0.003
碱度 (以NH ₃ 计) (ppm) Alkalinity (as NH ₃)	≤3	<3
杂醇油 Fusel Oil	通过测试 Passes test	通过 Pass
酮类, 异丙醇 Ketones, Isopropyl Alcohol	通过测试 Passes test	通过 Pass
铅 (mg/kg) Lead	≤0.5	<0.02
甲醇 (mg/L) Methanol	通过测试 Passes test	通过 Pass
不挥发物 (%) Nonvolatile Residue	≤0.003	0.0006
水溶性试验 Solubility in Water	通过测试 Passes test	通过 Pass
硫酸试验 Substances Darkened by Sulfuric Acid	通过测试 Passes test	通过 Pass
高锰酸钾试验 Substances Reducing Permanganate	通过测试 Passes test	通过 Pass
检验结论: 产品按客户要求进行检测, 结果符合要求。 Conclusion: The products are tested according to the customer's requirements, and the results meet the requirements.		
批准 Approver: [Redacted]	审核 Verifier: [Redacted]	编制 writer: [Redacted]



Appendix 1.4 Sunresin Statement



Sunresin-China biggest special resin manufacturer

Certificate

Product: Sunresin resins
Manufacturer: Sunresin New Materials Co. Ltd., Xi'an

To whom it may concern:

We hereby certify that main components of sunresin resins are DVB-styrene.
The procedure of production was strictly made accordingly to standard of FDA 21CFR § 173.65, AP(97)1 and § 173.25.

The product is confirmed suiling for using in food processing.

Sunresin New Materials Co. Ltd., Xi'an

July 29th 2020



Appendix 1.5 Anion Resin



北京清析技术研究院

测试报告

Test Report

报告编号	BT0041406-2
样品名称	Anion Exchange Resin 阴离子交换树脂
样品标识	
测试类别	委托测试
委托单位	Sunresin New Materials Co., Ltd., Xi'an 西安蓝晓科技新材料股份有限公司
签发日期	2020年04月21日

编制: 王筱 审核: 董屹然 签发: 唐文

地址: 北京市海淀区王庄路1号, 清华同方科技大厦B座
服务热线: 010-89471010 E-mail: icl@singhua-research.com

Appendix 1.6 Adsorption Resin

PONY 谱尼测试
Pony Testing International Group

JC3M-2
扫描二维码
关注谱尼测试

MA
160000343608

检测报告

(Test Report)

No. GNAW6CUA1F4005334

样品名称 (Sample Description)	食品工业用吸附树脂 (Adsorption resin for food industrialuse)
委托单位 (Applicant)	西安蓝晓科技新材料股份有限公司 (Sunresin New Materials Co.Ltd.Xi'an)

PONY 谱尼测试
Pony Testing International Group
www.ponytest.com

PONY 谱尼测试
Pony Testing International Group

检测结果

(Test Results)

No. GNAW6CUA1F4005334 第 1 页, 共 2 页 (page 1 of 2)

样品名称 (Sample Description)	食品工业用吸附树脂 (Adsorption resin for food industrial use)	样品规格 (Sample Specification)	—
委托单位 (Applicant)	西安蓝晓科技新材料股份有限公司 (Sunresin New Materials Co.Ltd.Xi'an)	商标 (Trade Mark)	seplite
到样日期 (Received Date)	2019-03-12	生产日期或批号 (Manufacturing Date or Lot No.)	2019-36P
检测日期 (Test Date)	2019-03-12~2019-03-20	样品等级 (Sample Grade)	食品级 (Food grade)
样品状态 (Sample Status)	固态 (Solid)	检测类别 (Test Type)	委托检测 (Commissioning Test)
检测项目 (Test Items)	见下页 See next page	检测环境 (Test Environment)	符合要求 (To meet the requirements)
检测方法 (Test Methods)	见下页 See next page		
所用主要仪器 (Main Instruments)	气相色谱仪 GC 等		
备注 (Note)	1. 样品来源: 客户提供 Sample From: Customer supply 2. 以上样品信息由委托单位提供 The information of sample was provided by the applicant. 3. 该报告中检测方法由委托单位指定。 The testing methods mentioned in this report were designated by the client. 4. 限值标准: GB/T 24395-2009 The limit basis of GB/T 24395-2009		
 PONY 专用章 (Special Stamp of PONY TESTING INTERNATIONAL GROUP)	编制人 (Edited by)	张瑜	
	审核人 (Checked by)	王素红	
	批准人 (Approved by)	杨和荣	
	签发日期 (Issued Date)	2019 年 03 月 22 日	

© Hotline 400-819-5688 谱尼测试集团股份有限公司
 www.ponytest.com 公司地址: 北京市西城区车公庄大街 66 号院 1 号楼 4 层至 5 层 101 电话: 010-83055000 传真: 010-82619629
 PONY-REG-1403-3-2019A 检测地址: 北京市朝阳区车公庄路 66 号院 1 号楼

PONY 谱尼测试
Pony Testing International Group

检测结果
(Test Results)

No. GNAW6CUA1F4005334 第 2 页, 共 2 页 (page 2 of 2)

样品名称和编号 (Sample Description and Number)	检测项目 (Test Items)	单位 (Unit)	限值 (Limit)	检测结果 (Test Results)	单项结论 (Evaluation)	检测方法 (Test Methods)
A1F4005334 食品工业用吸 附树脂 (Adsorption resin for food industrial use)	重金属 (以 Pb 计, 干基) 质量分数 Heavy metals (Pb, dry basis) mass fraction	%	≤0.0015	<0.0015	符合 (Pass)	GB/T 24396-2009 5.2
	苯 (Benzene)	mg/kg	≤2	未检出 (Not detected) (<0.6)	符合 (Pass)	GB/T 24396-2009 5.3
	1,2-二氯乙烯 (1,2-dichloroethane)	mg/kg	≤2	未检出 (Not detected) (<0.6)	符合 (Pass)	GB/T 24396-2009 5.3
	丙烯腈 (Acrylonitrile)	mg/kg	≤10	未检出 (Not detected) (<3.0)	符合 (Pass)	GB/T 24396-2009 5.3
	氯苯 (Chlorobenzene)	mg/kg	≤10	未检出 (Not detected) (<3.0)	符合 (Pass)	GB/T 24396-2009 5.3
	二乙烯苯 (Divinyl benzene)	mg/kg	≤10	未检出 (Not detected) (<3.0)	符合 (Pass)	GB/T 24396-2009 5.3
	甲苯 (Toluene)	mg/kg	≤20	未检出 (Not detected) (<6.0)	符合 (Pass)	GB/T 24396-2009 5.3
	苯乙烯 (Styrene)	mg/kg	≤20	未检出 (Not detected) (<6.0)	符合 (Pass)	GB/T 24396-2009 5.3
	二甲苯 (Xylene)	mg/kg	≤20	未检出 (Not detected) (<6.0)	符合 (Pass)	GB/T 24396-2009 5.3
	甲基丙烯酸甲酯 (Methyl methacrylate)	mg/kg	≤20	未检出 (Not detected) (<6.0)	符合 (Pass)	GB/T 24396-2009 5.3

以下空白
(End of Report)

☎ Hotline 400-819-5688 www.ponytest.com PONY-8196-5013-2019A

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 公司地址: 北京市海淀区知春路 66 号院 1 号楼 4 层至 5 层 10F
 检测地址: 北京市海淀区碧云路 66 号院 1 号楼
 电话: 010-82395090 传真: 010-82619629

Appendix 2 Certificates of Analysis for Multiple Batches of SoPure Stevia™

Appendix 2.1 Certificate of Analysis for SoPure Stevia™ Batch 20190106 TSG95

Appendix 2.2 Certificate of Analysis for SoPure Stevia™ Batch 20191101 TSG95

Appendix 2.3 Certificate of Analysis for SoPure Stevia™ Batch 20200101 TSG95

Appendix 2.4 Certificate of Analysis for SoPure Stevia™ Batch 20200203 TSG95

Appendix 2.5 Certificate of Analysis for SoPure Stevia™ Batch 20200301 TSG95

Appendix 2.1 Certificate of Analysis for SoPure Stevia™ Batch 20190106 TSG95



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CERTIFICATE OF ANALYSIS

Product Name: Steviol Glycosides (Total Steviol Glycosides ≥95%)
Product Code: TSG95 **Manufacture Date:** 2019.01.12
Lot Number: 20190106 TSG95 **Expiration Date:** 2022.01.11

Item	Method	Specification	Result
Appearance	Visual	White to off-white fine powder	White Powder
Taste	Organoleptic	Sweet	Sweet
Total Steviol Glycosides (Dry Basis, %)	JECFA 2017	Not less than 95.0	96.5
Loss On Drying (%)	JECFA 2017	Not more than 6.0	3.9
Ash (%)	JECFA 2017	Not more than 1.0	0.04
pH, 1% in Water	JECFA 2017	Not less than 4.5; Not more than 7.0	5.5
Arsenic (As)	AAS ChP2015 Part4 (2321)	Not more than 1.0 ppm	Not Detected
Cadmium (Cd)	AAS ChP2015 Part4 (2321)	Not more than 1.0 ppm	Not Detected
Lead (Pb)	AAS ChP2015 Part4 (2321)	Not more than 0.5 ppm	Not Detected
Mercury (Hg)	AAS ChP2015 Part4 (2321)	Not more than 0.1 ppm	Not Detected
Residual Solvents	JECFA 2017	Methanol, Not more than 200 ppm	<50 ppm
		Ethanol, Not more than 5000 ppm	<25 ppm
Total Aerobic Bacteria	ChP 2015 Part4 (1105)	Not more than 10 ³ cfu/g	<10 cfu/g
Mold& Yeast	ChP 2015 Part4 (1105)	Not more than 10 ² cfu/g	<10 cfu/g
E.Coli	ChP 2015 Part4 (1106)	Negative/ g	Negative
Salmonella	ChP 2015 Part4 (1106)	Negative/25g	Negative

Analyzed By : 张全香 Checked By: 薛雪 Approved By: 徐智秀

Appendix 2.2 Certificate of Analysis for SoPure Stevia™ Batch 20191101 TSG95



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Http://www.zcht.cc



CERTIFICATE OF ANALYSIS

Product Name: Steviol Glycosides (Total Steviol Glycosides ≥95%)
Product Code: TSG95 **Manufacture Date:** 2019.10.26
Lot Number: 20191101 TSG95 **Expiration Date:** 2022.10.25

Item	Method	Specification	Result
Appearance	Visual	White to off-white fine powder	White Powder
Taste	Organoleptic	Sweet	Sweet
Total Steviol Glycosides (Dry Basis, %)	JECFA 2017	Not less than 95.0	96.6
Loss On Drying (%)	JECFA 2017	Not more than 6.0	3.4
Ash (%)	JECFA 2017	Not more than 1.0	0.06
pH, 1% in Water	JECFA 2017	Not less than 4.5; Not more than 7.0	4.9
Arsenic (As)	AAS ChP2015 Part4 (2321)	Not more than 1.0 ppm	Not Detected
Cadmium (Cd)	AAS ChP2015 Part4 (2321)	Not more than 1.0 ppm	Not Detected
Lead (Pb)	AAS ChP2015 Part4 (2321)	Not more than 0.5 ppm	Not Detected
Mercury (Hg)	AAS ChP2015 Part4 (2321)	Not more than 0.1 ppm	Not Detected
Residual Solvents	JECFA 2017	Methanol, Not more than 200 ppm	<50 ppm
		Ethanol, Not more than 5000 ppm	<25 ppm
Total Aerobic Bacteria	ChP 2015 Part4 (1105)	Not more than 10 ³ cfu/g	10 cfu/g
Mold& Yeast	ChP 2015 Part4 (1105)	Not more than 10 ² cfu/g	<10 cfu/g
E.Coli	ChP 2015 Part4 (1106)	Negative/ g	Negative
Salmonella	ChP 2015 Part4 (1106)	Negative/25g	Negative

Analyzed By :  Checked By:  Approved By: 

Appendix 2.3 Certificate of Analysis for SoPure Stevia™ Batch 20200101 TSG95



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 Http://www.zcht.cc



CERTIFICATE OF ANALYSIS

Product Name: Steviol Glycosides (Total Steviol Glycosides ≥ 95%)
Product Code: TSG95 **Manufacture Date:** 2020.01.19
Lot Number: 20200101 TSG95 **Expiration Date:** 2023.01.18

Item	Method	Specification	Result
Appearance	Visual	White to off-white fine powder	White Powder
Taste	Organoleptic	Sweet	Sweet
Total Steviol Glycosides (Dry Basis, %)	JECFA 2017	Not less than 95.0	95.7
Loss On Drying (%)	JECFA 2017	Not more than 6.0	4.0
Ash (%)	JECFA 2017	Not more than 1.0	0.07
pH, 1% in Water	JECFA 2017	Not less than 4.5; Not more than 7.0	5.6
Arsenic (As)	AAS ChP2015 Part4 (2321)	Not more than 1.0 ppm	Not Detected
Cadmium (Cd)	AAS ChP2015 Part4 (2321)	Not more than 1.0 ppm	Not Detected
Lead (Pb)	AAS ChP2015 Part4 (2321)	Not more than 0.5 ppm	Not Detected
Mercury (Hg)	AAS ChP2015 Part4 (2321)	Not more than 0.1 ppm	Not Detected
Residual Solvents	JECFA 2017	Methanol, Not more than 200 ppm	<50 ppm
		Ethanol, Not more than 5000 ppm	51 ppm
Total Aerobic Bacteria	ChP 2015 Part4 (1105)	Not more than 10 ³ cfu/g	<10 cfu/g
Mold& Yeast	ChP 2015 Part4 (1105)	Not more than 10 ² cfu/g	<10 cfu/g
E.Coli	ChP 2015 Part4 (1106)	Negative/ g	Negative
Salmonella	ChP 2015 Part4 (1106)	Negative/25g	Negative

Analyzed By: 张全香 Checked By: 薛雪 Approved By: 徐智秀

Appendix 2.4 Certificate of Analysis for SoPure Stevia™ Batch 20200203 TSG95



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Http://www.zcht.cc

CERTIFICATE OF ANALYSIS



Product Name: Steviol Glycosides (Total Steviol Glycosides ≥95%)
 Product Code: TSG95 * Manufacture Date: 2020.02.22
 Lot Number: 20200203 TSG95 Expiration Date: 2023.02.21

Item	Method	Specification	Result
Appearance	Visual	White to off-white fine powder	White Powder
Taste	Organoleptic	Sweet	Sweet
Total Steviol Glycosides (Dry Basis, %)	JECFA 2017	Not less than 95.0	95.4
Loss On Drying (%)	JECFA 2017	Not more than 6.0	4.4
Ash (%)	JECFA 2017	Not more than 1.0	0.05
pH, 1% in Water	JECFA 2017	Not less than 4.5; Not more than 7.0	5.4
Arsenic (As)	AAS ChP2015 Part4 (2321)	Not more than 1.0 ppm	Not Detected
Cadmium (Cd)	AAS ChP2015 Part4 (2321)	Not more than 1.0 ppm	Not Detected
Lead (Pb)	AAS ChP2015 Part4 (2321)	Not more than 0.5 ppm	Not Detected
Mercury (Hg)	AAS ChP2015 Part4 (2321)	Not more than 0.1 ppm	Not Detected
Residual Solvents	JECFA 2017	Methanol, Not more than 200 ppm	<50 ppm
		Ethanol, Not more than 5000 ppm	25 ppm
Total Aerobic Bacteria	ChP 2015 Part4 (1105)	Not more than 10 ³ cfu/g	<10 cfu/g
Mold& Yeast	ChP 2015 Part4 (1105)	Not more than 10 ² cfu/g	<10 cfu/g
E.Coli	ChP 2015 Part4 (1106)	Negative/ g	Negative
Salmonella	ChP 2015 Part4 (1106)	Negative/25g	Negative

Analyzed By : 张全香

Checked By: 薛雪

Approved By: 徐雪秀

Appendix 2.5 Certificate of Analysis for SoPure Stevia™ Batch 20200301 TSG95



ZHUCHENG HAOTIAN PHARM CO.,LTD.
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 Http://www.zcht.cc



CERTIFICATE OF ANALYSIS

Product Name: Steviol Glycosides (Total Steviol Glycosides ≥95%)
Product Code: TSG95 **Manufacture Date:** 2020.02.15
Lot Number: 20200301 TSG95 **Expiration Date:** 2023.02.14

Item	Method	Specification	Result
Appearance	Visual	White to off-white fine powder	White Powder
Taste	Organoleptic	Sweet	Sweet
Total Steviol Glycosides (Dry Basis, %)	JECFA 2017	Not less than 95.0	96.3
Loss On Drying (%)	JECFA 2017	Not more than 6.0	3.6
Ash (%)	JECFA 2017	Not more than 1.0	0.05
pH, 1% in Water	JECFA 2017	Not less than 4.5; Not more than 7.0	5.7
Arsenic (As)	AAS ChP2015 Part4 (2321)	Not more than 1.0 ppm	Not Detected
Cadmium (Cd)	AAS ChP2015 Part4 (2321)	Not more than 1.0 ppm	Not Detected
Lead (Pb)	AAS ChP2015 Part4 (2321)	Not more than 0.5 ppm	Not Detected
Mercury (Hg)	AAS ChP2015 Part4 (2321)	Not more than 0.1 ppm	Not Detected
Residual Solvents	JECFA 2017	Methanol, Not more than 200 ppm	<50 ppm
		Ethanol, Not more than 5000 ppm	178 ppm
Total Aerobic Bacteria	ChP 2015 Part4 (1105)	Not more than 10 ³ cfu/g	<10 cfu/g
Mold& Yeast	ChP 2015 Part4 (1105)	Not more than 10 ² cfu/g	<10 cfu/g
E.Coli	ChP 2015 Part4 (1106)	Negative/ g	Negative
Salmonella	ChP 2015 Part4 (1106)	Negative/25g	Negative

Analyzed By: 张全香 Checked By: 薛雪 Approved By: 徐智秀

Appendix 3 Representative Chromatograms for Multiple Batches of SoPure Stevia™

Appendix 3.1 Representative Chromatogram for SoPure Stevia™ Batch 20190106 TSG95

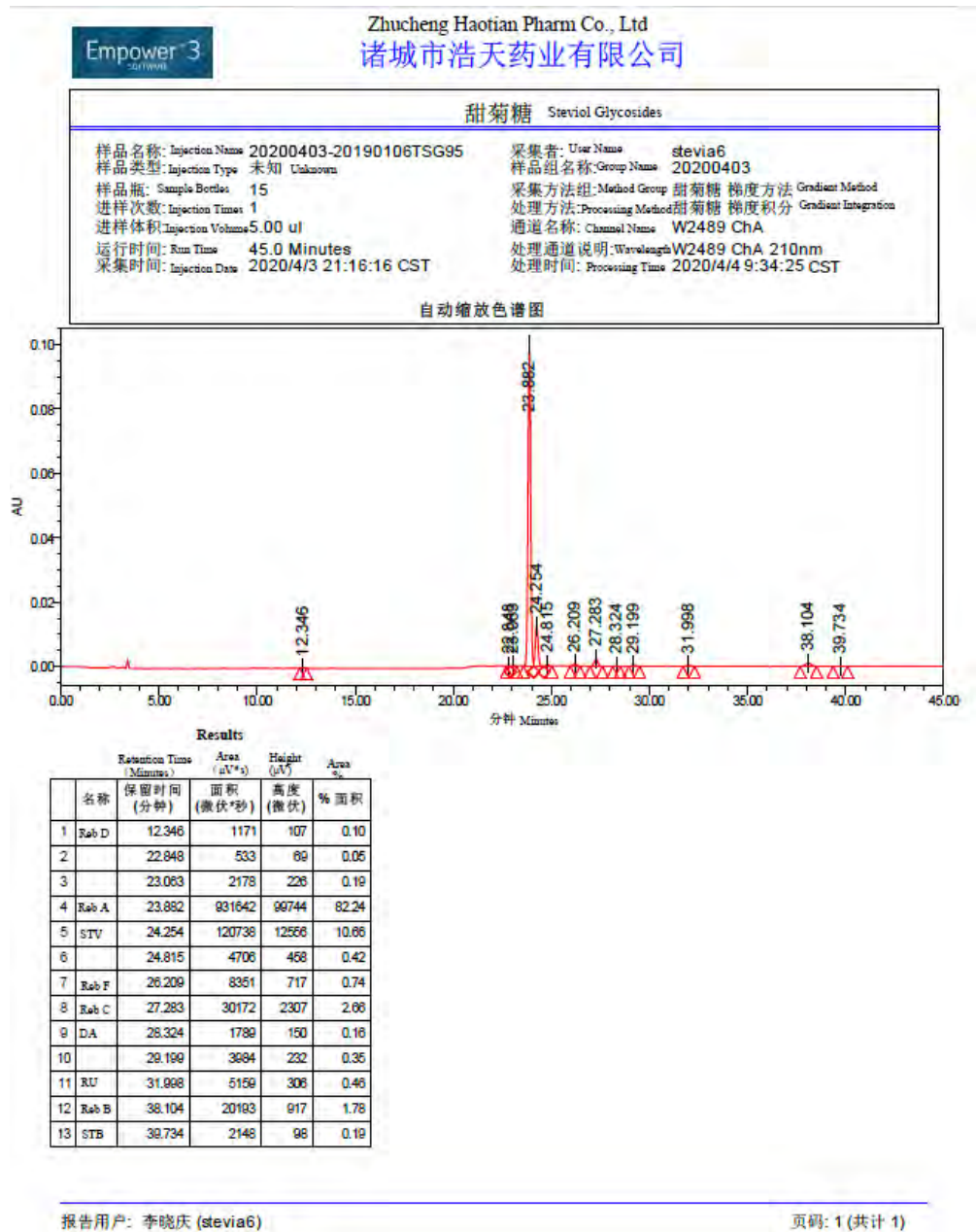
Appendix 3.2 Representative Chromatogram for SoPure Stevia™ Batch 20191101 TSG95

Appendix 3.3 Representative Chromatogram for SoPure Stevia™ Batch 20200101 TSG95

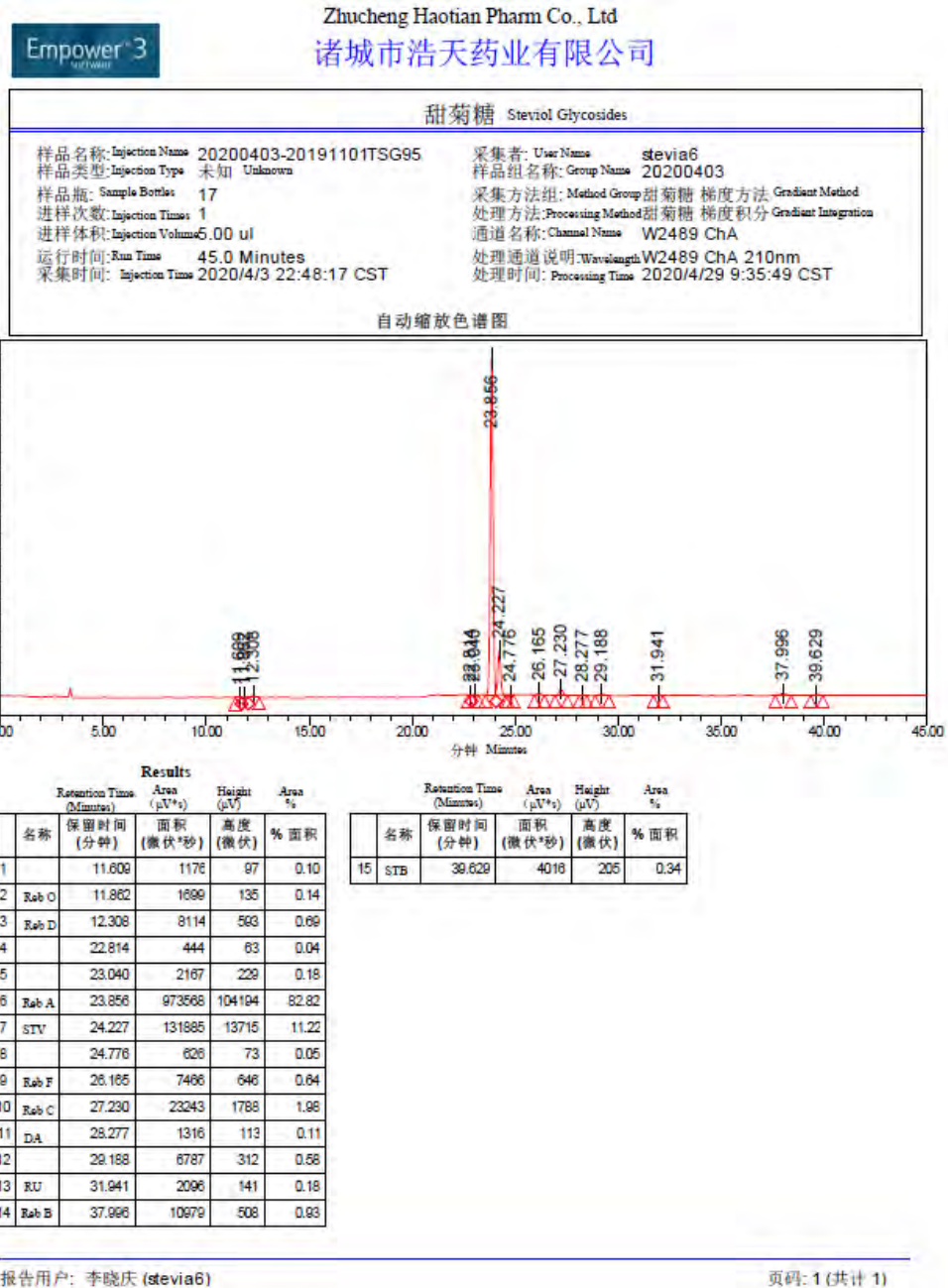
Appendix 3.4 Representative Chromatogram for SoPure Stevia™ Batch 20200203 TSG95

Appendix 3.5 Representative Chromatogram for SoPure Stevia™ Batch 20200301 TSG95

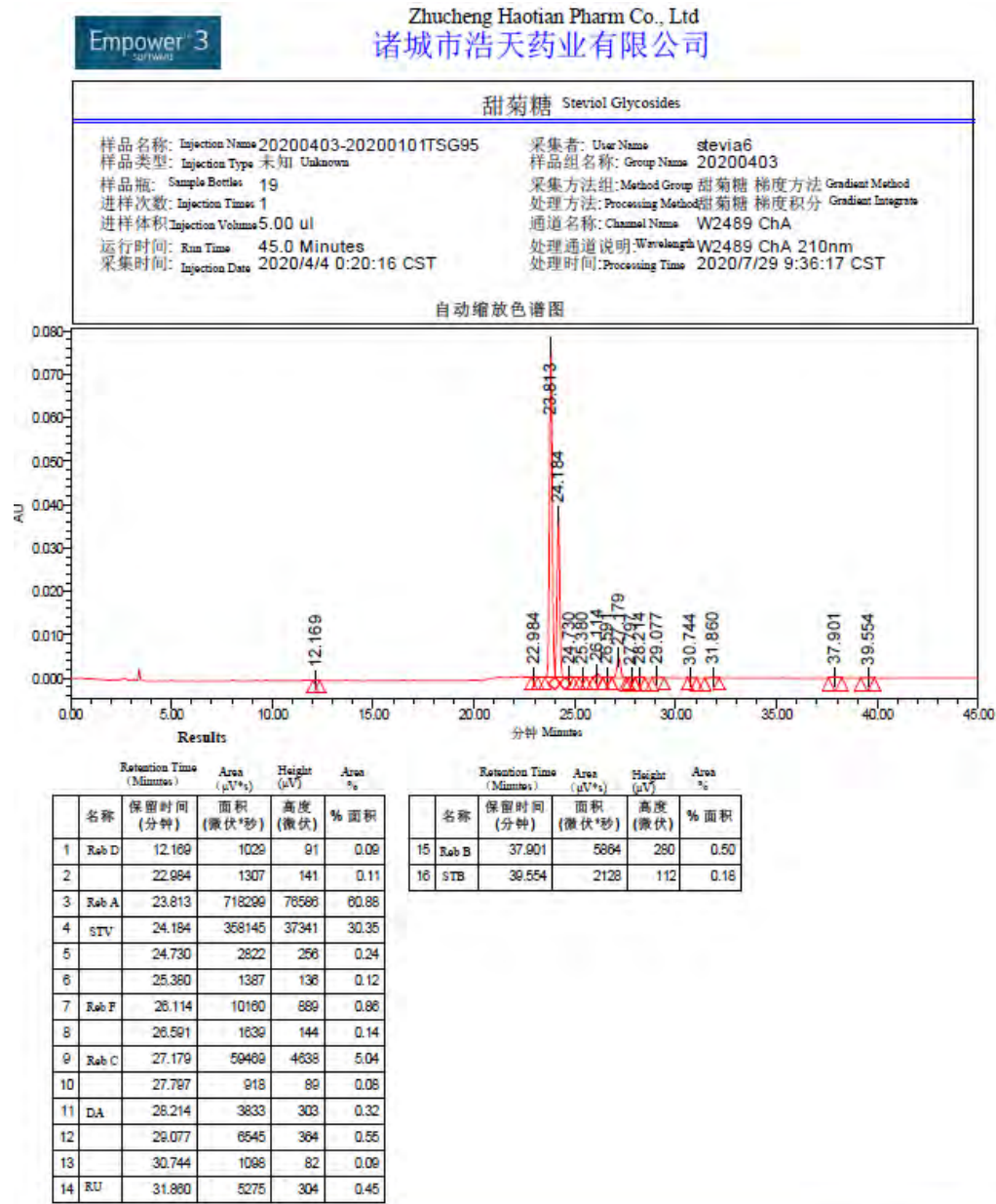
Appendix 3.1 Representative Chromatogram for SoPure Stevia™ Batch 20190106 TSG95



Appendix 3.2 Representative Chromatogram for SoPure Stevia™ Batch 20191101 TSG95



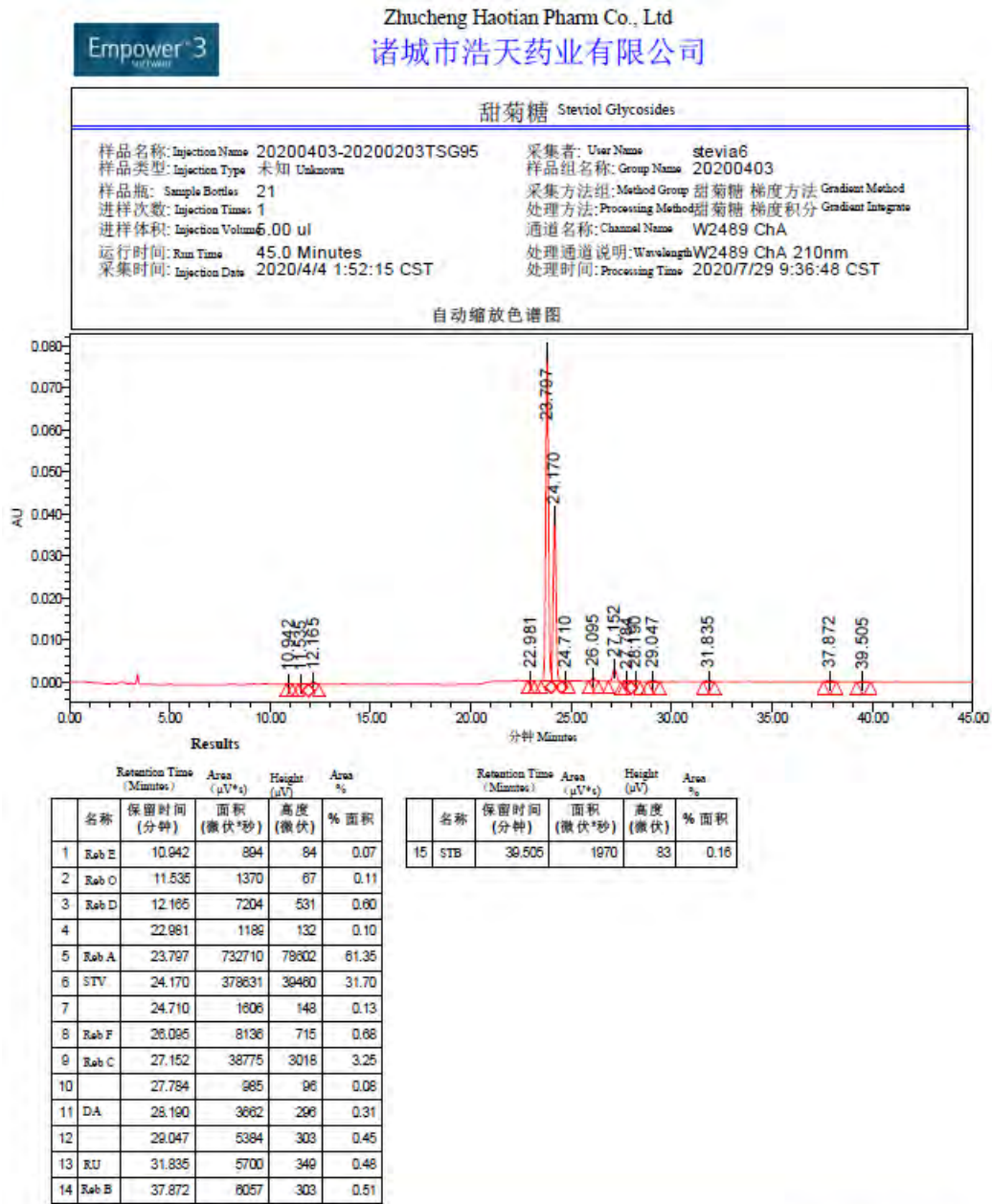
Appendix 3.3 Representative Chromatogram for SoPure Stevia™ Batch 20200101 TSG95



报告用户: 李晓庆 (stevia6)

页码: 1 (共计 1)

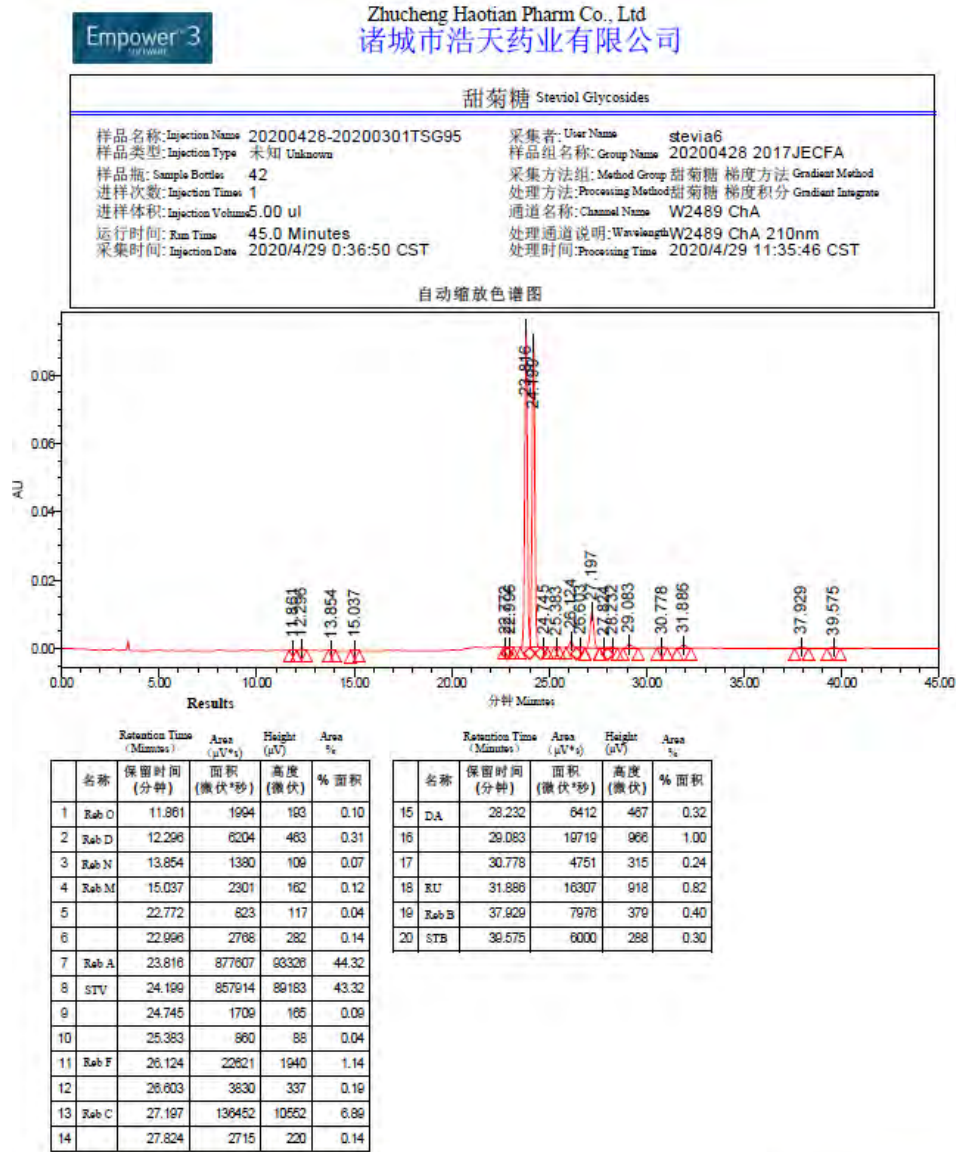
Appendix 3.4 Representative Chromatogram for SoPure Stevia™ Batch 20200203 TSG95



报告用户: 李晓庆 (stevia6)

页码: 1 (共计 1)

Appendix 3.5 Representative Chromatogram for SoPure Stevia™ Batch 20200301 TSG95



报告用户: 李晓庆 (stevia6)

页码: 1 (共计 1)

Appendix 4 SoPure Stevia™ Pesticide Testing Reports


Appendix 4.1 Representative Pesticide Testing Report for Stevia Leaf Raw Material

Appendix 4.2 Representative Pesticide Testing Report for SoPure Stevia™



HCl, salt (0.01) → Hexachlorobenzene (HCB) (0.01) Methoxychlor (0.2) → Penfluron (0.01) Phentolam (0.01) → Proxymetone (0.01) → Propoxone (0.01) → Isoflurane (0.05) → Telatrololol (0.01)	Heptachlor (0.01) Iacloprate (0.05) Mirex (0.01) → Deltamethrin-methyl (0.01) Phoson (0.01) → Prohexidion (0.05) Pyrethrin (0.01) Terephonol (0.01) Tokofluor-methyl (0.01)	Heptachlor (sum) () Iocarbazine (0.01) Myclotolol (0.01) → Fenoxonazole (0.01) Phoson (sum) () Promethrin (0.01) → Quinaprinol (0.01) Tebuthrin (0.01) Telatrololol (0.01)	Heptachlor epoxide, cis- (0.01) Inderprate (0.01) Naprofenolol (0.01) → Permethrin (0.01) → Piperonyl butoxide (0.01) Propoxion (0.05) Quinone (0.01) Tebutolol (0.01) Triclorolol (0.01)	Heptachlor epoxide, trans- (0.01) → Isodiphenyl-methyl (0.01) Nibutyl-oxipropyl (0.01) Permethrin (0.01) Permethrin-methyl (0.01) Prophenol (0.01) Quinone (sum) () Tebutolol (0.01) → Trifluorolol (0.01)	Heptachlor (0.01) Lindane (gamma-HCH) (0.01) Permethrin (0.01) → Fenoxonazole (0.01) → Proxymetone (0.01) → 3-AT (0.2) → Terephonol (0.01) → Vitexololol (0.01)
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SIGNATURE



Kevin Fu
 Authorized Signatory

EXPLANATORY NOTE

LOQ: Limit of Quantification
 < LOQ: Below Limit of Quantification
 N/A means Not applicable

Sum compounds: results are calculated from the results of each quantified compound as set by regulation
 The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
 The analytical result herein is applicable for the sample(s) tested only.
 This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
 The result(s) is(are) only for internal use by the client and not for publicly available as evidence. Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
 The Eurofins General Terms and Conditions apply to this analytical report.
 For and on behalf of Eurofins Technology Service (Qingdao) Co., Ltd.

END OF REPORT

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Appendix 5 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 (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.

1. 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 consumption 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.

2. 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).

3. 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 90th 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.

4. 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 90th percentile 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.

5. 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).

6. 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.”

7. 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 6 Studies on Steviol Glycosides Preparations

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

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

1. *In vivo* and *In vitro* Studies

- 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).
- Various animal studies that show stevioside is not readily absorbed from the GI tract:
 - Rats - Wingard Jr. et al. (1980); Nakayama et al. (1986); Koyama et al. (2003b);
 - Hamsters - Hutapea et al. (1999);
 - Pigs - Geuns et al. (2003a); and
 - Chickens - Geuns et al. (2003b).
- *In vitro* metabolism studies show that 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).
- *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.
- Koyama et al. (2003b) showed steviol can be converted to various glucuronides.
- 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} or 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.
- 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).
- Since the individual steviol glycosides show similar pharmacokinetics in the rat 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 per 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 studies that investigated the acute toxicity of stevioside (96% pure) is presented in Table 6.1.

Table 6.1. Acute Toxicity of Stevioside (Purity 96%) Given Orally to Rodents

Species	Sex	LD ₅₀ (g/kg bw) (LD ₅₀) (LD ₅₀) (LD ₅₀) (LD ₅₀) (LD ₅₀)	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)

bw – body weight; Brahmachari et al. (2011)– gram; kg -- kilogram

No lethality was noted within 14 days after 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

- Akashi and Yokoyama (1975) dosed rats with up to 2,500 mg per kg bw per day of stevioside for 3 months and reported no adverse effects.
- Mitsuhashi (1976) added up to 7% stevioside to the diets of F344 rats for 3 months and report no adverse effects.
- Aze et al. (1990) added stevioside at 0, 0.31, 0.62, 1.25, 2.5, 5% to the diets of F344 rats for 13 weeks and reported no adverse effects. The apparent NOAEL was >5% dietary stevioside.
- The Awney et al. (2011) study revealed toxicity in rats dosed at 15 and 1,500 mg per kg stevioside, 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

- 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 A) 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.
- 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%).
- 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 body weight gain decreases in both sexes. Kidney weights were significantly lower in 5% stevioside males; ovary, kidney and brain weights were significantly increased in 5% stevioside females; and there were decreased survival rates in males receiving 5% stevioside. However, stevioside was not carcinogenic at any level. The apparent NOAEL was the dietary level of 2.5%.
- No treatment-related increase in tumor incidence was seen in any of these studies.

E. Reproductive & Developmental Toxicity Studies

- 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 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 (Yodyingyud and Bunyawong, 1991). The NOAEL was determined to be 2,500 mg per kg bw per day.
- 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.
- 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).

F. Genotoxicity Studies

The following key genotoxicity studies have been conducted on stevia extracts and stevioside and showed negative responses:

- Bacterial mutagenicity studies negative for mutagenic response:
 - Medon et al. (1982);
 - Pezzuto et al. (1985);
 - Suttajit et al. (1993);
 - Matsui et al. (1996); and
 - Klongpanichpak et al. (1997).
- 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); and
 - Sasaki et al. (2002)
- Mouse bone marrow/liver micronucleus studies negative for mutagenic response:
 - Oh et al. (1999)
- In two separate reviews by Carakostas et al. (2008) and Brusick (2008), 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. Cytotoxicity

Abolhasani et al. (2020) evaluated the *in vitro* cytotoxicity of stevioside on cancerous liver (HepG2), colon (HT29), and breast (MCF7) cells, as well as normal kidney cells (Hek293), compared to cisplatin. Stevioside was reported to display higher cell growth inhibition on the HepG2 cell line and was not observed to have high toxicity on the Hek293 normal cell line. The authors concluded that stevioside “showed less cytotoxic effects compared to cisplatin.”

H. Clinical Studies & Other Reports in Humans

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 on 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 6.2.

Table 6.2. 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 <i>S. rebaudiana</i> 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 <i>Stevia rebaudiana</i> increased glucose tolerance. The extract decreased plasma glucose levels during the test and after overnight fasting in all volunteers. No adverse effects were reported.
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 years) + 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 years) + 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 a ↓ 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, or 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.

Author/ Year	Substance Tested	Total Daily Dose	Population Characteristics	Study Design and Duration	Noted Effects Safety parameter Results
Gregersen et al. (2004)	Stevioside - 91% + 9% other stevia glycosides	1 g stevioside or 1 g starch	12 patients with type 2 diabetes total	Acute paired cross-over study, single dose study	18% ↓ in glucose concentrations: Systolic and diastolic blood pressure were unchanged. No adverse effects
Temme et al. (2004)	Stevioside 97%	750 mg/kg bw/day (288 mg/kg bw steviol)	4 male and 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 and Type 2 diabetics, 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 week) mg/kg bw/day	Patients with mild hypertension	Randomized 24 week study	No changes in systolic BP, diastolic BP. No adverse effects
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) Age: 20-70 years	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

ALT – alanine aminotransferase; AST – aspartate aminotransferase; BP – blood pressure; bw – body weight; Brahmachari et al. (2011)– gram; GGT – gamma-glutamyltransferase; h – hour; HbA1c – glycated hemoglobin; kg – kilogram; mg – milligram

I. Other Studies

- 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.
 - 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.

- Treatment with the steviol glycosides tested over 24 hours resulted in minor increases in CYP3A29 mRNA expression (< 2.0-fold), while “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*.
- Zhao et al. (2020) studied the effect and mechanism of stevioside on preventive and therapeutic cardiac fibrosis caused by hyperglycemia in male C57BL/6 mice.
 - Stevioside supplementation reduced the expression of the cardiac fibrosis producing lysyl oxidase family (LOX) and weakened the collagen cross-linking lysyl oxidase-like 2 (LOXL2) caused by hyperglycemia.
 - Stevioside supplementation promoted the elimination of existing fibrosis via the regulation of matrix metalloproteinase (MMP 2/9) and tissue inhibitors of metalloproteinase (TIMP2/4).
 - No adverse effects were reported.

Part 2. Preparations That Are Primarily Rebaudioside A

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

- Slotter (2008a) examined the potential of rebaudioside A toxicity in rats at up to 2,000 mg per kg bw per day.
 - Low levels of rebaudioside A were detected in the peripheral blood of rats post-administration of 2,000 mg per kg bw per day.
 - Estimates of absorbed dose for rebaudioside A of 0.6 µg per mL in plasma (corresponding to 0.02%) were based on amounts measured in urine collected over 24 hours in comparison to the daily administered dietary dose.
 - Mean fecal rebaudioside A and measured hydrolysis products, expressed as Total Rebaudioside A Equivalents, compared with daily administered dose results in an estimated dose recovery of approximately 84%.
- Zhou et al. (2019) investigated the interaction of organic anion transporter 3 (OAT3)-mediated uptake of the rebaudioside A metabolite, steviol acyl glucuronide, with selected prescription drugs.
 - The inhibitory potency of therapeutic drugs (those frequently prescribed for treating hyperglycemia, hyperlipidemia, and hyperuricemia, including probenecid and glimepiride) was examined against human renal excretor - organic anion transporter 3 (hOAT3) and rat organic anion transporter 3 (rOAT3) for uptake of steviol acyl glucuronide.

- OAT3-mediated uptake of steviol acyl glucuronide was examined *in vitro* using hOAT3 and rOAT3 transfected human embryonic kidney 293 (HEK203) cells. Both probenecid and glimepiride were potent inhibitors of hOAT3 and rOAT3, with no apparent species differences observed.
- Pharmacokinetic studies in male Sprague Dawley rats revealed both probenecid and glimepiride significantly elevated plasma steviol acyl glucuronide concentrations, particularly between 6 and 8 hours after oral administration of rebaudioside A.
- The inhibition of OAT3 is a potential mechanism for the interaction between steviol acyl glucuronide and probenecid or glimepiride, which can alter pharmacokinetic and safety profiles of steviol acyl glucuronide and steviol glycosides—specifically rebaudioside A.
- The authors conclude that this interaction might be clinically relevant, and that care should be given to populations with concomitant use of stevia leaf extracts and probenecid or glimepiride.

B. 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 for males and 11,728 mg per kg for 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 $\geq 2,000$ 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 rebaudioside 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).

C. 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); and
 - Rumelhard et al. (2016).
- Mouse lymphoma (L5178Y/TK+/) studies negative for mutagenic response:
 - Clarke (2006); and
 - Williams and Burdock (2009).
- Human lymphocyte study negative for mutagenic response: Rumelhard et al. (2016)
- Chromosome aberration studies negative for mutagenic response:
 - Chinese hamster lung fibroblasts - Nakajima (2000a); and
 - Human lymphocytes - Williams and Burdock (2009).
- Mouse micronucleus studies negative for mutagenic response:
 - Nakajima (2000b) (BDF1 mouse bone marrow);
 - Krsmanovic and Huston (2006);
 - Williams and Burdock (2009); and
 - 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)

D. Reproductive & Developmental Studies on Rebaudioside A

- Curry et al. (2008) conducted a two-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 F₀ or F₁ 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 F₁ generation following weaning (beginning on postnatal day 21). The F₀ and F₁ 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.
- Cho et al. (2018) investigated the impact of stevia and obesity on fertility and reproductive outcomes in Sprague Dawley rats. Rats were administered 2-3 mg per kg bw per day rebaudioside A in drinking water starting two weeks prior to mating and throughout lactation. The authors reported that obese rats supplemented with rebaudioside A displayed a lower fertility index than untreated obese rats (53.3% vs. 85.7%, respectively); however, the rate of successful pregnancies was higher in obese rats supplemented with rebaudioside A than untreated obese rats (100% vs. 60.7%). No adverse effects or animal deaths were reported.
- Nettleton et al. (2020) investigated the impact of maternal low-dose rebaudioside A consumption on adiposity, glucose tolerance, gut microbiota, and the mesolimbic pathway in obese dams and their offspring. Pregnant obese rats and their offspring were fed high fat/sucrose diet plus 3 mg per kg bw per day rebaudioside A (Sigma-Aldrich) through 18 weeks postpartum. The authors noted that rebaudioside A consumption reduced the fertility of dams. The study supports findings that low-calorie sweeteners may not be metabolically inert.

E. Clinical Studies on Rebaudioside A

A summary of the clinical studies conducted on rebaudioside A is presented in Table 6.3.

Table 6.3. 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 (n=100) Age: 18-73 years	Primarily female 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 <i>Stevia rebaudiana</i> 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 years	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.

DBP – diastolic blood pressure; mg – milligram; SBP – systolic blood pressure

F. Safety of Rebaudioside A

There have been a 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 safe as long as there is a combined purity of not less than 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 minimal 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 in 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 lower 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 3 *in vitro* and 2 *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; and
 - 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 Other Steviol Glycosides Preparations

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

- 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.
- Purkayastha et al. (2014) compared the anaerobic *in vitro* metabolism of rebaudiosides A, B, D, and M with human fecal homogenates.
 - The rebaudiosides were hydrolyzed to steviol within 24 hours, with the majority of metabolism occurring within the first 8 hours.
 - Metabolism of rebaudiosides took longer at higher concentrations (2.0 mg per mL vs. 0.2 mg per mL).
 - There were no marked differences in rate or extent of hydrolysis observed between male and female fecal homogenates or individual rebaudiosides.
- Purkayastha et al. (2016) investigated the metabolic fate of steviolbioside, dulcoside A, and rebaudiosides A, B, C, D, E, F, and M in an *in vitro* study using pooled human fecal homogenates over the course of 24 to 48 hours.
 - The glycosidic side chains ---containing glucose, rhamnase, xylose, fructose, and those with deoxy-glucose including combinations of $\alpha(1-2)$, $\beta-1$, $\beta(1-2)$, $\beta(1-3)$, and $\beta(1-6)$ linkages ---were mostly degraded to steviol within 24 hours.
 - The rate of metabolism was slower at higher concentrations (2.0 mg per mL vs. 0.2 mg per mL).
 - No appreciable differences in metabolism were observed between fecal homogenates obtained from males and females or those obtained from different ethnicities.

- Purkayastha and Kwok (2020) investigated the *in vitro* metabolic fate of steviol glycosides in fecal homogenates collected from adults and children.
 - Steviol glycosides obtained from stevia leaf extract (composed of more than 20 steviol glycosides, with Reb D and Reb M as the principal components), bioconversion reaction product (composed of Reb D and Reb M), minor steviol glycosides extracted from a stevia leaf extract (composed of Reb AM, Reb W2, Reb U2, Reb V, Reb N, and Reb O), enzyme modified steviol glycosides, and rebaudioside A standard were used as test samples.
 - All steviol glycosides preparations tested “shared qualitatively similar *in vitro* metabolic fates.”
 - The authors concluded that “safety data for individual steviol glycosides can be used to support safety of all steviol glycosides produced by extraction and enzymatic conversion of stevia leaf extract.”

B. Toxicity Studies

- One study showed a toxic response and was conducted by Nunes et al. (2007a). In the Nunes study, rats were dosed with 4 mg per mL steviol glycosides in drinking water (estimated 80 to 500 mg per kg bw per day) for 45 days. Positive findings were reported in the liver, brain, and spleen, but most notably the liver. This study is considered to be an outlier in critical reviews conducted by Geuns (2007), Williams (2007), and Brusick (2008). The authors responded to these critiques (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.
- Silva et al. (2018) addressed the genotoxic activity of stevia (Svetia™, purity not reported¹³).
 - 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.
 - 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 of 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.

¹³ 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) indicates that the trademarked material is a blend of cane sugar and 97% pure rebaudioside A.

C. Other Studies

- Sánchez-Delgado et al. (2019) studied the effects of steviol glycosides in a seven-week study on healthy young adults aged 18-30 years old. Thirty-eight patients were assigned to one of three study groups and “washed out” for one week prior to study initiation.
 - For six weeks, study participants were administered one of the following dosage regimes:
 - Sucrose (8 X 5 g packets per day)
 - Sucralose (8 X 5 g packets per day)
 - Steviol glycosides (4 X 1 g packets per day)
 - Note: the authors did not indicate if any bulking agents were present in the packets.
 - Results were as follows:
 - Subjects in the sucrose treatment group showed increased triglycerides and cholesterol.
 - Subjects in the sucralose treatment group showed increased body weight.
 - Subjects in the steviol glycosides treatment group show decreased fat mass, decreased triglycerides, and decreased tumor necrosis factor- α .
 - The authors concluded that steviol glycosides may have positive effects on metabolic parameters.
- Halasa et al. (2020) published a case study vignette on the investigation of the presence of steviol glycosides metabolites in plasma, cerebrospinal fluid, amniotic fluid, and cord blood samples from as early as 2004. The end date was not provided.
 - Steviol glucuronide was detected primarily in the plasma.
 - Seven of the 38 adults (18%) had detectable steviol glucuronide concentrations, while two of 13 (15%) amniotic fluid samples and one of 15 (7%) cord blood samples were observed to contain steviol glucuronide.

Part 4. Studies on Crude Stevia Extracts

In several studies, pharmacological and biochemical effects of crude extracts of stevia leaves and purified steviol glycosides have been investigated.

- 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, and 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).
- 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.

- Wang and Wu (2019) studied the angiotensin-converting enzyme (ACE) inhibiting activity of an ethanolic extract of stevia leaves and purified steviol glycosides from the ethanol extract.
 - Steviol glycosides were reported to have double the ACE inhibitory activity of the ethanolic extract from stevia leaves.
 - Sensory tests in decaffeinated coffee, decaffeinated tea, and peanut protein beverages prepared with steviol glycosides demonstrated the preparations were well-accepted.
 - Steviol glycosides had a significant antihypertensive effect in spontaneously hypertensive rats. The authors suggest that the effect was dosage-dependent.
 - No adverse effects were reported.
- Assi et al. (2020) studied the efficacy of stevia extract alone and in combination with the commonly used sulfonyleurea, glimepiride, in a trial to introduce a new effective therapeutic regimen for type 2 diabetes mellitus.
 - Rats with type 2 diabetes were treated orally with 300 mg per kg per day stevia extract for 21 days.
 - Results indicated that treatment with stevia extract showed good control of blood glucose levels and that a significant elevation in insulin release to glimepiride was observed.
 - The authors reported that stevia extract reduced blood glucose, triglycerides, cholesterol, ALT, AST, urea, creatinine, tumor necrosis factor, and malondialdehyde levels, while improving insulin and adiponectin levels.
 - No adverse effects were reported.
- Ray et al. (2020) studied the effects of *Stevia rebaudiana* on glucose homeostasis, blood pressure, and inflammation.
 - No hypersensitivities or allergies were reported since 2008, and that the few prior reports were for “improperly filtered stevia extracts.”
 - No significant adverse effects were noted from any study included in the review.

Part 5. Studies on Principal Metabolite: Steviol

A. Acute Toxicity Studies

- Toskulkac et al. (1997) administered single doses of steviol (90%) to rats and hamsters:
 - Rat, oral LD₅₀ >15 g per kg; and
 - Hamster, oral LD₅₀ 5.2 g per kg bw in males and 6.1 g per kg bw in females.
 - Histopathological examination of the kidneys of hamsters 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) treated groups of pregnant golden hamsters with steviol (90%) at doses of 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:
 - Compadre et al. (1988);
 - Procinska et al. (1991); and
 - Klongpanichpak et al. (1997).
- 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:
 - Matsui et al. (1996) (mouse);
 - Temcharoen et al. (2000) (rat);
 - Temcharoen et al. (2000) (mouse); and
 - 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:
 - 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.

- 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.
- 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.
- Terai et al. (2002) – Steviol was found to be mutagenic in Aroclor-induced rat liver S9 fraction.
- 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, and
 - 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.

E. Other Studies

- Kurek et al. (2020) reported on the effect of steviol on cytotoxicity, adipogenesis, ROS concentration, and gene expression in the murine 3T3-L1 cell line.
 - There was no observed effect on the proliferation of cells, lipid accumulation, or intracellular ROS generation at steviol concentrations up to 100 μ M.
 - Furthermore, it was reported that steviol reduced the expression of genes regulating the adipogenesis and lipogenesis process.

Appendix 7 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).
- 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 use as a natural health product is permitted 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 were 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 Enzyme Modified Steviol Glycosides

1. U.S. Regulatory History

As of August 19, 2020, FDA has issued 64 “no questions” letters on GRAS Notices on rebaudioside A, rebaudioside D, rebaudioside M, or steviol glycosides, including those undergoing enzyme treatment (FDA, 2020).

In addition, the Flavor and Extract Manufacturers Association (FEMA) has included several steviol glycosides preparations that are used to formulate flavors on their GRAS lists as shown in Table 7.1.

Table 7.1. 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, <i>Stevia rebaudiana</i> , Rebaudioside A 60%	4771	Marnett et al. (2013)
Steviol glycoside extract, <i>Stevia rebaudiana</i> , Rebaudioside A 80%	4772	Marnett et al. (2013)
Steviol glycoside extract, <i>Stevia rebaudiana</i> , Rebaudioside C 30%	4796	Cohen et al. (2015a); Cohen et al. (2015b)
Steviol glycoside extract, <i>Stevia rebaudiana</i> , Rebaudioside A 22%	4805	Cohen et al. (2015a); Cohen et al. (2015b)
Steviol glycoside extract, <i>Stevia rebaudiana</i> 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	4895	Cohen et al. (2020)
Glucosylated steviol glycosides, 70-80%	4909	Cohen et al. (2020)
Glucosylated steviol glycosides, 40%	4910	Cohen et al. (2020)
Stevia extract stevioside, 70%	4911	Cohen et al. (2020)
Rebaudioside D 95%	4921	Cohen et al. (2020)
Rebaudioside M 95%	4922	Cohen et al. (2020)
Glucosylated steviol glycosides, 90%	4931	Cohen et al. (2020)
Rebaudioside E ≥85%	4936	Cohen et al. (2020)
Rebaudioside I 95%	4937	Cohen et al. (2020)

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, 2019c).
- 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, 2019b).
- Most recently, on June 27, 2019, Health Canada’s Food Directorate modified the List of Permitted Sweeteners to allow for the use of steviol glycosides from various sources in “standardized flavoured milks” (Health Canada, 2019a).

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 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 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) and 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 (95th 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).
- 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 2009, France published its approval for the food uses of rebaudioside A with a purity of 97% (AFSSA, 2009a; AFSSA, 2009b).
- 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 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 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% rebaudioside A or stevioside.
- 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).
- On September 24, 2019, the EFSA Panel on Food Additives and Flavourings concluded that there is no safety concern for rebaudioside M produced via enzymatic bioconversion and recommended that the European Commission consider establishing specifications for the preparation (EFSA, 2019).
- On March 24, 2020, EFSA published a scientific opinion in response to a proposed amendment of the specifications for steviol glycosides, stating that all steviol glycosides share the same metabolic fate, and therefore the safety of 60 steviol glycosides identified in the leaves of *Stevia rebaudiana* Bertoni can be based on “read-across” from previously evaluated toxicological data. EFSA maintained that the ADI of 4 mg per kg bw applies to all 60 steviol glycosides. The EFSA Panel noted that the inclusion of more steviol glycosides, “whilst maintaining the assay value of not less than 95%, would allow less pure preparations” onto the market. The Panel stated that they “cannot conclude on the safety of the proposed amendment to the specifications of steviol glycosides (E 960) as [a] food additive if the purity assay value of not less than 95% for the total content of steviol glycosides is maintained.” Furthermore, the Panel noted that it is possible to manufacture steviol glycosides with a purity higher than 95% total steviol glycosides (EFSA, 2020).

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 rebaudioside M does not pose any “public health and safety issues” (FSANZ, 2015).
- On January 14, 2016, rebaudioside M was approved for use “as a food additive in accordance with the current permissions for steviol glycosides” (FSANZ, 2016).
- 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, 2016).
- 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 rebaudioside 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).

- On May 14, 2020, FSANZ published an approval report for a draft variation to amend the specification for steviol glycosides from *Stevia rebaudiana* Bertoni in section S3—35 of the Australia New Zealand Food Standards Code to include rebaudioside E produced by enzymatic conversion from stevia leaf extract. The approved draft variation allows for the use of high purity rebaudioside E ($\geq 85\%$ rebaudioside E; $\geq 95\%$ total steviol glycosides) within the existing permissions and limits for steviol glycosides (FSANZ, 2020a). Subsequently, on July 28, 2020, Amendment No. 193 was published to include rebaudioside E produced by enzymatic conversion from stevia leaf extract (FSANZ, 2020b).

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).

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Appendix 8 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 of 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 to 200 mg per kg and in plain soy beverages 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, 2016).

- 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 95th 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 95th percentile for children ranged from 1.0 to 12.7 mg per kg bw per day.
 - For adults, the mean and 97.5th 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.
- In 2015, Urban et al. 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.
- The effects of non-nutritive low-calorie sweeteners on gut microbiota were reviewed by Plaza-Diaz et al. (2020). It was noted that there have been no reports of negative interactions between steviol glycosides and colonic microbiota; however, it is possible that steviol glycosides modify the gut microbiota. The authors note that further studies are necessary to “clarify its specific effects.”
- A recent review by Ray et al. (2020) focused on the effects of *Stevia rebaudiana* on glucose homeostasis, blood pressure, and inflammation. The authors reported that no hypersensitivities or allergies were reported since 2008, and that the few prior reports were for “improperly filtered stevia extracts.” Furthermore, Ray et al. notes that additional randomized controlled trials are needed to confirm the beneficial effects of stevia. No significant adverse effects were noted from any study included in the review.

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Appendix 9 GRAS Associates Expert Panel Report



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THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF THE PROPOSED USES OF ZHUCHENG HAOTIAN PHARM CO., LTD'S SOPURE STEVIA™

October 14, 2020

Foreword

An independent panel of experts ("Expert Panel") was convened by GRAS Associates, LLC on behalf of their client, Zhucheng Haotian Pharm Co., Ltd. ("ZCHT"), to evaluate the safety and Generally Recognized as Safe (GRAS) status of ZCHT's proposed uses of SoPure Stevia™ high purity steviol glycosides preparations ($\geq 95\%$ total steviol glycosides) 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 in Appendices 5-8, of ZCHT's SoPure Stevia™ high purity steviol glycosides preparations GRAS 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 SoPure Stevia™ high purity steviol glycosides preparations 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 349, GRN 367, GRN 493, GRN 619, GRN 702, GRN 733, GRN 790, GRN 795, GRN 838, and GRN 839.

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 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). Furthermore, Purkayastha et al. (2014; 2016) reported that rebaudioside B and rebaudioside A are metabolized to steviol in a similar, concentration-dependent manner in a pair of *in vitro* fecal homogenate studies. Most recently, Purkayastha and Kwok (2020) concluded that samples of stevia leaf extract, bioconverted steviol glycosides, a preparation of minor steviol glycosides, and enzyme modified steviol glycosides shared qualitatively similar *in vitro* metabolic fates as rebaudioside A in pooled human fecal homogenate samples, leading the authors to conclude that "safety data for individual steviol glycosides can be used to support safety of all steviol glycosides produced by extraction and enzymatic conversion of stevia leaf extract."

The Expert Panel reviewed the recent publications on steviol glycosides including those by Abolhasani et al. (2020), Afonso et al. (2020), Assi et al. (2020), Halasa et al. (2020), Kurek et al. (2020), Nettleton et al. (2020), Plaza-Diaz et al. (2020), Ray et al. (2020), and Zhao et al. (2020), and did not identify any that raise safety concerns with regard to the use of steviol glycosides in conventional foods.

The acceptable daily intake (ADI) for steviol glycosides has been set largely based on a published chronic study in rats (Toyoda et al., 1997) and several published 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 ZCHT's manufacturing process for their SoPure Stevia™ high purity steviol glycosides preparations is similar to the processes described for previous GRAS Notices for steviol glycosides materials.

The GRAS Associates Expert Panel convened on behalf of ZCHT has reviewed the proposed uses for SoPure Stevia™ high purity steviol glycosides preparations. The highest 90th percentile consumption by any populations subgroup of SoPure Stevia™, based upon the lowest relative sweetness intensity and greatest calculated steviol equivalence, was calculated to be approximately 6.60 mg per kg body weight (bw) per day, which is equivalent to 2.26 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, SoPure Stevia™ high purity steviol glycosides preparations are 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 in-depth review publications (Geuns, 2007; Williams, 2007; Brusick, 2008; Waddell, 2011; Carakostas, 2012; Urban et al., 2013) 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 ZCHT's SoPure Stevia™ high purity steviol glycosides (≥95% total steviol glycosides) preparations given the following conditions:

- ZCHT's SoPure Stevia™ high purity steviol glycosides preparations continue to meet the designated specifications;
- The minimum sweetness intensity for SoPure Stevia™ high purity steviol glycosides preparations remain unchanged; and
- SoPure Stevia™ high purity steviol glycosides preparations are produced in accordance with Current Good Manufacturing Practices (CGMPs).

Conclusion

The Expert Panel critically reviewed the data provided by ZCHT for their SoPure Stevia™ high purity steviol glycosides preparations, 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 ZCHT's SoPure Stevia™ high purity steviol glycosides 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 ZCHT's SoPure Stevia™ high purity steviol glycosides preparations, manufactured as described in Part 2.b. of ZCHT's GRAS 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 ZCHT's SoPure Stevia™ high purity steviol glycosides preparations are generally recognized as safe (GRAS).

A large grey rectangular box redacts the signatures of the three panel members.

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

Kara Lewis, Ph.D.

Katrina V. Emmel, Ph.D.
Panel Chair



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- Zhao, R., Wang, J., Qin, L., Zhang, X. and Mei, Y. (2020) 'Stevioside improved hyperglycemia-induced cardiac dysfunction by attenuating the development of fibrosis and promoting the degradation of established fibrosis', *Journal of Functional Foods*, 68, pp. 103895.

END

END

FDA USE ONLY

GRN NUMBER 000983	DATE OF RECEIPT Nov 30, 2020
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration
**GENERALLY RECOGNIZED AS SAFE
(GRAS) NOTICE**

Transmit completed form and attachments electronically via the Electronic Submission Gateway (*see Instructions*); OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety (*HFS-200*), Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740-3835.

PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission (*Check one*)
 New Amendment to GRN No. _____ Supplement to GRN No. _____

2. All electronic files included in this submission have been checked and found to be virus free. (*Check box to verify*)

3a. For New Submissions Only: Most recent presubmission meeting (*if any*) with FDA on the subject substance (*yyyy/mm/dd*): N/A

3b. For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? (*Check one*)
 Yes If yes, enter the date of communication (*yyyy/mm/dd*): _____
 No

PART II – INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person Hank Wang	Position Technical Director	
	Company (<i>if applicable</i>) Nascent Health Sciences (US Division of ZCHT)		
	Mailing Address (<i>number and street</i>) 33 Wood Ave., Suite 600		
City Iselin	State or Province New Jersey	Zip Code/Postal Code 08830	Country United States of America
Telephone Number 845-418-4456	Fax Number n/a	E-Mail Address hank@nascent-health.com	
1b. Agent or Attorney (if applicable)	Name of Contact Person William J. Rowe	Position President	
	Company (<i>if applicable</i>) GRAS Associates		
	Mailing Address (<i>number and street</i>) 11810 Grand Park Ave., Suite 500		
City North Bethesda	State or Province Maryland	Zip Code/Postal Code 20852	Country United States of America
Telephone Number 519-341-3367	Fax Number 888-531-3466	E-Mail Address wrowe@nutrasource.ca	

PART III – GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

SoPure Stevia™ High Purity Steviol Glycosides

2. Submission Format: (Check appropriate box(es))

- Electronic Submission Gateway Electronic files on physical media with paper signature page
- Paper
- If applicable give number and type of physical media _____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in FDA's files by reference? (Check one)

- Yes (Proceed to Item 5) No (Proceed to Item 6)

5. The submission incorporates by reference information from a previous submission to FDA as indicated below (Check all that apply)

- a) GRAS Notice No. GRN _____
- b) GRAS Affirmation Petition No. GRP _____
- c) Food Additive Petition No. FAP _____
- d) Food Master File No. FMF _____
- e) Other or Additional (describe or enter information as above) _____

6. Statutory basis for determination of GRAS status (Check one)

- Scientific Procedures (21 CFR 170.30(b)) Experience based on common use in food (21 CFR 170.30(c))

7. Does the submission (including information that you are incorporating by reference) contain information that you view as trade secret or as confidential commercial or financial information?

- Yes (Proceed to Item 8)
- No (Proceed to Part IV)

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information (Check all that apply)

- Yes, see attached Designation of Confidential Information
- Yes, information is designated at the place where it occurs in the submission
- No

9. Have you attached a redacted copy of some or all of the submission? (Check one)

- Yes, a redacted copy of the complete submission
- Yes, a redacted copy of part(s) of the submission
- No

PART IV – INTENDED USE

1. Describe the intended use of the notified substance including the foods in which the substance will be used, the levels of use in such foods, the purpose for which the substance will be used, and any special population that will consume the substance (e.g., when a substance would be an ingredient in infant formula, identify infants as a special population).

Intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into foods in general, other than infant formulas and meat and poultry products, at per serving levels reflecting good manufacturing practices and principles, in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

2. Does the intended use of the notified substance include any use in meat, meat food product, poultry product, or egg product? (Check one)

- Yes No

PART V – IDENTITY

1. Information about the Identity of the Substance

	Name of Substance¹	Registry Used (CAS, EC)	Registry No.²	Biological Source (if applicable)	Substance Category (FOR FDA USE ONLY)
1	High purity steviol glycosides	N/A	N/A	<i>Stevia rebaudiana</i> leaf extract	
2					
3					

¹ Include chemical name or common name. Put synonyms (*whether chemical name, other scientific name, or common name*) for each respective item (1 - 3) in Item 3 of Part V (*synonyms*)

² Registry used e.g., CAS (*Chemical Abstracts Service*) and EC (*Refers to Enzyme Commission of the International Union of Biochemistry (IUB), now carried out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB)*)

2. Description

Provide additional information to identify the notified substance(s), which may include chemical formula(s), empirical formula(s), structural formula(s), quantitative composition, characteristic properties (*such as molecular weight(s)*), and general composition of the substance. For substances from biological sources, you should include scientific information sufficient to identify the source (*e.g., genus, species, variety, strain, part of a plant source (such as roots or leaves), and organ or tissue of an animal source*), and include any known toxicants that could be in the source.

SoPure Stevia™ High Purity Steviol Glycosides preparations are prepared from steviol glycosides extracts prepared from *Stevia rebaudiana* leaves that are further purified to yield the desired >95% total steviol glycosides finished products. SoPure Stevia™ is composed of rubusoside, steviolbioside, dulcoside A, rebaudioside B, stevioside, rebaudioside C, rebaudioside F, rebaudioside A, and rebaudioside D.

There are no known toxicants.

3. Synonyms

Provide as available or relevant:

1	High purity steviol glycosides
2	Stevia extract
3	

PART VI – OTHER ELEMENTS IN YOUR GRAS NOTICE
(check list to help ensure your submission is complete – check all that apply)

- Any additional information about identity not covered in Part V of this form
- Method of Manufacture
- Specifications for food-grade material
- Information about dietary exposure
- Information about any self-limiting levels of use (which may include a statement that the intended use of the notified substance is not-self-limiting)
- Use in food before 1958 (which may include a statement that there is no information about use of the notified substance in food prior to 1958)
- Comprehensive discussion of the basis for the determination of GRAS status
- Bibliography

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

Yes No

Did you include this other information in the list of attachments?

Yes No

PART VII – SIGNATURE

1. The undersigned is informing FDA that Zhucheng Haotian Pharm Co., Ltd
(name of notifier)
has concluded that the intended use(s) of SoPure Stevia™ High Purity Steviol Glycosides
(name of notified substance)
described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

2. Zhucheng Haotian Pharm Co., Ltd (name of notifier) agrees to make the data and information that are the basis for the determination of GRAS status available to FDA if FDA asks to see them.

Zhucheng Haotian Pharm Co., Ltd (name of notifier) agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so.

Xinxing, Zhucheng, Shandong Province, 262218, The People's Republic of China
(address of notifier or other location)

Zhucheng Haotian Pharm Co., Ltd (name of notifier) agrees to send these data and information to FDA if FDA asks to do so.

OR

The complete record that supports the determination of GRAS status is available to FDA in the submitted notice and in GRP No.

(GRAS Affirmation Petition No.)

**3. Signature of Responsible Official,
Agent, or Attorney**

Katrina Emmel

Digitally signed by Katrina Emmel
Date: 2020.10.19 10:19:15 -07'00'

Printed Name and Title

Katrina Emmel on behalf of William J. Rowe, President

Date (mm/dd/yyyy)

10/19/2020

PART VIII – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Appendices 1-9 in the body of the dossier	

OMB Statement: Public reporting burden for this collection of information is estimated to average XX hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, 1350 Piccard Drive, Room 400, Rockville, MD 20850. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.