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January 13, 2010

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Division of
Biotechnology and
GRAS Notice Review

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Robert L. Martin

Re: GRAS Notification – High Purity Rebaudioside A (95%-98%)

Dear Dr. Martin:

On behalf of Pyure Brands, LLC of Naples, FL, we are submitting for FDA review a GRAS notification for High Purity Rebaudioside A (95%-98%). 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,

(b) (6)

Robert S. McQuate, Ph.D.
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Enclosure: GRAS Notification – High Purity Rebaudioside A (95%-98%) (in triplicate)

GRAS ASSOCIATES, LLC

GRAS ASSESSMENT

HIGH PURITY REBAUDIOSIDE A (95% - 98%)

Food Usage Conditions for General Recognition of Safety

For

Pyure Brands, LLC
4532 Tamiami Trail E.
Suite #303
Naples, FL 34112 USA

Evaluation by

Richard C. Kraska, Ph.D., DABT
Robert S. McQuate, Ph.D.

January 13, 2010

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

High Purity Rebaudioside A having purity falling in the range of 95% to 98%, which meets the specifications for Pyure Brands, LLC Reb A products as described below, has been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination, made by experts qualified by scientific training and experience, is based on scientific procedures as described in the following sections. The evaluation accurately reflects the conditions of the stevia-derived sweeteners' intended uses in foods.

Signed:

(b) (6)

January 13, 2010
Date

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074

B. Name & Address of Notifier

Pyure Brands, LLC
4532 Tamiami Trail E., Suite #303
Naples, FL 34112

As the notifier, Pyure Brands, LLC accepts responsibility for the GRAS determination that has been made for High Purity Rebaudioside A compositions[2] as described in the subject notification; consequently, these Rebaudioside A preparations, i.e., having purities of no less than 95% and as high as 98% rebaudioside A, meeting the conditions described herein are exempt from pre-market approval requirements for food ingredients.

---

[1] See 62 FR 18938 (17 April 1997) which is accessible at http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm.

[2] Pyure Brands refers to its High Purity Rebaudioside A products with the trade name of Pyure Elite.

C. Common Name & Identity of the Notified Substance

High Purity Rebaudioside A, routinely shortened to reb A or Reb A, is the common name for the notified substance; also see Section III.A.

D. Conditions of Intended Uses in Food

The subject High Purity Rebaudioside A preparations are intended to be added as a general purpose non-nutritive sweetener into various food categories other than infant formulas and meat and poultry products at per serving levels that reflect good manufacturing practices principles in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, Pyure Brands High Purity Rebaudioside A products have been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074.

II. INTRODUCTION

A. Objective

At the request of Pyure Brands, LLC ("Pyure Brands"), GRAS Associates, LLC ("GA") has undertaken an independent safety evaluation of Pyure Brands' High Purity Rebaudioside A (Reb A) preparations with Reb A purity of 95% - 98%. The purpose of the evaluation is to ascertain whether or not the intended food uses of the subject Reb A as a non-nutritive general purpose sweetener are generally recognized as safe, i.e., GRAS, when incorporated into various food categories other than infant formulas and meat and poultry products.

B. Foreword

Pyure Brands provided GA with background information needed to enable the GRAS assessment to be undertaken. In particular, the information provided addressed the safety/toxicity of steviol glycosides; the history of use of stevia in food; and compositional details, specifications, and method of preparation of its high purity rebaudioside A. The notifier was asked to provide adverse reports, as well as those that supported conclusions of safety.

Safety/toxicity studies performed with animals were noted to have value, along with available human testing. Pyure Brands was also asked to supply past and present human food use information. Knowing how much steviol glycosides has been safely consumed, i.e., the so-called "dose" or use levels, is critical in extrapolating to safe exposures for rebaudioside A when consumed as a food ingredient. The composite safety/toxicity studies, in concert with exposure information, ultimately provide the specific scientific foundation for the GRAS determination.

Pyure Brands supplied the product specifications and chemical properties and some consumption/exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through December 2009. A GRAS assessment based on the composite safety information, that is, based on scientific procedures, was undertaken. Those references that were deemed pertinent to the objective at hand are listed in Section VIII.

C. Summary of Regulatory History of Stevia

Stevia-derived sweeteners are permitted as a food additive in South America and in several countries in Asia, including China, Japan, and Korea. As discussed more fully below, over the past year, the subject sweeteners have received food usage approvals in Australia, New Zealand, Switzerland, and Mexico. The US FDA has issued six "no objection letters" in response to the GRAS notifications filed on behalf of Reb A and steviol glycosides food uses.

In the US, steviol glycosides have been used as a dietary supplement since 1995 (Geuns, 2003). No application for dietary supplement use of purified rebaudioside A is known to have been

made. At least two GRAS petitions seeking authorization for the addition of stevioside or steviol glycosides to foods had been submitted to FDA since 1989, yet no authorizations had been issued by FDA in response to these filings, presumably because the previously available safety data---including purity considerations---for stevia, stevioside, or steviol glycosides were viewed as being inadequate. These petitions were subsequently withdrawn.

Individual GRAS notifications were submitted by Merisant and Cargill to FDA in May 2008 for rebaudioside A, both more highly purified forms of the steviol glycosides. FDA issued "no objection" letters for each of these GRAS notices on December 17, 2008. McNeil Nutritionals, LLC submitted a GRAS notification to FDA in December, 2008 for its purified steviol glycosides with rebaudioside A consisting of the principal component. FDA issued a "no objection" letter to McNeil Nutritionals on June 11, 2009. Blue California submitted its GRAS notification for highly purified Reb A in January, 2009, and FDA issued a "no objection" letter to Blue California on July 20, 2009. GRAS notifications were also submitted to FDA by Sweet Green Fields, LLC and by Wisdom Natural Brands for their stevia-derived sweetener products, and each firm received a "no objection" letter from FDA in August, 2009.[3] As of September 24, 2009, two additional GRAS notifications---one addressing high purity steviol glycosides and the other focusing on high purity rebaudioside A---were submitted to FDA jointly by Sunwin and WILD Flavors.

The Food Standards Australia New Zealand (FSANZ) has completed evaluation of an application for use of steviol glycosides in foods and has recommended to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) to amend the Australia New Zealand Food Standards Code to allow its use in food (FSANZ, 2008).

Steviol glycosides have been under a lengthy review by the Joint Expert Committee on Food Additives ("JECFA"). The original review was published in 2000 (WHO, 2000). A draft monograph was reviewed at the 51st, 63rd and 68th JECFA meetings. A temporary ADI (acceptable daily intake) of 0-2 mg/kg (on a steviol basis) was established at the 63rd meeting (WHO, 2006). In addition, food grade specifications were made final by JECFA (FAO, 2007a), although they were subsequently updated in 2008 (FAO, 2008). At the 69th meeting, the temporary status of the ADI was removed and the ADI was raised to 0-4 mg/kg bw/day (on a steviol basis) as a result of the JECFA review of recently completed clinical studies with steviol glycosides (WHO, 2008). JECFA has published final monograph addendum on steviol glycosides (WHO, 2009).

In August 2008, Switzerland's Federal Office for Public Health cited the favorable actions of JECFA in issuing its approval for the use of stevia as a sweetener (Switzerland Office of Public Health, 2008).

In September 2009, France published its approval for the food uses of Reb A with a purity of 97% (AFSSA, 2009).

[3] GRAS notification 252 which was submitted by Merisant, GRAS notification 253 which was submitted by Cargill, GRAS notification 275 which was submitted by McNeil Nutritionals, GRAS notification 278 which was submitted by Blue California, GRAS notification 282 which was submitted by Sweet Green Fields, and GRAS notification 287 which was submitted by Wisdom Natural Brands are listed on FDA's website at http://www.accessdata.fda.gov/scripts/fc/fcnNavigation.cfm?rpt=grasListing, along with their respective "no objection" letters.

The stevia-derived sweeteners are not presently permitted as an ingredient in conventional food in the EU, UK, Hong Kong, or Canada (Hawke, 2003). This likely reflects a lack of review of new data on the sweeteners rather than a continuing concern about safety.

Hong Kong maintains that stevia is not permitted as a sweetener, as cited on the government website (Hong Kong Government, 2002). The Hong Kong Government was reported to be waiting for the JECFA determination on the safety of steviol glycosides. However, no further official actions have been noted since JECFA's final resolution was reported in June 2008.

On September 24, 1998 in the UK, the Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food rejected an application for use of steviol glycosides as a sweetener in herbal teas because "the applicant had not provided all of the information necessary to enable an assessment to be made."[4]

Other international bodies have investigated the safety aspects of stevia and steviol glycosides use in foods. In 1999 in the EU, the Scientific Committee on Food for the European Commission concluded that "there are no satisfactory data to support the safe use of these stevia plants and leaves," as reported in a five-page opinion dated June 17, 1999 (European Commission, 1999a). The Committee reiterated "its earlier opinion that stevioside is not acceptable as a sweetener on the presently available data," in a seven-page opinion also dated June 17, 1999 (European Commission, 1999b).

The European Food Safety Authority (EFSA, 2008) is reexamining the safety of steviol glycosides in light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission for EFSA to evaluate steviol glycosides (EFSA, 2008). EFSA's Food Additives & Nutrient Sources (ANS) Panel has been given a deadline of March 31, 2010 to answer this request. Furthermore, three petitions have been submitted to EFSA that seek authorization for steviol glycoside food additive use, and it is anticipated that positive opinions will be issued by EFSA in 2010 or 2011 (Nutraingredients, 2009).

D. FDA Regulatory Framework

Steviol glycosides (or stevioside) have been used in dietary supplements in the US since 1995 (Geuns, 2003) and is widely available to consumers in the US through retail outlets and Internet purchases (Al-Achi, 2000).

In accordance with FDA regulation of foods, however, dietary supplements cannot legally be added to conventional foods. Such ingredients must undergo premarket approval by FDA as food additives or, alternatively, the ingredients to be incorporated into conventional foods must be determined to be generally recognized as safe (GRAS). The authority to make GRAS determinations is not restricted to FDA. In fact, GRAS determinations may be provided by experts who are qualified by scientific training and experience to evaluate the safety of food and food ingredients under the intended conditions of use.[5]

[4] See http://www.maff.gov.uk/food/novel/980924.html.
[5] See 21 CFR 170.3(i)(3).

In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process. At that time, the petitioning process was replaced with a notification procedure.[6] While outlining the necessary content to be considered in making a GRAS determination, FDA encouraged that such determinations be provided to FDA in the form of a notification. However, notifying FDA of such determinations is strictly voluntary.

[6] See Footnote 1.

III. CHEMISTRY & MANUFACTURE OF REBAUDIOSIDE A (95% - 98%)

A. Common or Usual Name

Rebaudioside A, also referred to as Reb A or reb A, is one of the common steviol glycosides found in nature. The common or usual name for the products that are the subject of this notification is High Purity Rebaudioside A (Reb A), where the rebaudioside A content of the commercial production is no less than 95% and can be as high as 98%. Pyure Elite is the commercial name used by Pyure Brands in referring to the notified substance that is the subject of this GRAS evaluation.

Steviol glycosides have been referred to as stevia, stevioside, and stevia glycoside in the scientific literature. JECFA adopted the term, steviol glycosides, for the family of steviol derivatives with sweetness properties that are derived from the stevia plant. Presently, the term, stevia, is used more narrowly to describe the plant or crude extracts of the plant, while stevioside is the common name for another one of the specific glycosides that is extracted from stevia leaves.

B. Chemistry of Rebaudioside A

The following description is taken from the original JECFA monograph (WHO, 2000).

> Stevioside is a glycoside of the diterpene derivative steviol (ent-13-hydroxykaur-16-en-19-oic acid). Steviol glycosides are natural constituents of the plant *Stevia rebaudiana Bertoni*, belonging to the Compositae family. The leaves of *S. rebaudiana Bertoni* contain eight different steviol glycosides, the major constituent being stevioside (triglucosylated steviol), constituting about 5-10% in dry leaves. Other main constituents are rebaudioside A (tetraglucosylated steviol), rebaudioside C, and dulcoside A. *S. rebaudiana* is native to South America and has been used to sweeten beverages and food for several centuries. The plant has also been distributed to Southeast Asia. Stevioside has a sweetening potency 250-300 times that of sucrose and is stable to heat. In a 62-year-old sample from a herbarium, the intense sweetness of *S. rebaudiana* was conserved, indicating the stability of stevioside to drying, preservation, and storage (Soejarto et al., 1982; Hanson and De Oliveira, 1993).

The two predominant sweetener components of stevia extracts have been identified as stevioside and rebaudioside A. The chemical identities and key chemical identifiers for the two major components are shown below.

Stevioside

Chemical Name:	13-[2-Oβ-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
Chemical formula:	$C_{38}H_{60}O_{18}$
Formula Weight:	804.88
CAS Number:	57817-89-7

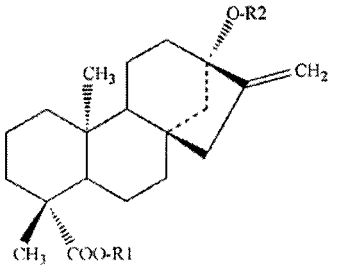
Rebaudioside A

Chemical Name:	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy] kaur-6-en-8-oic acid, β-D-glucopyranosyl ester

Chemical Formula: $C_{44}H_{70}O_{23}$
Formula Weight: 967.03
CAS Number: 58543-16-1

In the recent Chemical and Technical Assessment (FAO, 2007b), JECFA identified the sweetener components. They updated the list of common glycosides and their chemical structures, which are slightly different than compounds shown in other older publications (Nanayakkara et al., 1987; Suttajit et al., 1993). They are represented in Figure 1.

Figure 1. Chemical Structures of Various Steviol Glycosides Reproduced from FAO[a, b]



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

[a] From FAO, 2007b.
[b] The indicated C.A.S. No. for Rubusoside as reported in the cited reference is incorrect and should be 64849-39-4.

The structures of the components of stevia glycosides were also described in reviews by Kinghorn and Soejarto (1985), Kennelly (2002), and Geuns (2003). Non-sweet elements include the labdane diterpenes, triterpenes, sterols and flavonoid glycosides.

C. Manufacturing Processes

Various manufacturing processes yielding steviol glycosides have been described in the scientific and patent literature, and they are summarized below, along with Pyure Brands' manufacturing process for its Reb A 95% and Reb A 98%.

1. Scientific & Patent Literature

Typically, steviol glycosides are obtained by extracting leaves of *Stevia rebaudiana* Bertoni with hot water or alcohols (ethanol or methanol); the obtained extract is a dark particulate solution containing all the active principles plus leaf pigments, soluble polysaccharides, and other impurities. Some processes remove the "grease" from the leaves with solvents such as chloroform or hexane before extraction occurs (Kinghorn and Soejarto, 1985). There are dozens of extraction patents for the isolation of steviol glycosides. Kinghorn and Soejarto (1985) have categorized the extraction patents into those based on solvent, solvent plus a decolorizing agent, adsorption and column chromatography, ion exchange resin, and selective precipitation of individual glycosides. Methods using ultrafiltration, metallic ions, supercritical fluid extraction with CO_2 and extract clarification with zeolite are found within the body of newer patents.

At the 68th JECFA meeting in 2007, steviol glycosides were defined as the products obtained from the leaves of *Stevia rebaudiana Bertoni*. As cited by JECFA, the typical manufacture starts with extracting leaves with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with methanol to release the glycosides and the product is recrystallized with methanol. Ion-exchange resins may be used in the purification process. The final product is commonly spray-dried.

2. Pyure Brands Manufacturing Process for High Purity Rebaudioside A

The source of Reb A is the leaves of the *Stevia rebaudiana* (Bertoni) plant.

The steviol glycosides content is slightly influenced by several factors, including but not limited to: the temperatures while under cultivation; latitude where grown; and the timing of harvesting.

High purity Reb A (≥ 95%) is obtained by the extraction of Stevia leaves with water followed by adsorption onto resin where the glycosides are subsequently eluted with ethanol. The extract is filtered, desalinated, and concentrated before it is decolorized with activated carbon. The extract is dried, and the resulting solid is dissolved in water/ethanol solution. Finally, it is crystallized and dried, resulting in a high purity Reb A (95%) as the

final product. At least one additional recrystallization step is used to produce high purity Reb A (98%).

The overview of the production process is provided for Reb A (95%) in Figure 2 and for Reb A (98%) in Figure 3. The equipment used for processing is discussed in detail in Appendix B.

D. Product Specifications & Supporting Methods

1. JECFA Specifications for Steviol Glycosides

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

In 2007, JECFA recommended that the method of assay should include a minimum requirement of 95% of the total of 7 specific steviol glycosides on a dried weight basis (FAO, 2007a, see Appendix A-1). Stevioside and rebaudioside A are the major component glycosides of interest because of their sweetening property. The 5 other associated glycosides found in preparations of steviol glycosides accepted by the JECFA specification for the 95% requirement are rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B. These, however, are typically found at much lower levels than stevioside or rebaudioside A.

JECFA finalized food grade specifications at the 68th JECFA meeting, which were then published in FAO JECFA Monograph 4 (FAO, 2007a) and were subsequently revised (FAO, 2008). Steviol glycosides are described as a white to yellow powder, odorless to having a slight characteristic odor, and exhibiting a sweetness that is 200-300 times greater than sucrose. The ingredient must consist of a minimum of 95% of 7 specific steviol glycosides as specified in Appendix A-1 and A-2. The steviol glycosides are freely soluble in water and ethanol, and the 1 in 100 solutions exhibit pH values between 4.5 - 7.0. The product should not have more than 1% ash with no more than a 6% loss on drying at 105°C for 2 hours. Residual methanol levels should not exceed 200 mg/kg, and ethanol residues should not exceed 5000 mg/kg. Arsenic levels should not exceed 1 mg/kg as determined by the atomic absorption hydride technique. Lead levels should not exceed 1 mg/kg. The complete JECFA specifications including recommended analytical methods are contained in Appendix A.

2. Specifications for Pyure Brands' High Purity Rebaudioside A 95% & 98%

Pyure Brands has adopted product specifications for its High Purity Reb A products that meet or exceed JECFA recommendations. The specifications provided by Pyure Brands as compared to JECFA specifications for the final spray dried product are given in Table 1. Analyses demonstrating that 5 production batches of the subject materials meet these specifications are attached in Appendix B.

Figure 2. Overview of Primary Stevia Extract Production Processing for High Purity Reb A 95%

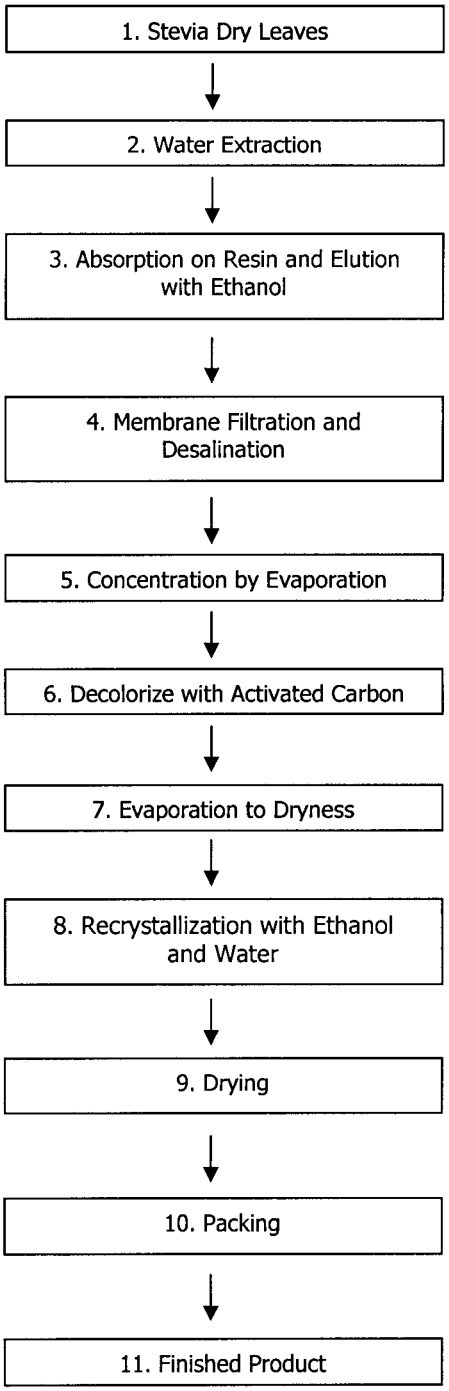


Figure 3. Overview of Primary Stevia Extract Production Processing for High Purity Reb A 98%

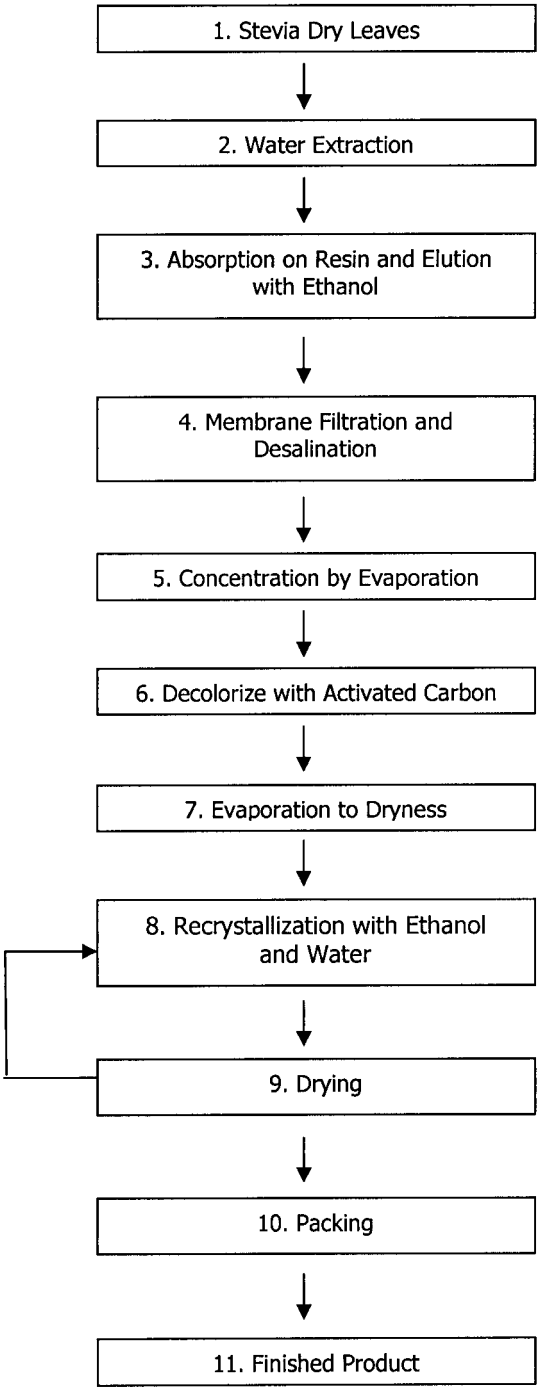


Table 1. Specifications for Pyure Brands Rebaudioside A 95% & 98% Products

PARAMETER	JECFA[a] SPECIFICATIONS REB A	PYURE BRANDS SPECIFICATIONS REB A (95%)	PYURE BRANDS SPECIFICATIONS REB A (98%)
Sweetness	200-300 times sweeter than sucrose	400 times sweeter than sugar	400 times sweeter than sugar
Total Glycosides (as St, Reb A, Reb B, Reb C Dulc A, Rub, and SB) on dry weight basis	NA	> 95%	> 99%
Moisture (loss on drying)	≤ 6%	< 6%	< 6%
Ash	≤ 1%	< 1%	< 1%
Solubility	Freely soluble in water	Freely soluble in water and ethanol	Freely soluble in water and ethanol
pH (1% solution)	4.5 - 7.0	4.5 - 7.0	4.5 - 7.0
Residual Methanol (ppm)	NA	<200	<200
Residual Ethanol (ppm)	NA	<5000	<5000
Lead	≤ 1 mg/kg	< 1 ppm	< 1 ppm
Arsenic	≤ 1 mg/kg	< 1 ppm	< 1 ppm
Total Plate Count	NA	< 2000 cfu/g	< 2000 cfu/g
Yeast and Mold	NA	≤ 100 cfu/g	≤ 100 cfu/g
Salmonella	NA	Negative	Negative
Total *E. coli*	NA	≤ 3 npm/g	≤ 3 npm/g
Fecal *E. coli*	NA	< 3 npm/g	< 3 npm/g

Abbreviations: St = Stevioside; Reb A = Rebaudioside A; Reb B = Rebaudioside B; Reb C = Rebaudioside C; Dulc A = Dulcoside A; Rub = Rubusoside; SB = Steviolbioside; NS = not specified; NA = not applicable.
a Prepared at 69th JECFA (2008).

E. Stability Data

Stevioside is a stable molecule over the pH range 3-9 and can be heated at 100°C for 1 hour, but rapidly decomposes at pH levels greater than 9 under these conditions (Kinghorn and Soejarto, 1985). It is speculated that steviobioside produced from stevioside by alkaline hydrolysis would be the major decomposition product obtained at pH 10 (Kinghorn and Soejarto, 1985).

Chang and Cook (1983) tested the stability of pure stevioside and rebaudioside A in carbonated phosphoric and citric acidified beverages and reported some degradation of both sweetening components after 2 months of storage at 37°C; however, there was no significant change at room temperature or below following 5 months of storage of stevioside and 3 months of storage of

rebaudioside A. He also reported that exposure to 1 week of sunlight did not affect stevioside, but resulted in approximately 20% loss of rebaudioside A. Heating at 60°C for 6 days resulted in 0-6% loss of rebaudioside A. As described more fully below, extensive stability testing results were compiled for inclusion in both the Merisant and Cargill GRAS notifications.

Detailed stability testing was conducted by Merisant on Reb A (1) as a powder, (2) as a pure sweetener in solution, and (3) on both cola-type and citrus carbonated beverages. No degradation was detected when the powder was stored at 105°C for 96 hours, and it was concluded that the powder was stable when stored for 26 weeks at 40±2°C with relative humidity of 75±5%. When considering Merisant's results of the stability investigations which include both published and unpublished testing results, it was determined that Reb A in carbonated citric acid beverages and phosphoric acid beverages showed no significant degradation during prolonged storage at refrigeration, normal ambient, or elevated ambient temperatures. Minimal loss of Reb A was detected after storage at 60°C, with considerable degradation noted after 13 hours at 100°C for carbonated beverage solutions and pure sweetener solutions (Merisant, 2008).

Cargill conducted detailed stability testing on Reb A as a powder under various storage conditions and under a range of pH and temperatures. In addition, Cargill assessed Reb A stability in several representative food matrices at room temperature and elevated temperatures. Stability profiles were created for table top sweetener applications, mock beverages including cola, lemon-lime, and root beer, yogurt, thermally processed beverages, and white cake. The stability testing revealed some degradation products that had not been detected in bulk Reb A. However, it was noted that these degradation products were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana* Bertoni. The degradation products all share the same steviol aglycone backbone structure as found in stevioside and rebaudioside A, but they differ by virtue of the glucose moities present.

Photostability studies were also conducted on the dry powder and mock beverages to ascertain Reb A behavior under defined conditions of fluorescent and near UV light exposure. Reb A was determined to be photostable under the defined conditions of analysis (Clos et al., 2008).

From the stability testing reported, it was concluded that Reb A is stable in various food matrices following several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, Reb A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

Pyure Brands High Purity Reb A 95% is comparable in composition to the Merisant materials, and the Pyure Brands High Purity Reb A 98% has a somewhat higher level of Reb A than the Merisant and Cargill materials, and the stability characteristics are expected to be equivalent to that exhibited by the materials studied by Merisant and Cargill. No formulation work or product development testing has given any indication of Reb A (95% - 98%) deterioration under any holding or handling conditions. Nonetheless, Pyure Brands has initiated stability testing on its High Purity Reb A products in concert with customer requirements, and such testing is in progress.

IV. INTENDED DIETARY USES

A. Intended Uses

Pyure Brands intends to market its High Purity Reb A (95% - 98%) as a table top sweetener and for incorporation into various food categories as a general purpose sweetener.[7] Its Reb A will function as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19). The 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 High Purity Reb A (95% - 98%) to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation.[8]

B. Food Uses As Addressed by JECFA, Merisant & Cargill

JECFA reviewed various estimates of possible consumption of steviol glycosides (WHO, 2006) as part of its safety deliberations. Estimated maximum use levels in various foods as evaluated by the Committee are summarized in Table 2.

Merisant listed expected levels of use for various food applications in their GRAS Notification. Their consumer estimates were largely based on food consumption survey data from 2003-2004 NHANES, a resource that reflects food intake over a two-day time period. Statistically weighted values were utilized to provide reliable quantitative findings that are representative of food consumption of actual "users" within the US population. The 2-day food surveys are known to overestimate actual consumption levels when compared to longer term food surveys, such as those based on 14-day surveys. On a per user basis, the mean daily consumption of Reb A was calculated to be 2.0 mg/kg bw/day, and that for the 90th percentile consumer was found to be 4.7 mg/kg bw/day. Specific food categories and use levels are given in Table 3.

Cargill utilized a different approach in estimating dietary intake figures for Reb A when incorporated as a general sweetener in a broad cross-section of processed foods (Cargill, 2008). Cargill reasoned that Reb A uses and use levels would be rather comparable to aspartame uses in the US with a few minor exceptions. They performed a side-by-side consumption analysis for Reb A versus aspartame, using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008). Their findings are considered further in Section IV.C and are tabulated in Table 4.

C. Estimated Daily Intake

Pyure Brands intends to incorporate its Pyure Elite High Purity Reb A into a broad selection of food categories at levels that comply with GMP uses. The very conservative consumer intake estimates provided by JECFA as shown above in Table 2 were utilized to gauge the potential human exposures of steviol glycosides and Reb A in foods as reported in the US and in other

[7] Pyure Brands has no plans to incorporate its High Purity Reb A (95% - 98%) into infant formulas or meat and poultry products.
[8] See 21 CFR 182.1(b)(1).

countries. Since Reb A is about twice as sweet as the mixed glycosides, these levels can be adjusted downward accordingly.

Table 2. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents

Food Type	Maximum Use Level Reported[a] (mg steviol glycosides /kg of food)	Maximum Use Level Calculated for rebaudioside A[b] mg rebaudioside A /kg of food	Maximum Use Level Calculated for rebaudioside A[b] mg steviol equivalents /kg of food
Desserts	500	250	83
Cold confectionery	500	250	83
Pickles	1000	500	167
Sweet corn	200	100	33
Biscuits	300	150	50
Beverages	500	250	83
Yogurt	500	250	83
Sauces	1000	500	167
Delicacies	1000	500	167
Bread	160	80	27

[a] Reproduced from WHO, 2006.

[b] Calculated by Expert Panel assuming twice the sweetness intensity for rebaudioside A and three-fold difference in molecular weight between rebaudioside A and steviol.

Table 3. Proposed Uses & Levels of Rebaudioside A by Merisant (2008)

Food Group	Reb A (ppm)
Tabletop sweeteners	30,000[a]
Sweetened ready-to-drink teas	90-450
Fruit juice drinks	150-500
Diet soft drinks	150-500
Energy drinks	150
Flavored water	150
Cereals (oatmeal, cold cereal, cereal bars)	150

[a] Reb A content of sachet prior to dilution and not representative of "as consumed."

Table 4. Summary of Estimated Daily Intake Assessments for Rebaudioside A & Calculation of Reb A Values from JECFA and FSANZ Estimates of the EDI

Scenarios	EDI		
	As Steviol[a] (mg/kg bw/day)	As Reb A[b] (mg/kg bw/day)	Total Daily Intake[c] (mg/day)
JECFA			
100% Reb A replacement of sugars	5.0	7.5	450
20-30% Reb A replacement of sugars	1.0 - 1.5	1.5 - 2.3	90 - 140
FSANZ			
100% Reb A replacement of sugars	0.3 - 1.0	0.5 - 1.5	30 - 90
MERISANT			
		2.0 - 4.7[d]	120 - 282
CARGILL			
		1.3 - 3.4[d]	78 - 204

[a] Published values for mixed steviol glycosides consumption listed in this column were used for the calculation of Reb A consumption values appearing in next two columns.
[b] Estimates for Reb A consumption were calculated from JECFA and FSANZ estimates as steviol by multiplying by 3 to correct for the molecular weight of Reb A compared to steviol and by subsequently dividing by 2 because of the increased inherent sweetness of Reb A compared to the mixed steviol glycosides.
[c] Total daily intake figures were calculated for a 60 kg adult.
[d] Published values are shown for comparison purposes.

In concert with the JECFA intake estimates, further consideration was given to anticipated human exposures as projected independently and with different approaches by both Merisant and Cargill in compiling their GRAS dossiers (Merisant, 2008 and Cargill, 2008). As noted below, the multiple approaches tended to converge to yield estimated daily intakes (EDIs) in the range of 1.3 – 4.7 mg/kg bw/day that, when compared to the acceptable daily intake (ADI), constitutes an integral component in the subject GRAS evaluation.

The Committee evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that were prepared for the European Commission by the Scientific Committee on Food.

JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that **steviol glycosides would replace all dietary sugars**, at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which

is 200:1. The intakes ranged from 1.3 mg/kg bw/day with the African diet to 3.5 mg/kg bw/day with the European diet.

JECFA also estimated the per capita exposure derived from disappearance (poundage) data supplied by Japan and China. The Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US. Table 5 summarizes the exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee.

Table 5. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)

ESTIMATE	EXPOSURE (mg/kg BW/DAY)
GEMS/Food (International)[a]	1.3--3.5 (for a 60 kg person)
Japan, Per Capita	0.04
Japan, Replacement Estimate[b]	3
US, Replacement Estimate[b]	5

[a] WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme.
[b] These estimates were prepared in parallel to those for the international estimates; it was assumed that all dietary sugars in diets in Japan and the US would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200:1.

JECFA concluded that the replacement estimates were highly conservative---that is, the calculated dietary exposure overestimates likely consumption---and that true dietary intakes of steviol glycosides (as steviol) would probably be **20 – 30% of these values** or 1.0 - 1.5 mg/kg bw/day on a steviol basis, **or 3.0 – 4.5 mg/kg bw/day for Reb A based on the molecular weight adjustment.** Furthermore, by adjusting for the 400-fold increased sweetness of Reb A relative to sucrose compared to the mixed steviol glycosides sweetness factor of 200-fold relative to sucrose assumed by JECFA, **the estimated dietary intake of Reb A would likely be about 1.5 – nearly 2.3 mg/kg bw/day.**

FSANZ (2008) similarly estimated steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario which resulted in estimated exposures of 0.3 - 1.0 mg/kg bw/day on a steviol basis, or **0.5 – 1.5 mg/kg bw/day for Reb A when making both the molecular weight and sweetness equivalency calculations**.

Merisant also calculated a dietary estimate for rebaudioside A of 2.0 mg/kg bw/day for the average consumer of the foods listed in Table 3 and 4.7 mg/kg bw/day for a 90th percentile consumer.

In another review conducted on behalf of Cargill and included in their GRAS notification, the intake of Reb A when used as a complete sugar replacement was estimated at 1.3 – 3.4 mg/kg bw/day when calculated as Reb A (Renwick, 2008). The estimated daily intake assessments have been compiled in Table 4, and we can see that **total daily consumption of Reb A for defined food categories and as a general purpose sweetener is expected to be 5 mg/kg bw/day or less**, for a total daily dietary exposure of 300 mg Reb A or less for an adult.

D. Other Information on Human Exposure to Stevia: Use as a Food Ingredient & Other Uses

The predominant use of steviol glycosides as a food ingredient has occurred in Brazil and Japan.[9] It is reported that 40% of the artificial sweetener market in Japan is stevia based and that steviol glycosides are commonly used in processed foods in Japan (Lester, 1999).

There are no reported uses of Reb A as a dietary supplement, but steviol glycoside usage as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand. It has wide use in China and Japan in food and in dietary supplements. In the US, stevia is available in packets containing 60 - 90 mg steviol glycoside for home supplement uses, such as in beverages or other foods. It is estimated that sales of stevia in the US reached $45 million in 2005 (The Food Institute Report, 2006). No estimates were located on the daily consumption levels of steviol glycosides consumed in the US *via* dietary supplements.

During the second quarter in 2008, as a result of selected firms obtaining independent GRAS determinations for the steviol glycoside-derived sweeteners, such materials have begun to be incorporated into foods in the US. In light of FDA's review of the Merisant, Cargill, McNeil Nutritionals, Blue California, Sweet Green Fields, and Wisdom Natural Brands GRAS notifications and issuance of "no objection" letters, the use of steviol glycoside-derived sweeteners such as rebaudioside A is anticipated to grow substantially in the US, and international uses are also expected to increase with the favorable JECFA determination at its 2008 meeting.

In South America, stevia is commonly used as a treatment for Type II diabetes (Hawke, 2003). However, elevated doses in the range of 1 gram per person per day or more were reported to be necessary to achieve this therapeutic effect (Gregersen et al., 2004).

[9] See Raintree Nutrition Tropical Plant Database (www.rain-tree.com/stevia.html).

V. SAFETY DATA FOR REBAUDIOSIDE A

A. Safety Data on Steviol Glycosides: Reviews by Expert Bodies & Other Scientists

The biological, toxicological, and clinical data on stevia and steviol glycosides have been assessed by a number of reviewers (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002) and most notably through the extended evaluation by JECFA (WHO, 2000, 2006, 2007, 2008) and a review by Food Standards Australia New Zealand (FSANZ, 2008) for use in food. The JECFA reviews, as well as the other reviews completed before 2008, primarily focused on mixtures of steviol glycosides typically and were not specific for purified rebaudioside A.

Some of the earliest studies on steviol glycosides were of limited value regarding safety assessments since the actual compositions of materials investigated and their questionable purities undermined drawing firm toxicological conclusions. For example, it had been reported that there was a decrease in fertility with crude stevia preparations and the mutagenic activity of the principle metabolite, steviol, was called into question. FDA was unwilling to authorize the use of stevia based on questions raised about safety by studies with materials of lesser purity and by studies with unusual protocols in *in vivo* and in *in vitro* systems usually employing high doses or high concentrations of test materials. These concerns included renal toxicity, effects on glucose metabolism, and inhibition of mitochondrial enzymes. However, over the last 15 years, the safety of steviol glycosides and rebaudioside A in particular were rather thoroughly studied with comprehensive and modern toxicology protocols using scientifically accepted dosing regimens of purified test substances. The results of these investigations are discussed below.

In addition, JECFA encouraged the further elucidation of clinical effects on blood pressure and glucose metabolism on hypertensive and diabetic individuals, respectively, in normal human subjects. By 2006, sufficient favorable data were generated for JECFA to generate a temporary ADI which was finalized in 2008. More details on the JECFA reviews are discussed in Section V.A.1. The key toxicology and clinical data on steviol glycosides (primarily stevioside) and the principle metabolite steviol reviewed by JECFA and other reviewers are summarized in Appendix C.

1. Summary of JECFA Reviews

In 1999, the 51st meeting of JECFA (WHO, 2000) expressed the following reservations about the safety data available at that time for steviol glycosides:

> The Committee noted several shortcomings in the information available on stevioside. In some studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was available on other constituents or contaminants. Furthermore, no studies of human metabolism of stevioside and steviol were available. In addition, data on long-term toxicity and carcinogenicity were available for stevioside in only one species. The mutagenic potential of steviol has been tested sufficiently only *in vitro*.

Additional data were subsequently provided on the metabolism of steviol glycosides. These data helped understand that the common steviol glycosides are converted to

steviol by intestinal bacteria and then rapidly converted to glucuronides that are excreted. The committee now had a molecular basis to become comfortable with studies on test materials which consisted of variable composition but were relatively high purity mixtures of the common steviol glycosides. The committee came to the conclusion that steviol glycosides are not mutagenic and that steviol is mutagenic in *in vitro* studies but not *in vivo*. The committee became convinced that purified steviol glycosides did not impair reproductive performance as did crude preparations of stevia and that there was sufficient chronic studies in rats with adequate no observed effect levels (NOEL) that could support a reasonable acceptable daily intake (ADI) in the range of doses that would be encountered by the use of steviol glycosides as a sugar substitute. The mutagenic, reproductive and chronic studies relied upon by JECFA are summarized in Appendix C. However, JECFA wanted more clinical data to rule out pharmacological effects at the expected doses. The following excerpt was taken from the report of the 63rd meeting (WHO, 2006):

> The Committee noted that most of the data requested at its fifty-first meeting, e.g., data on the metabolism of stevioside in humans, and on the activity of steviol in suitable studies of genotoxicity *in vivo*, had been made available. The Committee concluded that stevioside and rebaudioside A are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*.
>
> The NOEL for stevioside was 970 mg/kg bw/day in a long-term study (Toyoda et al., 1997) evaluated by the Committee at its fifty-first meeting. The Committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to about 12.5–25 mg/kg bw/day (equivalent to 5–10 mg/kg bw/day expressed as steviol). The evidence available at present was 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 therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg/kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg/kg bw/day (or 383 mg/kg bw/day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. The Committee noted that this temporary ADI only applies to products complying with the specifications.
>
> The Committee required additional information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics.

At the 68th meeting in 2007, JECFA concluded that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008 (WHO, 2007). Furthermore, sufficient data had been received to revise and finalize food additive specifications for steviol glycosides (FAO, 2007a). 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, 2007b).

> In response to the call for data on "stevioside" for the 63rd meeting of the Committee, submissions from several countries showed that the main components of the commercially available extracts of stevia are

stevioside and rebaudioside A, in various amounts ranging from about 10-70% stevioside and 20-70% rebaudioside A. The information indicated that most commercial products contained more than 90% steviol glycosides with the two main steviol glycosides comprising about 80% of the material. The 63rd JECFA required that the summed content of stevioside and rebaudioside A was not less than 70% and established a minimum purity of 95% total steviol glycosides. Analytical data showed that most of the remaining 5% could be accounted for by saccharides other than those associated with the individual steviol glycosides.

Noting that the additive could be produced with high purity (at least 95%) and that all the steviol glycosides hydrolyze upon ingestion to steviol, on which the temporary ADI is based, the 68th JECFA decided it was unnecessary to maintain a limit for the sum of stevioside and rebaudioside content. The Committee recognized that the newly revised specifications would cover a range of compositions that could include, on the dried basis, product that was at least 95% stevioside or at least 95% rebaudioside A.

At the 69th meeting in 2008, JECFA came to a final evaluation (WHO, 2008) based on their satisfaction with the completed clinical studies, and the Committee actually raised the ADI to 0 – 4 mg/kg bw/day and removed the "temporary" designation. In the final toxicology monograph addendum (WHO, 2009), the summary of the Committee's key conclusions were stated as follows:

From a long-term study with stevioside, which had already been discussed by the Committee at its fifty-first meeting, a NOEL of 970 mg/kg bw per day was identified. At its sixty-third meeting, the Committee set a temporary ADI of 0–2 mg/kg bw for steviol glycosides, expressed as steviol, on the basis of this NOEL for stevioside of 970 mg/kg bw per day (383 mg/kg bw per day expressed as steviol) and a safety factor of 200, pending further information. The further information was required because the Committee had noted that stevioside had shown some evidence of pharmacological effects in patients with hypertension or with type 2 diabetes at doses corresponding to about 12.5–25.0 mg/kg bw per day (5–10 mg/kg bw per day expressed as steviol).

The results of the new studies presented to the Committee at its present meeting have shown no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. The Committee concluded that the new data were sufficient to allow the additional safety factor of 2 and the temporary designation to be removed and established an ADI for steviol glycosides of 0–4 mg/kg bw expressed as steviol.

The Committee noted that some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides, but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.

2. Summary of FSANZ Review of Steviol Glycosides

Food Standards Australia New Zealand (FSANZ) completed a review of the safety of steviol glycosides for use as a sweetener in foods in 2008. The risk assessments undertaken by FSANZ concluded that steviol glycosides are well-tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal,

hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day. The FSANZ review discussed the adequacy of the existing database and several new studies, including the clinical studies reviewed by JECFA in the summer of 2007, most notably the work of Barriocanal et al., which was later published in 2008.

Prior to publishing their final report, which occurred after the JECFA meeting of 2008, FSANZ, in their draft document, also indicated that the new data in humans provides a basis for revising the uncertainty factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore, FSANZ established an ADI of 4 mg/kg bw/day for steviol glycosides as steviol equivalents, derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study (FSANZ, 2008).

B. Safety Data on Rebaudioside A

Only limited studies were available on rebaudioside A during the JECFA deliberations. Several toxicology studies have been recently reported on purified rebaudioside A, although it is uncertain whether or not these studies were considered by JECFA during its 2008 deliberations. These studies include additional mutagenicity data, comparative pharmacokinetic studies with stevioside in rats and humans, several subchronic studies in rats and one in dogs and additional reproduction and developmental studies in rats, as well as additional clinical studies.

1. Mutagenicity Studies

Reb A was evaluated for genotoxicity with a set of *in vitro* and *in vivo* assays covering mutation, chromosome damage and DNA strand breakage with consistent and uniformly negative results (Pezzuto et al, 1985; Nakajima, 2000a; Nakajima, 2000b; Sekihashi et al., 2002) as reviewed by Brusick (2008). An unpublished chromosome aberration assay of Reb A in cultured mammalian cells was submitted for JECFA review (Nakajima, 2000a). The JECFA review of this study indicated that no increases in chromosome aberrations were found.

More recently, in their GRAS Notification, Merisant submitted three unpublished studies on Reb A including a bacterial mutagenicity study (Wagner and Van Dyke, 2006), a mouse lymphoma study (Clarke, 2006) and a mouse micronucleus study (Krsmanovic and Huston, 2006). All three studies indicated lack of mutagenic or genotoxic activity. In addition, there is another set of published studies that indicate lack of mutagenicity *in vitro in Salmonella, E. coli,* and mouse lymphoma cells, lack of *in vitro* clastogenic effects in Chinese hamster V79 cells and the absence of *in vivo* effects in a mouse micronucleus assay and a rat study for unscheduled DNA synthesis (Williams and Burdock, 2009).

Table 6 summarizes the key mutagenicity testing results for Reb A. For a more comprehensive summary of mutagenicity studies on steviol glycosides, see Appendix C.

Table 6. Mutagenicity Studies on Rebaudioside A

End-Point	Test System	Material	Purity (%)	Concentration / Dose	Result	Reference
Bacterial Mutagenicity	5 Salmonella strains with and without exogenous metabolic activation system	Reb A	99.5	1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate	No mutagenic response	Wagner and Van Dyke (2006)
Bacterial Mutagenicity	4 Salmonella strains and 1 *E. coli* strain with and without exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg per plate	No mutagenic response	Williams and Burdock (2009)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence and presence of exogenous metabolic activation system	Reb A	99.5	Cloning conc. of 500, 1000, 2000, 3000, 4000 and 5000 µg/mL	No mutagenic or clastogenic response	Clarke (2006)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence and presence of exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Chromosome Aberration	Human lymphocytes in absence and presence of exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Mouse Micronucleus	Micronucleus study in groups of 5 male and 5 female ICR mice	Reb A	99.5	500, 1000 and 2000 mg/kg bw	No increase in micronuclei formation	Krsmanovic and Huston (2006)
Mouse Micronucleus	Micronucleus study in groups of 5 male and 5 female NMRI mice	Reb A	95.6	Up to 750 mg/kg bw	No increase in micronuclei formation	Williams and Burdock (2009)
Unscheduled DNA Synthesis	Unscheduled DNA synthesis in one group of 4 Wistar rats	Reb A	95.6	Up to 2000 mg/kg bw	No increase in unscheduled DNA synthesis	Williams and Burdock (2009)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevio-side, 52%; Reb A, 22%	250 - 2000 mg/kg bw	Negative[a]	Sekihashi et al. (2002)
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Reb A	NS	1.2 - 55 mg/mL	Negative[b]	Nakajima (2000a)
Micronucleus formation	BDF1 mouse bone marrow	Reb A	NS	500-2000 mg/kg bw per day for 2 days	Negative[c]	Nakajima (2000b)
Forward mutation	*S. typhimurium* TM677	Reb A	NS	10 mg/plate	Negative[b]	Pezzuto et al. (1985)

NS = Not specified.
[a] Sacrificed at 3 hours and 24 hours.
[b] With or without metabolic activation (source not specified in original monograph).
[c] Sacrificed at 30 hours after 2nd administration.

2. Subchronic Studies

Two repeated dose studies were conducted by the oral route in Wistar rats (Curry and Roberts, 2008). In a 4-week study, Reb A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000 and 100,000 ppm. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In the 13-week

study, Wistar rats were administered rebaudioside A at dietary concentrations of 0, 12,500, 25,000 and 50,000 ppm. Reductions in body weight gain attributable to initial taste aversion and lower caloric density of the diet were observed in high-dose male and females groups. Inconsistent reductions in serum bile acids and cholesterol were attributed to physiological changes in bile acid metabolism due to excretion of high levels of rebaudioside A *via* the liver. All other hepatic function test results and liver histopathology were within normal limits. Significant changes in other clinical pathology results, organ weights and functional observational battery test results were not observed. Macroscopic and microscopic examinations of all organs, including testes and kidneys, were unremarkable with respect to treatment-related findings. The NOAEL in the 13-week toxicity study was considered to be 50,000 ppm or approximately 4,161 and 4,645 mg/kg body weight/day in male and female rats, respectively.

A 90-day dietary toxicity study was conducted in Crl:CD(SD) rats with Reb A (99.5% purity) doses of 500, 1000 and 2000 mg/kg bw/day (Eapen, 2007). Each group consisted of 20/animals/sex. There were no treatment related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters. No treatment related macroscopic, organ weight or microscopic findings were reported. Significantly lower body weight gains were noted in the 2000 mg/kg bw/day group in males but not females. The body weight in males was 9.1% lower than the control group at the end of the dosing period (study week 13). The investigators did not consider this result to be adverse due to the small magnitude of difference from the control group value and were most likely due to the large proportion of the diet represented by the test material. The assigned NOAEL was ≥2000 mg/kg bw/day.

A 6-month dietary toxicity study in Beagle dogs was conducted to evaluate the potential toxic effects of Reb A (97.5% purity) at dosage levels of 0, 500, 1000 or 2000 mg/kg bw/day (Eapen, 2008). All groups consisted of 4 males and 4 females. During the course of the study, there were no unscheduled deaths. No treatment-related clinical observations were noted. Home cage, open field observations and functional observations and measurements were unaffected by the administration of rebaudioside A. There were no differences in hematology findings, serum chemistry findings, or urinalysis findings between groups. In addition, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. Based on the results of this study, the authors concluded that no systemic toxicity of rebaudioside A was observed at dosage levels up to 2000 mg/kg bw/day and the assigned NOAEL was ≥2000 mg/kg bw/day.

3. Reproduction & Developmental Studies

Rebaudioside A (97 % purity) was administered via the diet to male and female Han Wistar rats at 0, 7,500, 12,500, and 25,000 ppm for two generations (Curry, et al., 2008). Rebaudioside A treatment was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. No treatment-related effects of rebaudioside A were observed in either the F_0 or F_1 generations on reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology. The survival and general

condition of the F_1 and F_2 offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by rebaudioside A treatment. 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/kg body weight/day.

The results of the published studies are supported by the results of two unpublished studies with Reb A (2008a, b). In a two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were offered either basal diet or the test article, rebaudioside A (purity 95.7%), continuously in the diet for at least 70 consecutive days prior to mating (Sloter 2008a). Rebaudioside A doses were 0, 500, 1000 and 2000 mg/kg/day for the F_0 and F_1 generations. F_0 animals were approximately 7 weeks of age at the initiation of test diet exposure. The test diet was offered to the offspring selected to become the F_1 generation following weaning (beginning on postnatal day [PND] 21). The F_0 and F_1 males continued to receive rebaudioside A throughout mating, continuing through the day of euthanasia. The F_0 and F_1 females continued to receive rebaudioside A throughout mating, gestation and lactation until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females (estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints). A dose level ≥2000 mg/kg bw/day (highest dose administered) was assigned to be the NOAEL for parental systemic and reproductive toxicity.

Reb A was tested by gavage in an embryo/fetal development study in rats (Sloter, 2008b). Intrauterine growth and survival were unaffected by the test article, and there were no test article-related fetal malformations or developmental variations at any dosage level. In the absence of maternal or developmental toxicity a dose level ≥2000 mg/kg bw/day (highest dose administered) was considered to be the NOAEL for maternal and embryo/fetal developmental toxicity when Reb A was administered by oral gavage to pregnant rats.

4. Clinical Studies on Rebaudioside A

A randomized, double-blind trial evaluated the hemodynamic effects of four weeks' consumption of 1000 mg/day rebaudioside A (97% purity) versus placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Maki et al., 2008a). Subjects were predominantly female (76%, rebaudioside A and 82%, placebo) with a mean age of ~41 (range 18 to 73) years. At baseline, mean resting, seated SBP/DBP was 110.0/70.3 mm Hg and 110.7/71.2 mm Hg for the rebaudioside A and placebo groups, respectively. Compared with placebo, rebaudioside A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-hour ambulatory blood pressure responses. The authors concluded that these results indicate that consumption of as much as 1000 mg/day of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

Another trial evaluated the effects of 16 weeks of consumption of 1000 mg rebaudioside A (97% purity, n = 60), a steviol glycoside with potential use as a sweetener, compared to

placebo (n = 62) in men and women (33-75 years of age) with type 2 diabetes mellitus (Maki, et al., 2008b). Mean ± standard error changes in glycosylated hemoglobin levels did not differ significantly between the rebaudioside A (0.11 ± 0.06%) and placebo (0.09 ± 0.05%; p = 0.355) groups. Changes from baseline for rebaudioside A and placebo,

respectively, in fasting glucose (7.5 ± 3.7 mg/dL and 11.2 ± 4.5 mg/dL), insulin (1.0 ± 0.64 μU/mL and 3.3 ± 1.5 μU/mL), and Cpeptide (0.13 ± 0.09 ng/mL and 0.42 ± 0.14 ng/mL) did not differ significantly (p > 0.05 for all). Assessments of changes in blood pressure, body weight, and fasting lipids indicated no differences by treatment. Rebaudioside A was well-tolerated, and records of hypoglycemic episodes showed no excess versus placebo. The authors suggest that these result that chronic use of 1000 mg rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

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

Three recently completed studies have shed light on the absorption and fate of rebaudioside A in rats and humans.

The toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol were examined in rats for comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, rebaudioside A (Roberts and Renwick, 2008). Single, oral doses of the radiolabelled compounds were extensively and rapidly absorbed with plasma concentration-time profiles following similar patterns for stevioside and rebaudioside A. Elimination of radioactivity from plasma was essentially complete within 72 hours. All plasma samples had similar metabolite profiles; the predominant radioactive component in all samples was steviol, with lower amounts of steviol glucuronide(s) and low levels of one or two other metabolites. Rebaudioside A, stevioside, and steviol were metabolized and excreted rapidly, with the majority of the radioactivity eliminated in the feces within 48 hours. Urinary excretion accounted for less than 2% of the administered dose for all compounds in both intact and bile duct-cannulated rats, and the majority of the absorbed dose was excreted *via* the bile. After administration of the compounds to intact and bile duct-cannulated rats, radioactivity in the feces was present primarily as steviol. The predominant radioactive compound detected in the bile of all cannulated rats was steviol glucuronide(s), indicating de-conjugation in the lower intestine. The authors concluded that the overall data on toxicokinetics and metabolism indicate that Reb A and stevioside are handled in an almost identical manner in the rat after oral dosing.

This randomized, double blind, cross-over study assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of rebaudioside A and stevioside in healthy adult male subjects (Wheeler et al., 2008). Steviol glucuronide appeared in the plasma of all subjects after administration of rebaudioside A or stevioside, with median Tmax values of 12.0 and 8.00 hours post-dose, respectively. Steviol glucuronide was eliminated from the plasma, with similar $t_{1/2}$ values of approximately 14 hours for both compounds. Administration of rebaudioside A resulted in a significantly (approximately 22%) lower steviol glucuronide geometric mean Cmax value (1472 ng/ml) than administration of stevioside (1886 ng/mL). The geometric mean AUC0-t

value for steviol glucuronide after administration of rebaudioside A (30788 ng*hr/mL) was approximately 10% lower than after administration of stevioside (34090 ng*hr/mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72 hour collection period, accounting for 59% and 62% of the rebaudioside A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that both rebaudioside A and stevioside were hydrolyzed to steviol in the gastrointestinal tract prior to absorption. The majority of circulatory steviol was in the form of steviol glucuronide indicating rapid first-pass conjugation prior to urinary excretion. Only a small amount of steviol was detected in urine (rebaudioside A: 0.04%; stevioside: 0.02%). 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.

Another pharmacokinetic study was done as a toxicokinetic (TK) phase of a dietary study to determine the potential of rebaudioside A toxicity in rats at levels up to 2000 mg/kg bw/day (Sloter, 2008a). Reb A and total steviol were detected in peripheral blood of rats during daily administration of 2000 mg/kg bw/day of Reb A at extremely low levels, with mean plasma concentrations of approximately 0.6 and 12 ug/mL, respectively. Estimates of absorbed dose for Reb A and total steviol were approximately 0.02% and 0.06%, respectively, based on the amounts measured in urine collected over 24 hours in comparison to daily administered dietary dose to rats. Mean fecal Reb A and measured hydrolysis products expressed as *Total Reb A Equivalents* compared to daily administered dose results in an estimate of percent of dose recovered ≈ 84%.

VI. DISCUSSION OF GRAS CRITERIA & REVIEWED INFORMATION

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. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[10]

Amplification is provided in that the determination 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 about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."
>
> "General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information."[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.

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10 See 21 CFR 170.3(i).
11 See 21 CFR 170.30(a).
12 See Footnote 1.

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).

As noted below, the safety assessment to ascertain GRAS status for rebaudioside A with the defined food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

B. Panel Discussion on the Expert Safety Reviews of Steviol Glycosides

Steviol glycosides are unique compounds in that they have viable uses as a non-nutritive sweetener in foods.[13] The series of reviews by JECFA indicate the progression of knowledge on the toxicology of these compounds. Many early toxicology studies were conducted on crude extracts of stevia and there were also several studies with *in vivo* and *in vitro* models which explored the biological activity of stevia extracts at high doses or high concentrations. Several concerns were noted, including impairment of fertility, renal effects, interference with glucose metabolism and inhibition of mitochondrial enzymes. As more studies were done on purified glycosides, the toxicology profile of steviol glycosides eventually proved out to be rather unremarkable. A number of subchronic, reproductive and chronic studies have been conducted in laboratory animals. In general, the studies were adequately designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably the reproductive studies with purified steviol glycosides refuted the concern of effects on fertility that were initially reported with stevia leaves or crude extracts. All other concerns failed to manifest themselves at the doses employed in long-term rat studies.

As discussed in Section V, JECFA reasoned that there were adequate chronic studies in rats, particularly the study by Toyoda et al., (1997) on which to base an ADI with an adequate margin of safety. The committee was satisfied that the lack of carcinogenic response in these well-conducted studies justified their 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 which share the same basic metabolic and excretory pathway and that the use of high purity preparations of various steviol glycosides is safe to use as a sugar substitute. The additional clinical data subsequently presented allowed JECFA to establish a permanent ADI of 0 - 4 mg/kg bw/day (based on steviol equivalents) or 0 - 12 mg/kg bw for rebaudioside A over and above the temporary ADI of 0 - 2 mg/kg bw/day (based on steviol equivalents).

The Panel agrees with this reasoning. It should be noted that in a recent study, DNA damage was seen in a variety of organs in a comet assay in rats maintained on drinking water containing

[13] 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) recently published a comprehensive review 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 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 principle metabolite, steviol, but the pharmacological effects of steviol have not been comprehensively investigated.

4 mg/mL steviol glycosides for up to 45 days (Nunes et al., 2007). Several experts in the field have questioned the methodology used in this study (Geuns, 2007; Williams, 2007; Brusick, 2008). The Panel has reviewed the cited publications and agrees and discounts the importance of the Nunes study.

Regarding clinical effects noted in humans, in order to corroborate the observations in these studies that 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). As reviewed by FSANZ, new data presented to JECFA demonstrate the lack of pharmacological effects of steviol glycosides at 11 mg/kg bw/day in normal individuals or approximately slightly more than 4 mg/kg bw on the basis of steviol equivalents (Barriocanal et al., 2008). JECFA may also have had preliminary results associated with the recently published clinical studies on rebaudioside A (Maki et al., 2008a, b). The Panel has reviewed the clinical studies and concludes that there will no effects on blood pressure and glucose metabolism in humans at the doses of rebaudioside A expected from use in food as a non-nutritive sweetener.

Part of JECFA's review included anticipated dietary patterns and the use concentrations expected in various foods in order to calculate an estimated daily intake or EDI (WHO, 2003, 2006). For US consumption, based on the assumption of 100% substitution of steviol glycosides for sugar, an EDI of 5 mg/kg bw/day steviol was calculated. JECFA concluded that the replacement estimates were highly conservative and that this calculated intake of steviol glycosides (as steviol) would more likely be 20–30% of these values. Except for the scenario developed by JECFA with 100% replacement of sugars by steviol glycosides, and as discussed in Section IV.C and summarized in Table 4, the highest dietary estimate for use in foods for Reb A is 4.7 mg/kg bw/day. The Panel embraces the JECFA ADI of 4 mg/kg bw/day based on steviol equivalents which corresponds to 12 mg/kg bw/day for Reb A and notes that the estimates as contained in Table 4 of anticipated dietary intake are below the ADI.

C. Expert Panel Discussion of the Safety of Rebaudioside A

More than a dozen papers describing the results of a comprehensive research program on Reb A were published since July, 2008. Many of these studies formed the basis of the Cargill GRAS notification (GRN 253). Several other studies were sponsored by Merisant and similarly these were then submitted with their GRAS notification (GRN 252). Previously, only a limited number of toxicology studies specifically on Reb A were conducted. As in the previous section, JECFA, as a world renowned expert body for the evaluation of food ingredient safety, had concluded even before these new studies were completed that seven common steviol glycosides are safe for use as sweetener preparations when present in any combination as long as the combined purity of 95% or more was established.

The presumed strategy of the most recent research on Reb A was to conduct a limited number of well-designed and executed toxicology studies on the specific compound and to demonstrate in rats and in humans that it is handled pharmacokinetically similarly to stevioside, which is the steviol glycoside on which most previous pharmacokinetic research was conducted. This was done to justify using the JECFA generated ADI without having to conduct a chronic study in rats

with rebaudioside A. In addition, the Merisant group upgraded the mutagenicity and genotoxicity data available on rebaudioside A with three assays that FDA generally considers to be most predictive for carcinogenicity potential. The Cargill group conducted two clinical studies to assure that rebaudioside A does not have potentially problematic pharmacological effects on blood glucose and blood pressure as was demonstrated for stevioside.

The most recent research on rebaudioside A was summarized by Carakostas et al. (2008). These reviews summarized the findings of the Cargill research program as follows:

- 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.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes, et al., 2007) 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.
- The pharmacokinetic similarity between rebaudioside A and stevioside justifies the use of the ADI established by JECFA that was determined on studies employing stevioside as the main component as the ADI for rebaudioside A.
- The dietary levels expected from consumption of rebaudioside A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers

The Panel concurs that both the JECFA and Renwick (2008) consumption estimates very conservatively represent a potential high user of rebaudioside A if this non-nutritive sweetener becomes widely available in food. As part of this GRAS evaluation, the Panel adopts the JECFA EDI for application to Pyure Brands' High Purity Reb A (95% - 98%).

In consideration of the aggregate safety information available, the Panel has concluded that JECFA has conducted an expert evaluation 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/kg bw/person as steviol equivalents, which is equivalent to 12 mg/kg bw/day for rebaudioside A on a weight basis. The Panel agrees that unwanted pharmacological effects are not likely to occur at this level and that high consumers of Reb A are not likely to exceed this level. Therefore, the Panel adopts the JECFA-derived ADI as a safe exposure for rebaudioside A and that food uses meeting the specifications within the limits determined by this esteemed international body of food safety experts can be considered to be generally recognized as safe (GRAS) within the meaning of the Food, Drug, and Cosmetic Act.

D. Discussion of Concerns Raised by UCLA Researchers & the Center for Science in the Public Interest (CSPI)

In August of 2008, two UCLA researchers published a criticism of the GRAS Assessment by Cargill (Kobylewski and Eckhert, 2008). They were recruited for this task by CSPI, long known as

a "public watchdog" on food ingredient safety. The basic deficiencies contained within the toxicology review generated by the UCLA group can be summarized as follows:

- There are insufficient mutagenicity and genotoxicity data on rebaudioside A compared to comparable data available for stevioside to confirm that rebaudioside A is not likely to have carcinogenic properties.
- The metabolism of rebaudioside A is too different from stevioside to rely on the rat chronic studies on stevioside to set an ADI for rebaudioside A.
- The carcinogenic potential of both stevioside and rebaudioside A should be examined in a second rodent species. They suggest that a mouse study is needed according to FDA Redbook guidelines.[14]

1. Panel's Overall Conclusions on UCLA & CSPI Concerns

The Panel has reviewed the UCLA paper, as well as the Reb A studies submitted by Merisant and Cargill as part of their GRAS notifications to FDA. CSPI has challenged the safety determination for Reb A based to a great extent on the UCLA toxicology review. The Panel recognizes that one can always avoid making food ingredient safety decisions by asking for more data, and CSPI has adopted this position.

Based on the review of the UCLA evaluation and the composite safety information on steviol glycosides and Reb A and for the reasons summarized below, the Panel disagrees with the conclusions of the UCLA study.

The pharmacokinetic work shows that stevioside and rebaudioside A are not absorbed *per se* but are converted to steviol in the GI tract. This occurs more slowly for rebaudioside A due to the fact that it has a disaccharide side chain instead of a monosaccharide side chain present in stevioside. In both humans and rats, the steviol is rapidly converted to the glucuronide. The glucuronide is not further metabolized but is efficiently excreted. In the rat, elimination occurs in the bile to the large intestine. In humans, elimination of the glucuronide occurs both in bile and urine. The UCLA group indicates that this is a profound difference and suggests that this makes the rat a poor model for the extrapolation of an ADI. The Panel disagrees with this concern. It is more important that the glucuronide is not expected to be toxic and is not further metabolized and is efficiently eliminated. The route of elimination is different, but elimination is elimination. In addition, the mouse lymphoma and mouse micronucleus studies conducted by Merisant and Williams and Burdock (2009), as well as several mouse micronucleus studies conducted by others, give no indication that there are any undiscovered carcinogenic pathways that may operate in the mouse that are not observed in the rat, and, therefore, do not indicate a need for a mouse carcinogenicity study on Reb A, steviol glycosides, or steviol.

Consequently, the Panel rejects the concerns of the two UCLA authors.

[14] See Toxicological Principles for the Safety Assessment of Food Ingredients Redbook 2000 (http://www.fda.gov/Food/Guidance ComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/Redbook/default.htm), and Guidance for Industry Summary Table of Recommended Toxicological Testing for Additives Used in Food (http://www.fda.gov/Food/Guidance ComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/ucm054658.htm).

The Panel further notes that JECFA is composed of dozens of scientists that are experts on food ingredient safety that have established ADIs for food ingredients over the last 40 years.

Both Merisant and Cargill took rather rigorous scientific approaches to demonstrate the safety of rebaudioside A. The studies were equally well conducted. The safety profiles compiled by Merisant and Cargill differ somewhat, yet the results are complementary and are mutually reinforcing of rebaudioside A safety.[15]

The Cargill studies 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. The Merisant notification augmented genotoxicity data in three systems recognized by FDA as good predictors of carcinogenic potential. Two of these assays were conducted in mouse systems. Additional mutagenicity and genotoxicity studies have been published on rebaudioside A (Williams and Burdock, 2009). Merisant added a subchronic study in dogs and a teratology study in rats. Both Cargill and Merisant relied on the JECFA ADI for steviol glycosides as determined largely by published chronic studies in rats. Both groups justified the use of the ADI on pharmacokinetic arguments showing the similarity of stevioside and rebaudioside A metabolism and excretion.

The Panel endorses the conclusion of JECFA and the Cargill and Merisant Expert Panels in that there are a sufficient number of good quality health and safety studies to support the determination that the intended use of purified preparations of steviol glycosides, including Reb A, when added to food at levels up to full replacement of sugar on a sweetness equivalency basis, meets FDA's definition of safe.

E. Common Knowledge Elements of GRAS Determination

The first common knowledge element for a GRAS determination is that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing published, peer-reviewed scientific journals. The majority of studies reviewed as part of this safety assessment have been accepted for publication in the scientific literature as reported in Section V. 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. Thus, these studies become generally available to the scientific community. JECFA only reviewed a limited number of studies conducted specifically on rebaudioside A. The collection of supporting data on rebaudioside A has recently been enhanced by the 2008 studies cited earlier. The newest clinical studies that address JECFA's concern on unwanted pharmacological effects with steviol glycosides

[15] We note that the UCLA group did not review the Merisant studies.

(Barriocanal et al., 2008) and with rebaudioside A (Maki et al., 2008 a, b) are now published in the peer-reviewed scientific literature.

To be sure, the Panel recognizes that the safety of steviol glycoside in human foods has been the subject of interest for many years. In addition to the reported substantial history of consumption of stevia, especially in South America and Asia, many scientific studies have been conducted and published. Some of the studies have raised concerns about the safety, and the Panel has given careful attention to such concerns. The overriding evidence has diminished the Panel's concerns based on better study designs, better execution, or simply updated investigations that better reflect state-of-the art toxicological principles and findings.

The remaining common knowledge element for a GRAS determination is that there must be a basis to conclude that there is consensus among qualified scientists about the safety of the substance with its intended use. The JECFA opinion largely meets the common knowledge test on its own. The Panel is cognizant of the scientific rigor and broad base of scientific expertise that resides with the prestigious JECFA. JECFA is composed of expert scientists from various regulatory agencies around the world, as well as other scientists chosen because of their specific expertise on various classes of food ingredients. In addition, FDA participated in the JECFA deliberations.

The JECFA conclusion has been reviewed and validated by other respected regulatory agencies including FSANZ, the Switzerland Office of Public Health, and France's Agence Francais De Securite Sanitaire Des Alimenta (FSANZ, 2008, Switzerland Office of Public Health, 2008, and AFSSA, 2009). A number of other well-respected scientists have indicated that steviol glycosides are safe for human consumption at doses in the range of the JECFA ADI (Xili et al., 1992; Toyoda et al., 1997; Geuns, 2003; Williams, 2007).

The common knowledge element has been embellished by the many respected scientists that participated in the Cargill-sponsored new research conducted on rebaudioside A, most notably Brusick and Renwick. An assertion of "general recognition of safety" was made by Carakostas et al. (2008). In summary, there are many diverse groups of scientists from all corners of the globe that together provide strong fulfillment of the consensus requirement. Of particular significance from the perspective of establishing consensus for the safety of high purity steviol glycosides are the mid-December 2008 "no objection" determinations by FDA for the GRAS notifications for rebaudioside A as submitted by Merisant and Cargill and the more recent comparable findings by FDA with the additional GRAS notifications cited elsewhere.

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 the GRAS conclusion on rebaudioside A as outlined in this evaluation. The broader scientific community has concluded that past concerns expressed by others over the years (Huxtable, 2002) and earlier safety issues noted by FDA have been resolved by newer data on more purified test materials and the rigid specifications for purity published by JECFA for steviol glycosides, including rebaudioside A. Indeed, scientists from FDA are members of JECFA and have not objected to the safety decision on steviol glycosides. There is also a wider consensus that the body of new research on rebaudioside A is sufficient as opposed to the small group of scientists that argue that more studies need to be done before the sweetener is made available in the US.

VII. CONCLUSIONS[16]

Pyure Brands' High Purity Rebaudioside A (95% - 98%) as expressed on a dry weight basis is Generally Recognized As Safe when consumed as a non-nutritive sweetener when: (1) it is produced in accordance with FDA Good Manufacturing Practices requirements; (2) it meets or exceeds the JECFA purity specifications for steviol glycosides; and (3) it is consumed within the designated JECFA ADI of 12 mg/kg bw/day on a rebaudioside A basis. In order to remain within the designated ADI, it is important to observe good manufacturing practices principles in that the quantity of a substance added to food shall not exceed the amount reasonably required to accomplish its intended technical effect.

This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

(b) (6)

Richard C. Kraska, Ph.D., DABT

(b) (6)

Robert S. McQuate, Ph.D.

January 13, 2010

[16] The detailed educational and professional credentials for the individuals serving on the Expert Panel can be found on the GRAS Associates website at www.gras-associates.com. Richard C. Kraska, Ph.D., DABT and Robert S. McQuate, Ph.D. have extensive technical backgrounds in the evaluation of food ingredient safety. Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Kraska served as Chair of the Panel.

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APPENDIX A

JECFA Steviol Glycosides Specifications & Analytical Method

A-1 JECFA Steviol Glycosides Specifications & Analytical Method - 2007

A-2 JECFA Steviol Glycosides Specifications & Analytical Method - 2008

APPENDIX A-1

STEVIOL GLYCOSIDES

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding tentative specifications prepared at the 63rd JECFA (2004), in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A temporary ADI of 0-2 mg/kg bw (expressed as steviol) was established at the 63rd JECFA (2004).

SYNONYMS INS no. 960

DEFINITION The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with methanol to release the glycosides and product is recrystallized with methanol. Ion-exchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property Associated glycosides include rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

Chemical name

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-6-en-8-oic acid, β-D-glucopyranosyl ester

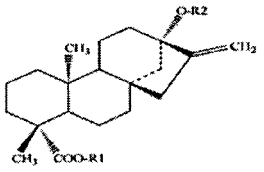
C.A.S. number

Stevioside:	57817-89-7
Rebaudioside A.	58543-16-1

Chemical formula

Stevioside.	$C_{38}H_{60}O_{18}$
Rebaudioside A:	$C_{44}H_{70}O_{23}$

Structural formula The seven named steviol glycosides:



Compound name	*R1*	*R2*
Stevioside	β-Glc	β-Glc-β-Glc(2→1)
Rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside C	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
Dulcoside A	β-Glc	β-Glc-α-Rha(2→1)
Rubusoside	β-Glc	β-Glc
Steviolbioside	H	β-Glc-β-Glc(2→1)
Rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides.
Glc and Rha represent, respectively, glucose and rhamnose sugar moieties.

Formula weight — Stevioside: 804.88; Rebaudioside A: 967.03

Assay — Not less than 95% of the total of the seven named steviol glycosides, on the dried basis.

DESCRIPTION — White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.

FUNCTIONAL USES — Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) — Freely soluble in water and in ethanol

Stevioside and rebaudioside A — The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.

pH (Vol. 4) — Between 4.5 and 7.0 (1 in 100 solution)

PURITY

Total ash (Vol. 4) — Not more than 1%

Loss on drying (Vol. 4) — Not more than 6% (105°, 2h)

Residual solvents (Vol. 4) — Not more than 200 mg/kg methanol
(Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)

Arsenic (Vol. 4) — Not more than 1 mg/kg
Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities).

METHOD OF ASSAY Determine the percentages of the individual steviol glycosides by high pressure liquid chromatography (Volume 4).

Standards
Stevioside, >99.0% purity and rebaudioside A, >97% purity (available from Wako pure Chemical Industries, Ltd. Japan).

Mobile phase
Mix HPLC-grade acetonitrile and water (80:20). Adjust the pH to 3.0 with phosphoric acid (85% reagent grade). Filter through 0.22 µm Millipore filter or equivalent.

Standard solutions
(a) Accurately weigh 50 mg of dried (105°, 2 h) stevioside standard into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
(b) Repeat with previously dried rebaudioside A standard.

Sample solution
Accurately weigh 60-120 mg of dried (105°, 2 h) sample into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with the mobile phase.

Chromatography Conditions
Column: Supelcosil LC-NH2 or equivalent (length: 15-30 cm; inner diameter: 3.9-4.6 mm)
Mobile phase: A 80:20 mixture of acetonitrile and water (see above)
Flow rate: Adjust so that the retention time of rebaudioside A is about 21 min.
Injection volume: 5-10 µl
Detector: UV at 210 nm
Column temperature: 40°

Procedure
Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample solution and of the standard solutions.

The retention times relative to rebaudioside A (1.00) are:

0.45-0.48 for stevioside	0.12-0.16 for rubusoside
0.25-0.30 for dulcoside A	0.35-0.41 for steviolbioside
0.63-0.69 for rebaudioside C	0.73-0.79 for rebaudioside B

Measure the peak areas for the seven steviol glycosides from the sample solution (the minor components might not be detected). Measure the peak area for stevioside for the standard solution.

Calculate the percentage of each of the seven steviol glycosides, *X*, in the sample from the formula:

$$\%X = [W_S/W] \times [f_X A_X/A_S] \times 100$$

where

W_S is the amount (mg) of stevioside in the standard solution

W is the amount (mg) of sample in the sample solution

A_S is the peak area for stevioside from the standard solution

A_X is the peak area of *X* for the sample solution

f_X is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 (stevioside), 0.98 (dulcoside A), 1.20 (rebaudioside A), 1.18 (rebaudioside C), 0.80 (rubusoside), 0.80 (steviolbioside), and 1.00 (rebaudioside B).

Calculate the percentage of total steviol glycosides (sum the seven percentages).

APPENDIX A-2

STEVIOL GLYCOSIDES

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding specifications prepared at the 68th JECFA (2007), published in FAO JECFA Monographs 5 (2008). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

SYNONYMS INS no. 960

DEFINITION The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

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Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-6-en-8-oic acid, β-D-glucopyranosyl ester

C A.S. number

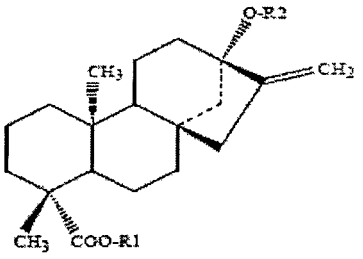
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: $C_{38}H_{60}O_{18}$
Rebaudioside A: $C_{44}H_{70}O_{23}$

Structural Formula

The seven named steviol glycosides:



Compound name	*R1*	*R2*
Stevioside	β-Glc	β-Glc-β-Glc(2→1)
Rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside C	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
Dulcoside A	β-Glc	β-Glc-α-Rha(2→1)
Rubusoside	β-Glc	β-Glc
Steviolbioside	H	β-Glc-β-Glc(2→1)
Rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides. Glc and Rha represent, respectively, glucose and rhamnose sugar moieties.

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Stevioside and rebaudioside A	The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.
pH (Vol 4)	Between 4.5 and 7.0 (1 in 100 solution)

PURITY

Total ash (Vol. 4)	Not more than 1%
Loss on drying (Vol. 4)	Not more than 6% (105°, 2h)
Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I in Volume 4, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

METHOD OF ASSAY Determine the percentages of the individual steviol glycosides by high pressure liquid chromatography (Volume 4).

Standards
Stevioside, >99.0% purity and rebaudioside A, >97% purity (available from Wako pure Chemical Industries, Ltd. Japan).

Mobile phase
Mix HPLC-grade acetonitrile and water (80:20). Adjust the pH to 3.0 with phosphoric acid (85% reagent grade). Filter through 0.22 µm Millipore filter or equivalent.

Standard solutions
(a) Accurately weigh 50 mg of dried (105°, 2 h) stevioside standard into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
(b) Repeat with previously dried rebaudioside A standard.

Sample solution
Accurately weigh 60-120 mg of dried (105°, 2 h) sample into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with

the mobile phase.

Chromatography Conditions

Column: Supelcosil LC-NH_2 or equivalent (length: 15-30 cm; inner diameter: 3.9-4.6 mm)
Mobile phase: A 80:20 mixture of acetonitrile and water (see above)
Flow rate: Adjust so that the retention time of rebaudioside A is about 21 min.
Injection volume: 5-10 µl
Detector: UV at 210 nm
Column temperature: 40°

Procedure

Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample solution and of the standard solutions.

The retention times relative to rebaudioside A (1.00) are:

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Calculate the percentage of each of the seven steviol glycosides, *X*, in the sample from the formula:

$$\%X = [W_s/W] \times [f_x A_x/A_s] \times 100$$

where

W_s is the amount (mg) of stevioside in the standard solution
W is the amount (mg) of sample in the sample solution
A_s is the peak area for stevioside from the standard solution
A_x is the peak area of *X* for the sample solution
f_x is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 (stevioside), 0.98 (dulcoside A), 1.20 (rebaudioside A), 1.18 (rebaudioside C), 0.80 (rubusoside), 0.80 (steviolbioside), and 1.00 (rebaudioside B).

Calculate the percentage of total steviol glycosides (sum the seven percentages).

APPENDIX B

CHEMISTRY REPORT FOR GRAS NOTICE FOR PYURE BRANDS' HIGH PURITY REB A

B-1 Chemistry Report for GRAS Notice for Rebaudioside A (95%)

B-2 Chemistry Report for GRAS Notice for Rebaudioside A (98%)

APPENDIX B-1

CHEMISTRY REPORT FOR GRAS NOTICE FOR REBAUDIOSIDE A (REB A (95%))

Pyure™ Brands, llc.

DECEMBER 23, 2009

CHEMISTRY REPORT FOR GRAS NOTICE FOR REBAUDIOSIDE A (REB A)

SUBMITTED BY:

Pyure Brands, LLC
4532 Tamiami Trail E. Suite #303
Naples, FL 34112 USA

CONTACT FOR TECHNICAL INFORMATION:

Pyure Brands, LLC
4532 Tamiami Trail E. Suite #303
Naples, FL 34112 USA

DATE:

October 23, 2009

CONTENTS

I. PURPOSE OF REPORT

II. PRODUCT DESCRIPTION

III. PRODUCTION PROCESS
- a. FLOW CHART
- b. EQUIPMENT USED

IV. DESCRIPTION OF METHOD (Data and Maps for 5 Batches of Samples)
- a. STEVIOL GLYCOSIDE SPECIFICATIONS
- b. CALIBRATION CURVE
- c. CHROMATOGRAMS
- d. INSPECTION REPORTS

V. CERTIFICATE OF ANALYSIS (ETHANOL)

VI. PESTICIDE RESIDUE REPORT

VII. JECFA STANDARD

VIII. APPENDIX A

IX. END OF REPORT

PURPOSE:

This is a report by Pyure Brands, LLC located in Naples, FL USA.

The purpose is to demonstrate that five (5) batches of Pyure Brands, LLC stevia (Rebaudioside A 95%+), meets the specifications and guidelines set forth by JECFA.

Pyure™ Brands, llc.

PRODUCT DESCRIPTION:

Pyure Brands, LLC
Address: 4532 Tamiami Trail E. Suite #303 Naples, FL 34112 USA
Tel: +1 (305) 509.5096
Fax: +1 (888) 226.2490
Web: www.pyuresweet.com
Email: info@pyurebrands.com

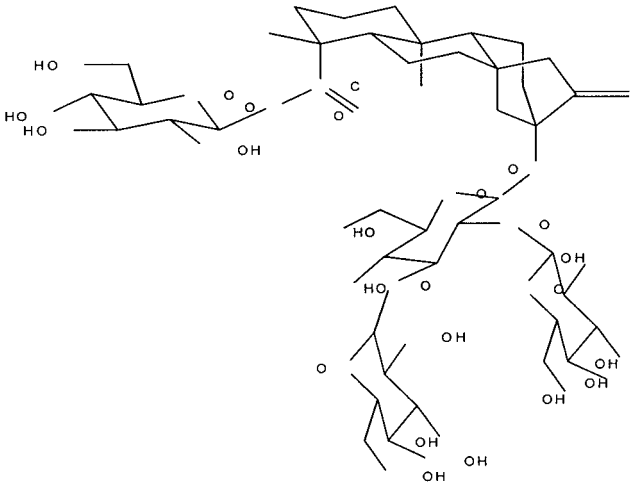
Rebaudioside A

The subject of this GRAS notice is Rebaudioside A (common or usual name Reb A). Reb A is a steviol glycoside of the Stevia plant.

Chemical name for Reb A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D- glucopyranosyl) oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester.

Molecular formula for Reb A: $C_{44}H_{70}O_{23}$
Molecular weight for Reb A: 967.

The structural formula for Reb A is shown as below:



Reb A is a natural sweetener native to the Guarani tribes of Paraguay and Brazil. It is a pure diterpene glycoside extracted from the *Stevia rebaudiana* (Bertoni) plant, which belongs to the Asteraceae family. Reb A is the second most abundant glycoside in the Stevia leaf and is considered more suitable for use to sweeten foods and beverages than stevioside due to its greater solubility in water and better overall taste profile. Reb A is generally know to taste about 200-300 times sweeter than sugar and is also non-nutritive.

It is structurally similar to other steviol glycosides, including stevioside. In many parts of the world including Japan, South Korea, Israel, Mexico, Paraguay, Brazil and Argentina, steviol glycosides (including extracts of the leaves, stevioside and Reb A) are used to sweeten foods and beverages.

The Reb A 95% manufactured by Pyure Brands, LLC is up to 400 times sweeter than cane sugar and has characteristics of a white powder with a cool and sweet taste. The melting point of this Reb A 95% is 198°C to 202°C and has a high temperature resistance. It is easy for moisture absorption in the air and soluble in water and alcohol.

The Reb A 95% has the characteristics of high sweetness and low calorie and can be used as a food additive in food, beverage, health care and medical industries, etc. It contains no odor and has no known carcinogens. The Reb A 95% has shown promise in helping prevent symptoms from hypertension, diabetes mellitus, treating obesity, heart disease and tooth decay, to name a few. Reb A is sweeter than cane sugar and is an excellent, all-natural alternative providing many healthful benefits.

PRODUCTION PROCESS:

Tel: +1 (305) 509.5096
Fax: +1 (888) 226.2490
Web: wwwpyuresweet.com
Email: info@pyurebrands.com

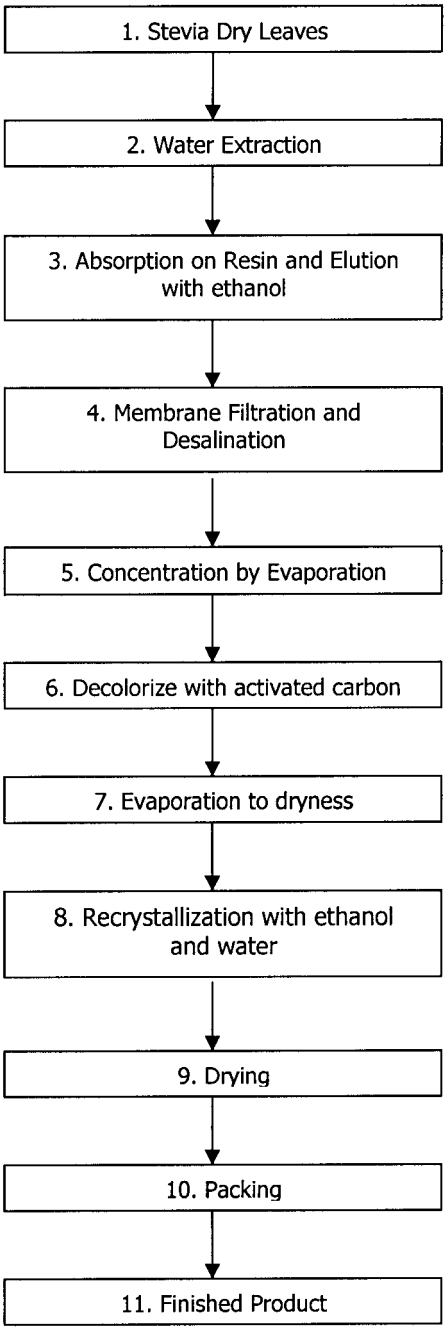
Rebaudioside A 95%

The source of Reb A is the leaves of the *Stevia rebaudiana* (Bertoni) plant.

The steviol glycoside content is slightly influenced by several factors (included but not limited to): the temperature at the particular time of the year of cultivation; latitude; and the timing of harvesting.

High purity Reb A (≥ 95%) is obtained by the extraction of Stevia leaves with water followed by adsorption onto resin where the glycosides are eluted with ethanol. The extract is filtered and desalinated and is concentrated before it is decolorized with activated carbon. The extract is dried and the resulting solid is dissolved in water/ethanol solution. Finally it is crystallized and dried, resulting in a high-purity Reb A (95%) as the final product.

Flow Chart:


1. Stevia Dry Leaves
2. Water Extraction
3. Absorption on Resin and Elution with ethanol
4. Membrane Filtration and Desalination
5. Concentration by Evaporation
6. Decolorize with activated carbon
7. Evaporation to dryness
8. Recrystallization with ethanol and water
9. Drying
10. Packing
11. Finished Product

Equipment Used In Production Process:

Name/Description	Type	Year Purchased	Condition	Employee Responsible
Steeping Tank	4000cm*1500cm*1000cm	2004	Good	Jingwen Li
Setting Tank	2500cm*3500cm	2004	Good	Weiming Wang
Plate and Frame Filter Press	800m2	2004	Good	Fuzan Wang
Resin exchanging column	800cm*2400cm	2004	Good	Fupeng Wang
Concentration Unit	10T	2004	Good	Shuangan Wang
Membrane Filtration	3T/h	2004	Good	Hongwei Li
Spraying and Drying Tower	GLZ-150	2004	Good	Yifei Cao
Boiler	4T	2004	Good	Wenhua Zhuang

DESCRIPTION OF METHOD:

DATA AND MAPS FOR THE 5 BATCHES OF SAMPLES:

To determine that samples meet JECFA specifications, the five different batches were analyzed by and according to JECFA methods.

The five batches of samples are manufactured by Pyure Brands, LLC and are tested by HPLC in the JECFA method.

HPLC SYSTEM: Purchased from Dionex, Inc. (NASDAQ: DNEX) www.dionex.com
Model purchased is type U3000 (UltiMate 3000 Series Pump Systems).

STANDARD PRODUCT OF RA: Purchased from Wako Pure Chemical Industries, Ltd. of Japan; a wholly owned subsidiary of Wako Chemicals USA, Inc. www.wako-chem.co.jp www.wakousa.com

TEST METHOD FOR STANDARD CURVE:
The 0.5mg/ml, 1.0mg/ml and 1.2mg/ml standard solution are separately prepared. Under the same conditions, they are tested by HPLC and get the standard curve.

PURITY CALCULATION:
{RA (standard)/RA(sample)} x {S(sample)/S(standard)} x 100%

LOSS ON DRYING:
Drying under 105°C for 2 hours thermostat drying box, then cooling and finally weighing.

PREPARATION OF SAMPLE INJECTION:
Accurately weigh a 50mg dried sample and then dilute it to 100ml in a volumetric flask. Dissolve with mobile phase and dilute to volume with the mobile phase.
Note: Reason for difference in retention time: the five batches were tested on different dates and there are reasonable errors about the blending proportion of the mobile phase.

Specifications Steviol Glycosides

PARAMETER	SPECIFICATION	20091108	20091109	20091110	20091112	20091114	METHOD REFERENCE
Chemical Specifications							
Total Glycosides (as St, Reb A, Reb B, Reb C, Dulc A, Rub, and SB) on dry weight basis	> 98%	97.20	95.5	96.8	96.8	95.4	JECFA
Sweetness Intensity	Specify range as time sugar sweetness	400	400	400	400	400	JECFA
Residual ethanol	<5000 ppm	<3500ppm	<3500ppm	<3500ppm	<3500ppm	<3500ppm	<3500ppm
Moisture (loss on drying)	< 6%	1.70	1.75	1.76	1.68	1.80	JECFA
Ash	<1%	0.02	0.02	0.03	0.03	0.02	JECFA
Solubility	soluble in water and ethanol	>4.8 g/100ml	>4.8 g/100ml	>4.8 g/100ml	>4.8 g/100ml	>4.8 g/100ml	JECFA
pH (1% soln)	4.5-7.0	5.81	5.78	5.70	5.81	5.62	JECFA
Lead	< 1 ppm	0.03	0.03	0.03	0.03	0.03	JECFA
Arsenic	< 1 ppm	0.1	0.1	0.1	0.1	0.1	JECFA
Microbiological Specifications							
Aerobic Plate Count	<2000 cfu/g	20	20	20	20	20	
Mold	<100 cfu/g	□10	□10	□10	□10	□10	
Yeast	< 100 cfu/g	□10	□10	□10	□10	□10	
Salmonella	Negative	Not found	Not found	Not found	Not found	Not found	
Total *E. coli*	< 3 npm/g	□3npm/g	□3npm/g	□3npm/g	□3npm/g	□3npm/g	
Fecal *E. coli*	< 3 npm/g	Not found	Not found	Not found	Not found	Not found	

Abbreviations: St=Stevioside; Reb A=Rebaudioside A; Reb B=Rebaudioside B; Reb C=Rebaudioside C; Dulc A=Dulcoside A; Rub= Rubusoside; SB=Steviolbioside

Calibration Curve

Operator:Administrator Timebase:HPLC Sequence:ARA98

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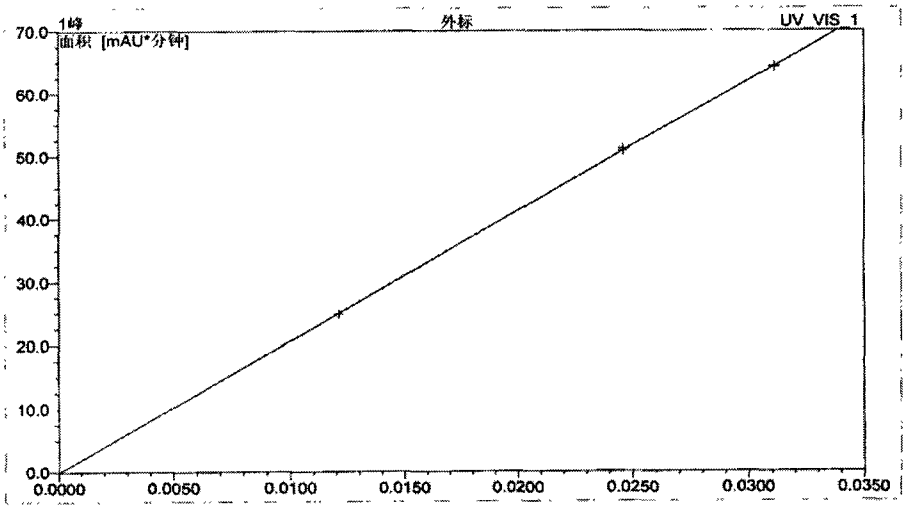
2009-10-24 4:20 下午

15 RA98(Calibration Curve)

Sample Name:	**RA98(Calibration Curve)**	*Injection Volume:*	**20.0**
Vial Number:	**15**	*Channel:*	**UV_VIS_1**
Sample Type:	**standard**	*Wavelength:*	**210**
Control Program:	**2008-12**	*Bandwidth:*	**n.a.**
Quantif. Method:	**甜菊糖**	*Dilution Factor:*	**1.0000**
Recording Time:	**2009-9-26 14:27**	*Sample Weight:*	**0.0311**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Cal.Type	Points	Coeff.Det. %	Offset	Slope	Curve
1	19.85	1峰	Lin	5	99.9900	0.0000	2066.6655	0.0000
Average:					99.9900	0.0000	2066.6655	0.0000

defltLCMS/Calibration(Curr.Peak)

Chromeleon (c) Dionex 1996-2001
Version 6.80 Build 2212

Note: The unit for the x axis of the calibration curve is in minutes (□钟). It is located at the top-left corner of the chromatograph. The sample amount for the calibration curve is 50mg, 100mg and 120mg, separately.

DESCRIPTION OF METHOD (cont): Chromatogram #1:

Batch #20091108

Operator:Administrator Timebase:HPLC Sequence:ARA98

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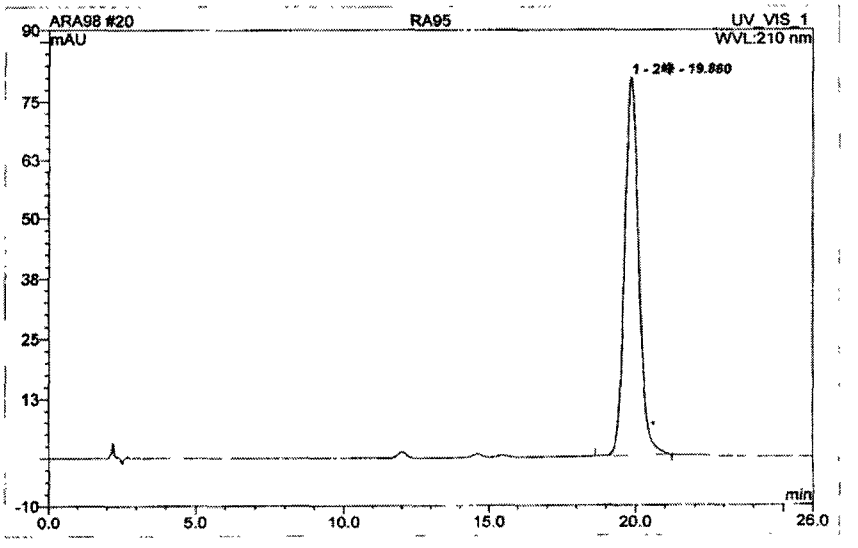
2009-11-23 10:38 上午

20 RA95

Sample Name:	**RA95**	*Injection Volume:*	**20.0**
Vial Number:	**20091108**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**210**
Control Program:	**2008-12**	*Bandwidth:*	**n.a.**
Quantif. Method:	**甜菊糖**	*Dilution Factor:*	**1.0000**
Recording Time:	**2009-11-20 14:45**	*Sample Weight:*	**0.0220**
Run Time (min):	**26.00**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	19.86	2峰	79.669	2650.546	100.00	0.972	BMB
Total:			79.669	2650.546	100.00	0.972	

defitLCMS/Integration

Chromeleon (c) Dionex 1996-2001
Version 6.80 Build 2212

DESCRIPTION OF METHOD (cont): Chromatogram #2:

Batch #20091109

Operator:Administrator Timebase:HPLC Sequence:ARA98

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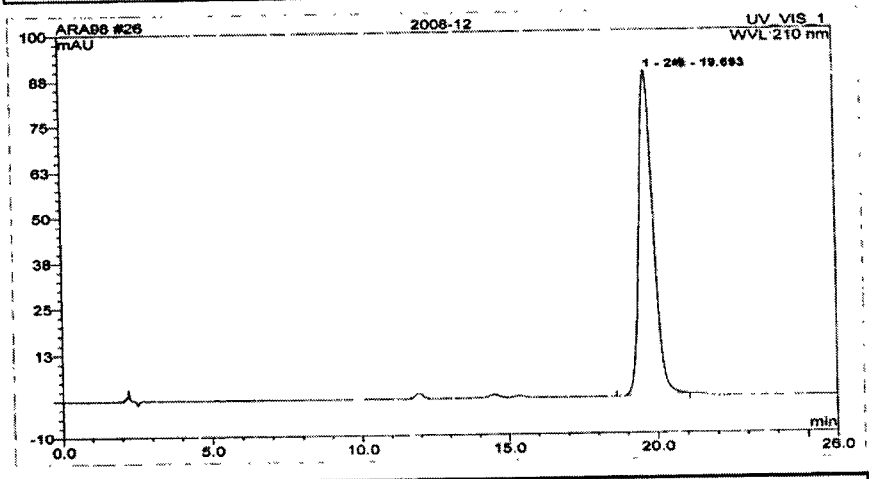
2009-11-21 4:19 下午

26 2008-12

Sample Name:	2008-12	*Injection Volume.*	20.0
Vial Number:	20091109	*Channel:*	UV_VIS_1
Sample Type:	unknown	*Wavelength*	210
Control Program:	2008-12	*Bandwidth:*	n.a.
Quantif. Method:	甜菊糖	*Dilution Factor*	1.0000
Recording Time:	2009-11-21 15:44	*Sample Weight:*	0.0229
Run Time (min):	26.00	*Sample Amount:*	1.0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	19.69	2#	88.505	48.285	100.00	0.955	BMB
Total:			88.505	48.285	100.00	0.955	

defitLCMS/Integration

Chromeleon (c) Dionex 1996-2001
Version 6 80 Build 2212

DESCRIPTION OF METHOD (cont): Chromatogram #3:

Batch #20091110

Operator:Administrator Timebase:HPLC Sequence:ARA98

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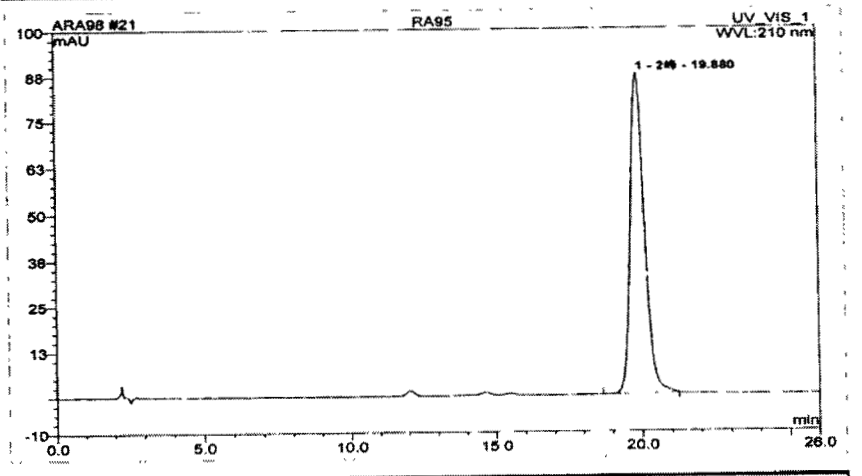
2009-11-21 3:24 下午

21 RA95

Sample Name:	RA95	*Injection Volume:*	20.0
Vial Number:	20091110	*Channel:*	UV_VIS_1
Sample Type:	unknown	*Wavelength:*	210
Control Program:	2006-12	*Bandwidth:*	n.a.
Quantif. Method:	甜菊糖	*Dilution Factor:*	1.0000
Recording Time:	2009-11-20 15:13	*Sample Weight:*	0.0242
Run Time (min):	26.00	*Sample Amount:*	1.0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	19.88	2峰	87.386	2904.856	100.00	0.968	BMB
Total:			87.386	2904.856	100.00	0.968	

defitLCMS/Integration

Chromeleon (c) Dionex 1996-2001
Version 6.80 Build 2212

DESCRIPTION OF METHOD (cont): Chromatogram #4:

Batch #20091112

Operator:Administrator Timebase:HPLC Sequence:ARA98

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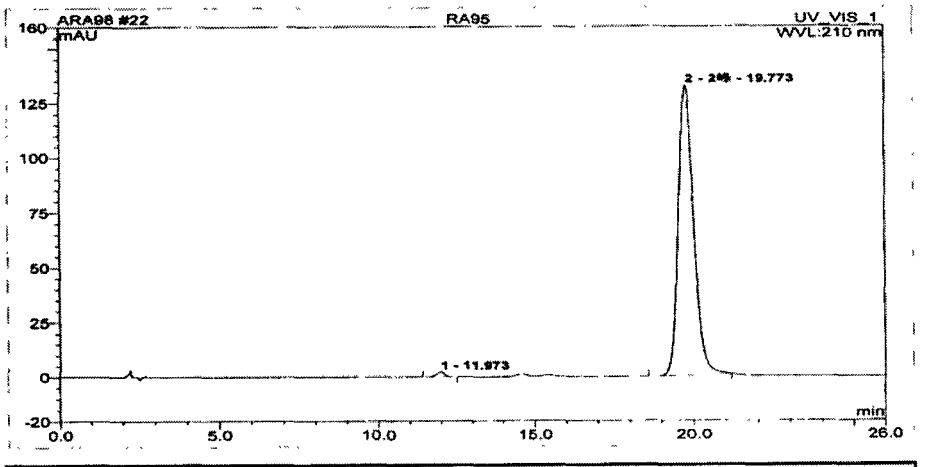
2009-11-21 3:30 PM

22 RA95

Sample Name:	RA95	*Injection Volume:*	20.0
Vial Number:	20091112	*Channel:*	UV_VIS_1
Sample Type:	unknown	*Wavelength:*	210
Control Program:	2008-12	*Bandwidth:*	n.a.
Quantif. Method:	甜菊糖	*Dilution Factor:*	1.0000
Recording Time:	2009-11-20 15:46	*Sample Weight:*	0.0366
Run Time (min):	26.00	*Sample Amount:*	1.0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11.97	n.a.	2.224	42.207	0.95	n.a.	BMB
2	19.77	2峰	132.394	4393.747	99.05	0.968	BMB
Total:			134.617	4435.955	100.00	0.968	

defltLCMS/Integration

Chromeleon (c) Dionex 1996-2001
Version 6.80 Build 2212

DESCRIPTION OF METHOD (cont): Chromatogram #5:

Batch #20091114

Operator:Administrator Timebase:HPLC Sequence:ARA98

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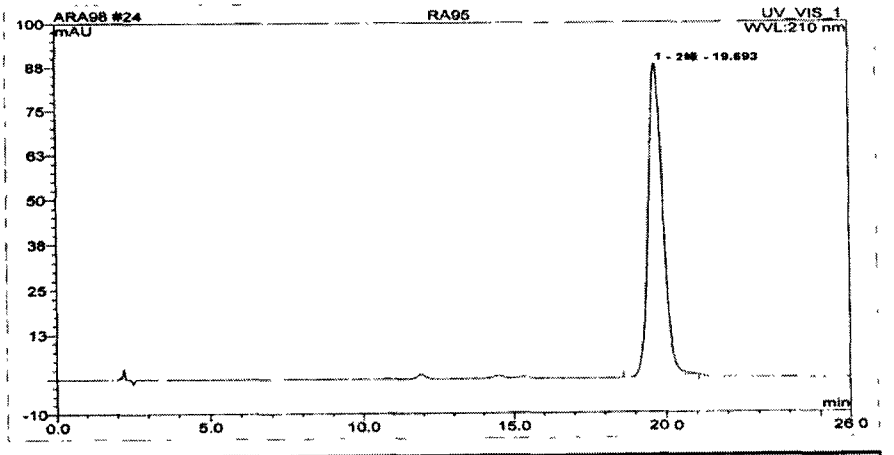
2009-11-21 3.39 下午

24 RA95

Sample Name:	RA95	*Injection Volume*	20.0
Vial Number:	20091114	*Channel:*	UV_VIS_1
Sample Type:	unknown	*Wavelength:*	210
Control Program:	2008-12	*Bandwidth*	n.a.
Quantif. Method.	甜菊糖	*Dilution Factor.*	1.0000
Recording Time.	2009-11-21 14:31	*Sample Weight:*	0.0227
Run Time (min):	26.00	*Sample Amount:*	1.0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	19.69	2峰	87.626	2867.817	100.00	0.954	BMB
Total:			87.626	2867.817	100.00	0.954	

defiltLCMS/Integration

Chromeleon (c) Dionex 1996-2001
Version 6.80 Build 2212

Note: Translation in Appendix A

Pyure Brands, LLC
Address: 4532 Tamiami Trail E. Suite #303 Naples, FL 34112 USA
Tel: +1 (305) 509.5096 Fax: +1 (888) 226.2490 Web: www.pyuresweet.com

□□□□□□

INSPECTION REPORT

□□□□□□□□ Product Name: **Stevia**	□□□□□**R-A95%** Product specifications:
□□□20091108 Batch Number:	□□□500 kg BatchQuantity:
□□□□□2009-11-08 Date of production:	□□□□□50g Sample size:
□□□□□JECFA Ref standard:	□□□□□2009-11-20 Analysis Date:

□□□□

Analytical Results

□ □ **Items**	□□ **Standard**	□ □ **Results**
1、 □□□%□(HPLC) Total Content%:	JECFA	99.34
2、 R-A□□%□ R-A Content%□	JECFA	97.20
3、 □□□ Sweetness:	JECFA 200-400	400
4、 □□□□□ Specific optical rotation :	GB8270-1999 -30°□ -38°	-31
5、 □□□□□ Specific absorbance:	GB8270-1999 ≤0.05	0.023
6、 □□□ Ash%:	JECFA ≤0.1%	0.03
7、 □□□ Moisture%:	JECFA ≤6.0%	2.26
8□ □□□□□ Ethanol residual :PPM	JECFA <5000PPM	2395
9□ PH□□□□□□%□ PH(%of solution)	JECFA 4.0-7.0	5
10□□□□AS□□□ Arsenic(AS):PPM	JECFA < 1ppm	0.10
11□□□ Lead : PPM	JECFA < 1ppm	0.03
12□□□□□□(□□□□) Microbial Index	<1000/g	<20/g
13□□□□ Appearance:	JECFA WHITE POWDER	□□□□ WHITE POWDER
1、 □□□□□ Storage condition:	GB8270-1999	□□ DRY
2、 □□□ Conclude:	□□□□□□□□□□□□ Qualified	
3、 □□□□□ Storage life:	2□ TWO YEARS	
4、 □□□ Remark:	\	

□□□Analyst□□ □□ □□ (Auditing)□□□□

Pyure Brands, LLC
Address: 4532 Tamiami Trail E. Suite #303 Naples, FL 34112 USA
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□□□□□□

INSPECTION REPORT

□□□□□□□□ Product Name: **Stevia**	□□□□□**R-A95%** Product specifications:
□□□20091109 Batch Number:	□□□500 kg Batch Quantity:
□□□□□2009-11-09 Date of production:	□□□□□50g Sample size:
□□□□□JECFA Ref standard:	□□□□□2009-11-21 Analysis Date:

□□□□

Analytical Results

□ □ **Items**	□□ **Standard**	□ □ **Results**
8、 □□□%□(HPLC) Total Content%:	JECFA	99.15
9、 R-A□□%□ R-A Content%□	JECFA	95.50
10、 □□□ Sweetness:	JECFA 200-400	400
11、 □□□□□ Specific optical rotation :	GB8270-1999 -30°□ -38°	-32
12、 □□□□□ Specific absorbance:	GB8270-1999 ≤0.05	0.021
13、 □□□ Ash%:	JECFA ≤0.1%	0.02
14、 □□□ Moisture%:	JECFA ≤6.0%	2.18
8□ □□□□□ Ethanol residual :PPM	JECFA <5000PPM	2400
9□ PH□□□□□□%□ PH(%of solution)	JECFA 4.0-7.0	5
10□□□□AS□□□ Arsenic(AS):PPM	JECFA < 1ppm	0.10
11□□□ Lead : PPM	JECFA < 1ppm	0.03
12□□□□□□(□□□□) Microbial Index	<1000/g	<20/g
13□□□□ Appearance:	JECFA WHITE POWDER	□□□□ WHITE POWDER
5、 □□□□□ Storage condition:	GB8270-1999	□□ DRY
6、 □□□ Conclude:	□□□□□□□□□□□ Qualified	
7、 □□□□□ Storage life:	2□ TWO YEARS	
8、 □□□ Remark:	\	

□□□Analyst□□ □□ □□ (Auditing)□□□□

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□□□□□□

INSPECTION REPORT

□□□□□□□□ Product Name: **Stevia**	□□□□□**R-A95%** Product specifications:
□□□20091110 Batch Number:	□□□500 kg BatchQuantity:
□□□□□2009-11-10 Date of production:	□□□□□50g Sample size:
□□□□□JECFA Ref standard:	□□□□□2009-11-20 Analysis Date:

□□□□ **Analytical Results**

□ □ **Items**	□□ **Standard**	□ □ **Results**
15、 □□□%□(HPLC) Total Content%:	JECFA	99.21
16、 R-A□□%□ R-A Content%□	JECFA	96.80
17、 □□□ Sweetness:	JECFA 200-400	400
18、 □□□□□ Specific optical rotation :	GB8270-1999 -30°□ -38°	-32
19、 □□□□□ Specific absorbance:	GB8270-1999 ≤0.05	0.022
20、 □□□ Ash%:	JECFA ≤0.1%	0.02
21、 □□□ Moisture%:	JECFA ≤6.0%	2.33
8□ □□□□□ Ethanol residual :PPM	JECFA <5000PPM	2380
9□ PH□□□□□□%□ PH(%of solution)	JECFA 4.0-7.0	5
10□□□□AS□□□ Arsenic(AS):PPM	JECFA < 1ppm	0.10
11□□□ Lead : PPM	JECFA < 1ppm	0.03
12□□□□□□(□□□□) Microbial Index	<1000/g	<20/g
13□□□□ Appearance:	JECFA WHITE POWDER	□□□□ WHITE POWDER
9、 □□□□□ Storage condition:	GB8270-1999	□□ DRY
10、 □□□ Conclude:	□□□□□□□□□□□□ Qualified	
11、 □□□□□ Storage life:	2□ TWO YEARS	
12、 □□□ Remark:	\	

□□□Analyst□□ □□ □□ (Auditing)□□□□

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□□□□□□

INSPECTION REPORT

□□□□□□□□ Product Name: **Stevia**	□□□□□**R-A95%** Product specifications:
□□□20091112 Batch Number:	□□□500 kg BatchQuantity:
□□□□□2009-11-12 Date of production:	□□□□□50g Sample size:
□□□□□JECFA Ref standard:	□□□□□2009-11-20 Analysis Date:

□□□□ **Analytical Results**

□ □ **Items**	□□ **Standard**	□ □ **Results**
22、 □□□%□(HPLC) Total Content%:	JECFA	99.22
23、 R-A□□%□ R-A Content%□	JECFA	96.80
24、 □□□ Sweetness:	JECFA 200-400	400
25、 □□□□□ Specific optical rotation :	GB8270-1999 -30°□ -38°	-31
26、 □□□□□ Specific absorbance:	GB8270-1999 ≤0.05	0.022
27、 □□□ Ash%:	JECFA ≤0.1%	0.03
28、 □□□ Moisture%:	JECFA ≤6.0%	2.35
8□ □□□□□ Ethanol residual :PPM	JECFA 5000PPM	2400
9□ PH□□□□□□%□ PH(%of solution)	JECFA 4.0-7.0	5
10□□□□AS□□□ Arsenic(AS):PPM	JECFA < 1ppm	0.10
11□□□ Lead : PPM	JECFA < 1ppm	0.03
12□□□□□□(□□□□) Microbial Index	<1000/g	<20/g
13□□□□ Appearance:	JECFA WHITE POWDER	□□□□ WHITE POWDER
13、 □□□□□ Storage condition:	GB8270-1999	□□ DRY
14、 □□□ Conclude:	□□□□□□□□□□□□ Qualified	
15、 □□□□□ Storage life:	2□ TWO YEARS	
16、 □□□ Remark:	\	

检验（Analyst）：姬红 审核 (Auditing)：王福刚

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□□□□□□

INSPECTION REPORT

□□□□□□□□ Product Name: **Stevia**	□□□□□**R-A95%** Product specifications:
□□□20091114 Batch Number:	□□□500 kg BatchQuantity:
□□□□□2009-11-14 Date of production:	□□□□□50g Sample size:
□□□□□JECFA Ref standard:	□□□□□2009-11-21 Analysis Date:

□□□□ **Analytical Results**

□ □ **Items**	□□ **Standard**	□ □ **Results**
29、 □□□%□(HPLC) Total Content%:	JECFA	99.14
30、 R-A□□%□ R-A Content%□	JECFA	95.40
31、 □□□ Sweetness:	JECFA 200-400	400
32、 □□□□□ Specific optical rotation :	GB8270-1999 -30°□ -38°	-32
33、 □□□□□ Specific absorbance:	GB8270-1999 ≤0.05	0.022
34、 □□□ Ash%:	JECFA ≤0.1%	0.03
35、 □□□ Moisture%:	JECFA ≤6.0%	2.27
8□ □□□□□ Ethanol residual :PPM	JECFA <5000PPM	2410
9□ PH□□□□□□%□ PH(%of solution)	JECFA 4.0-7.0	5
10□□□□AS□□□ Arsenic(AS):PPM	JECFA < 1ppm	0.10
11□□□ Lead : PPM	JECFA < 1ppm	0.03
12□□□□□□(□□□□) Microbial Index	<1000/g	<20/g
13□□□□ Appearance:	JECFA WHITE POWDER	□□□□ WHITE POWDER
17、 □□□□□ Storage condition:	GB8270-1999	□□ DRY
18、 □□□ Conclude:	□□□□□□□□□□□□ Qualified	
19、 □□□□□ Storage life:	2□ TWO YEARS	
20、 □□□ Remark:	\	

□□□Analyst□□ □□ □□ (Auditing)□□□□

CERTIFICATE OF ANALYSIS:

ETHANOL

Jining Sanjiu Commercial and Trading Co., Ltd.

Appearance	colorless and clear
Hazen	7
Odor	no strange smell
Taste	Purity
Ethanol(20°C),%(V/V)	96.2
Sulfuric acid test, No,	42
Oxidation test□min	26
Aldehyde (calculate with acetaldehyde) ,mg/L	12
N-propyl alcohol, mg/L	88
Fusel oil, mg/L	18
Methanol, mg/L	98
Acid (calculate with acetic acid	12
Non-volatile matter, mg/L	18
Heavy metal (calculate with Pb), mg/L	---

FAX -

济宁三九商贸有限公司	食用酒精出厂质量证明书	Q/LJJ-JL-8.2.4-15-03-01 NO.
产品原料 玉米 执行标准 GB10343-2002 产品等级		数量（T）
商方名称 济宁 车号		出厂时间

产品质量检验结果

外观	无色透明
色度，号	7
气味	无异味
口味	纯正
乙醇（20℃），%（V/V）	96.2°
硫酸试验，号	42
氧化试验，min	26
醛（以乙醛计），mg/L	12
正丙醇，mg/L	88
杂醇油（以异丁醇，异戊醇计），mg/L	18
甲醇，mg/L	98
酸（以乙酸计），mg/L	12
不挥发物，mg/L	18
重金属（以 Pb 计），mg/L	/

备注 符合GB10343-2002食用酒精. 报告日期： 审核： 检验：

PESTICIDE RESIDUE REPORT: PART 1 of 7

SGS Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

The following sample(s) was/were submitted by/ on behalf of the client as:
Sample information (sample name, batch No., manufacture) stated by the client to be:

code	SGS job No.	Sample name	Batch No.
#1	QDFDO090401282FD.1	Stevia Sugar	2009.3.10

SGS reference No.: SH9048816/CHEM
Date of receipt: Mar 23, 2009
Testing period: Mar 24, 2009 ~ Apr 03, 2009

TEST (S) REQUESTED:
Selected test(s) as requested by applicant:
--To determine the Pesticide Residue Content in the submitted samples.

TEST RESULT(S):

No.	Test Items		Test method	Unit	Test limit	Test result
1	Alachlor	甲草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
2	Aldrin	艾氏剂	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
3	Benalaxyl	苯霜灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
4	Benfluralin	乙丁氟灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
5	Benfuresate	呋草磺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
6	a-BHC	a-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
7	β-BHC	β-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
8	γ-BHC	林丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
9	δ-BHC	δ-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND

Remark:
1. The results apply only to the samples as supplied.
2. The test was carried out by a SGS laboratory.
3. ND=Not detected.
4. NR= Not recoveried

Signed for and on behalf of SGS
(b) (6)
Authorized Signature

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QDFD 0 1 7 8 2

PESTICIDE RESIDUE REPORT: PART 2 of 7

SGS

Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

No.	Test Items		Test method	Unit	Test limit	Test result
10	Bifenthrin	联苯菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
11	Bromophos-ethyl	乙基溴硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
12	Bromopropylate	溴螨酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
13	Butachlor	丁草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
14	Chlorbenside	氯杀螨	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
15	cis-Chlordane	顺式-氯丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
16	trans-Chlordane	反式-氯丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
17	Chlorfenapyr	虫螨腈	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
18	Chlorfenson	杀螨酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
19	Chlorfenvinphos	毒虫畏	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
20	Chlorobenzilate	乙酯杀螨醇	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
21	Chlorpyrifos	毒死蜱	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
22	Chlorpyrifosmethyl	甲基毒死蜱	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
23	Chlozolinate	烹氯得	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
24	λ-Cyhalothrin	λ-氯氟氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
25	o,p'-DDD	o,p'-滴滴滴	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
26	p,p'-DDD	p,p'-滴滴滴	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
27	o,p'-DDE	o,p'-滴滴伊	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
28	p,p'-DDE	p,p'-滴滴伊	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
29	o,p'-DDT	o,p'-滴滴涕	With reference to US FDA PAM, GB/T 19648-2005	mg/kg	0.01	ND
30	p,p'-DDT	p,p'-滴滴涕	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
31	Diazinon	二嗪磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
32	Dicofol	三氯杀螨醇	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
33	Dieldrin	狄氏剂	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND

Page 2 of 7

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QD... 17283

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PESTICIDE RESIDUE REPORT: PART 3 of 7

SGS

Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

No.	Test Items		Test method	Unit	Test limit	Test result
34	Dimethylvinphos	甲基毒虫畏	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
35	Disulfoton	乙拌磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
36	a-Endosulfan	a-硫丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
37	β-Endosulfan	β-硫丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
38	EPN	苯硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
39	Ethion	乙硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
40	Ethofumesate	乙氧呋草黄	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
41	Ethoprophos	灭线磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
42	Fenitrothion	杀螟硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
43	Fenpropathrin	甲氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
44	Fenthion	倍硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
45	Flusilazole	氟硅唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
46	Fonofos	地虫硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
47	Furalaxyl	呋霜灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
48	Heptachlor	七氯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
49	Heptenophos	庚烯磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
50	Hexachlorobenzene	六氯苯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
51	Isofenphos	异柳磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
52	Nitrothal-isopropyl	稻瘟酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
53	Paclobutrazol	多效唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
54	Penconazole	戊菌唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
55	Pendimethalin	二甲戊乐灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
56	Pentachloroanisole	五氯茴香醚	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
57	Phenthoate	稻丰散	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND

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QDFD 017284

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PESTICIDE RESIDUE REPORT: PART 4 of 7

SGS

Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

No.	Test Items		Test method	Unit	Test limit	Test result
58	Phorate	甲拌磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
59	Pirimiphos-ethyl	乙基嘧啶磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
60	Pirimiphos-methyl	甲基嘧啶磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
61	Profenophos	丙溴磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
62	Prometryn	扑草净	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
63	Propiconazole	丙环唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
64	Propyzamide	炔苯酰草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
65	Quinalphos	喹硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
66	Safrotin	巴胺磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
67	Salithion	蔬果磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
68	Tecnazene	四氯硝基苯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
69	Terbufos	特丁硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
70	Tetrachlorvinphos	杀虫畏	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
71	Tolclofos-methyl	甲基立枯磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
72	Trifluralin	氟乐灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
73	Fensulfothion	丰索磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
74	Triazophos	三唑磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
75	Acephate	乙酰甲胺磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
76	Acetamiprid	啶虫脒	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
77	Acetochlor	乙草胺	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
78	Anilofos	莎稗磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
79	Atrazine	莠去津	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
80	Azinophos-methyl	保棉磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND

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PESTICIDE RESIDUE REPORT: PART 5 of 7

SGS

Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

No.	Test Items		Test method	Unit	Test limit	Test result
81	Bendiocarb	恶虫威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
82	Bitertanol	联苯三唑醇	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
83	Buprofezin	噻嗪酮	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
84	Carbaryl	甲萘威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
85	Carbendazim	多菌灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	Trace
86	Carbofuran	虫螨威(克百威)	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
87	Coumaphos	蝇毒磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
88	Deltamethrin	溴氰菊酯	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
89	Dichlorvos	敌敌畏	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
90	Diethofencarb	乙霉威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
91	Dimethoate	乐果	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
92	Endosulfan sulfate	硫丹硫酸酯	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
93	Esprocarb	戊草丹	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
94	Ethiofencarb	乙硫苯威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
95	Fipronil	氟虫腈	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
96	Haloxyfop-ethoxyethyl	氟吡乙禾灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
97	Haloxyfop-methyl	氟吡甲禾灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
98	Iprovalicarb	丙森锌	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.10	ND
99	Isoprocarb	异丙威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
100	Isoprothiolane	稻瘟灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
101	Lenacil	环草啶	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
102	Linuron	利谷隆	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
103	Malathion	马拉硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
104	Mefenacet	苯噻酰草胺	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND

(b) (6)

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QDFD 017256

PESTICIDE RESIDUE REPORT: PART 6 of 7

SGS

Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

No.	Test Items		Test method	Unit	Test limit	Test result
105	Metalaxyl	甲霜灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
106	Metamitron	灭它通	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
107	Methamidophos	甲胺磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
108	Methidathion	杀扑磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
109	Mevinphos	速灭磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
110	Monocrotophos	久效磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
111	Omethoate	氧化乐果	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
112	Oxadixyl	恶霜灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
113	Oxamyl	杀线威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
114	Oxydemeton-methyl	砜吸磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
115	Parathion	对硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
116	Phosalone	伏杀硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
117	Phosmet	亚胺硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
118	Phoxim	辛硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
119	Pirimicarb	抗蚜威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
120	Promecarb	猛杀威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
121	Propargite	炔螨特	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
122	Propoxur	残杀威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
123	Pyrazophos	吡菌磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
124	Pyridaphenthion	哒嗪硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
125	Simazine	西玛津	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
126	Tebuconazole	戊唑醇	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND

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PESTICIDE RESIDUE REPORT: PART 7 of 7

SGS

Test Report **No: QDFDO090401282FD-1** **Date: Apr 13, 2009**

No.	Test Items		Test method	Unit	Test limit	Test result
127	Tebufenozide	虫酰肼	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.10	ND
128	Tebufenpyrad	吡螨胺	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
129	Thiophanate-methyl	甲基硫菌灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	Trace
130	Tolfenpyrad	唑虫酰胺	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND

(b) (6)

Sample description: sample in the plastic bag.

Page 7 of 7
*** End of Report***

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JECFA METHOD

JECFA Standard

STEVIOL GLYCOSIDES

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding specifications prepared at the 68th JECFA (2007), published in FAO JECFA Monographs 5 (2008). An
ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the
69th JECFA (2008).

SYNONYMS INS no. 960

DEFINITION The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B generally present in preparations of steviol glycosides
at levels lower than stevioside or rebaudioside A.

Chemical name

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D- glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-6-en-8-oic acid, β-D- glucopyranosyl ester

C.A.S. number

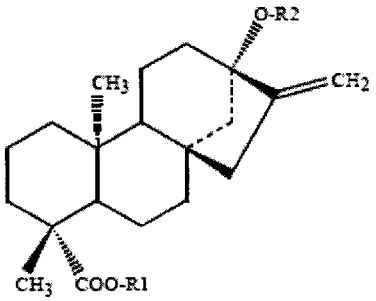
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: $C_{38}H_{60}O_{18}$
Rebaudioside A: $C_{44}H_{70}O_{23}$

Structural Formula

The seven named steviol glycosides:



Compound name	*R1*	*R2*
Stevioside	□-Glc	□-Glc-□-Glc(2□1)
Rebaudioside A	□-Glc	□-Glc-□-Glc(2□1) □-Glc(3□1)
Rebaudioside C	□-Glc	□-Glc-*a*-Rha(2□1) □-Glc(3□1)
Dulcoside A	□-Glc	□-Glc-*a*-Rha(2□1)
Rubusoside	□-Glc	□-Glc
Steviolbioside	H	□-Glc-□-Glc(2□1)
Rebaudioside B	H	□-Glc-□-Glc(2□1) □-Glc(3□1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides.
Glc and Rha represent, respectively, glucose and rhamnose sugar moieties.

Formula weight — Stevioside: 804.88
Rebaudioside A: 967.03

Assay — Not less than 95% of the total of the seven named steviol glycosides, on the dried basis.

DESCRIPTION — White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.

FUNCTIONAL USES — Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) — Freely soluble in water

Stevioside and rebaudioside A — The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A

pH (Vol. 4) — Between 4.5 and 7.0 (1 in 100 solution)

PURITY

Total ash (Vol. 4) — Not more than 1%

Loss on drying (Vol. 4) — Not more than 6% (105°, 2h)

Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000mg/kg ethanol (Method I in Volume 4, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

METHOD OF ASSAY

Determine the percentages of the individual steviol glycosides by high pressure liquid chromatography (Volume 4).

Standards
Stevioside, >99.0% purity and rebaudioside A, >97% purity (available from Wako pure Chemical Industries, Ltd. Japan).

Mobile phase
Mix HPLC-grade acetonitrile and water (80:20). Adjust the pH to 3.0 with phosphoric acid (85% reagent grade). Filter through 0.22 µm Millipore filter or equivalent.

Standard solutions
(a) Accurately weigh 50 mg of dried (105°, 2 h) stevioside standard into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
(b) Repeat with previously dried rebaudioside A standard.

Sample solution
Accurately weigh 60-120 mg of dried (105°, 2 h) sample into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with the mobile phase.

Chromatography Conditions
Column: Supelcosil LC-NH_2 or equivalent (length: 15-30 cm; inner diameter: 3.9-4.6 mm)
Mobile phase: A 80:20 mixture of acetonitrile and water (see above) Flow rate: Adjust so that the retention time of rebaudioside A is
about 21 min.
Injection volume: 5-10 µl Detector:
UV at 210 nm Column temperature:
40°

Procedure
Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample solution and of the standard solutions.

The retention times relative to rebaudioside A (1.00) are:

0.45-0.48 for stevioside 0.12-0.16 for rubusoside
0.25-0.30 for dulcoside A 0.35-0.41 for steviolbioside
0.63-0.69 for rebaudioside C 0.73-0.79 for rebaudioside B

Measure the peak areas for the seven steviol glycosides from the sample solution (the minor components might not be detected). Measure the peak area for stevioside for the standard solution.

Calculate the percentage of each of the seven steviol glycosides, *X*, in the sample from the formula:

$$\%X = [WS/W] \times [fXAX/AS] \times 100$$

where
WS is the amount (mg) of stevioside in the standard solution
W is the amount (mg) of sample in the sample solution
AS is the peak area for stevioside from the standard solution
A*X* is the peak area of *X* for the sample solution
f*X* is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 (stevioside), 0.98 (dulcoside A), 1.20 (rebaudioside A), 1.18 (rebaudioside C), 0.80 (rubusoside), 0.80 (steviolbioside), and 1.00 (rebaudioside B).

Calculate the percentage of total steviol glycosides (sum the seven percentages)

APPENDIX A:

APPENDIX A

1. □□□□□ Name of the sample
2. □□□□ Bottle No. of the sample
3. □□□□□ Type of the sample
4. □□□□□ Control Procedure
5. □□□□□ Quantitative methods
6. □□□□□ Record time
7. □□□□□ Run time
8. □□□□□ Volume
9. □□□ Path
10. □□□ Wave length
11. □□□ Band width
12. □□□□□ Diluting factor
13. □□□□□ Weight of the sample
14. □□□□ Inside scalar
15. No.

16. □□□□□ Retention time

17. □□□□ Name of peak

18. □□□ Height

19. □□□ Area

20. □□□□ Relative area

21. □□□□□ Relative quantity

-END OF REPORT-

APPENDIX B-2

CHEMISTRY REPORT FOR GRAS NOTICE FOR REBAUDIOSIDE A (REB A (98%))

Pyure™ Brands, llc.

DECEMBER 23, 2009

CHEMISTRY REPORT FOR GRAS NOTICE FOR REBAUDIOSIDE A (REB A)

SUBMITTED BY:

Pyure Brands, LLC
4532 Tamiami Trail E. Suite #303
Naples, FL 34112 USA

CONTACT FOR TECHNICAL INFORMATION:

Pyure Brands, LLC
4532 Tamiami Trail E. Suite #303
Naples, FL 34112 USA

DATE:

December 23, 2009

CONTENTS:

I. PURPOSE OF REPORT

II. PRODUCT DESCRIPTION

III. PRODUCTION PROCESS
   a. FLOW CHART
   b. EQUIPMENT USED

IV. DESCRIPTION OF METHOD (Data and Maps for 5 Batches of Samples)
   a. STEVIOL GLYCOSIDE SPECIFICATIONS
   b. CALIBRATION CURVE
   c. CHROMATOGRAMS
   d. INSPECTION REPORTS

V. CERTIFICATE OF ANALYSIS (ETHANOL)

VI. PESTICIDE RESIDUE REPORT

VII. JECFA STANDARD

VIII. APPENDIX A

IX. END OF REPORT

PURPOSE:

This is a report by Pyure Brands, LLC located in Naples, FL USA.

The purpose is to demonstrate that five (5) batches of Pyure Brands, LLC stevia (Rebaudioside A 98%+), meets the specifications and guidelines set forth by JECFA.

PRODUCT DESCRIPTION:

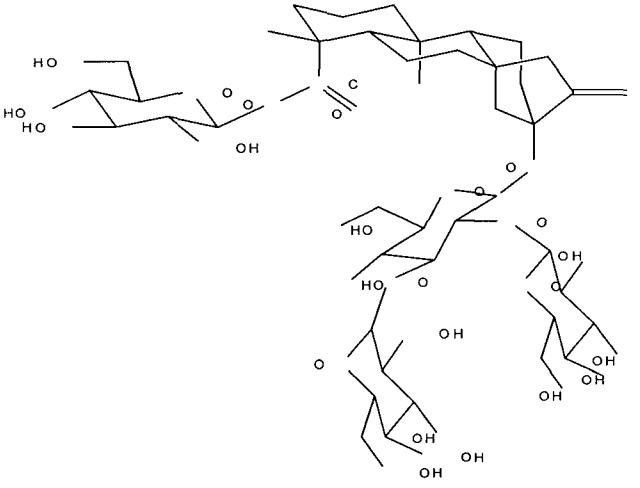
Pyure Brands, LLC
Address: 4532 Tamiami Trail E. Suite #303 Naples, FL 34112 USA
Tel: +1 (305) 509.5096
Fax: +1 (888) 226.2490
Web: www.pyuresweet.com
Email: info@pyurebrands.com

Rebaudioside A

The subject of this GRAS notice is Rebaudioside A (common or usual name Reb A). Reb A is a steviol glycoside of the Stevia plant.

Chemical name for Reb A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D- glucopyranosyl) oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester.

Molecular formula for Reb A: $C_{44}H_{70}O_{23}$
Molecular weight for Reb A: 967.03
The structural formula for Reb A is shown as below:



Reb A is a natural sweetener native to the Guarani tribes of Paraguay and Brazil. It is a pure diterpene glycoside extracted from the *Stevia rebaudiana* (Bertoni) plant, which belongs to the Asteraceae family. Reb A is the second most abundant glycoside in the Stevia leaf and is considered more suitable for use to sweeten foods and beverages than stevioside due to its greater solubility in water and better overall taste profile. Reb A is generally know to taste about 200-300 times sweeter than sugar and is also non-nutritive.

It is structurally similar to other steviol glycosides, including stevioside. In many parts of the world including Japan, South Korea, Israel, Mexico, Paraguay, Brazil and Argentina, steviol glycosides (including extracts of the leaves, stevioside and Reb A) are used to sweeten foods and beverages.

The Reb A 98% manufactured by Pyure Brands, LLC is up to 400 times sweeter than cane sugar and has characteristics of a white powder with a cool and sweet taste. The melting point of this Reb A 98% is 198°C to 202°C and has a high temperature resistance. It is easy for moisture absorption in the air and soluble in water and alcohol.

The Reb A 98% has the characteristics of high sweetness and low calorie and can be used as a food additive in food, beverage, health care and medical industries, etc. It contains no odor and has no known carcinogens. The Reb A 98% has shown promise in helping prevent symptoms from hypertension, diabetes mellitus, treating obesity, heart disease and tooth decay, to name a few. Reb A is sweeter than cane sugar and is an excellent, all-natural alternative providing many healthful benefits.

PRODUCTION PROCESS:

Pyure Brands, LLC
Address: 4532 Tamiami Trail E. Suite #303 Naples, FL 34112 USA
Tel: +1 (305) 509.5096
Fax: +1 (888) 226.2490
Web: wwwpyuresweet.com
Email: info@pyurebrands.com

Rebaudioside A 98%

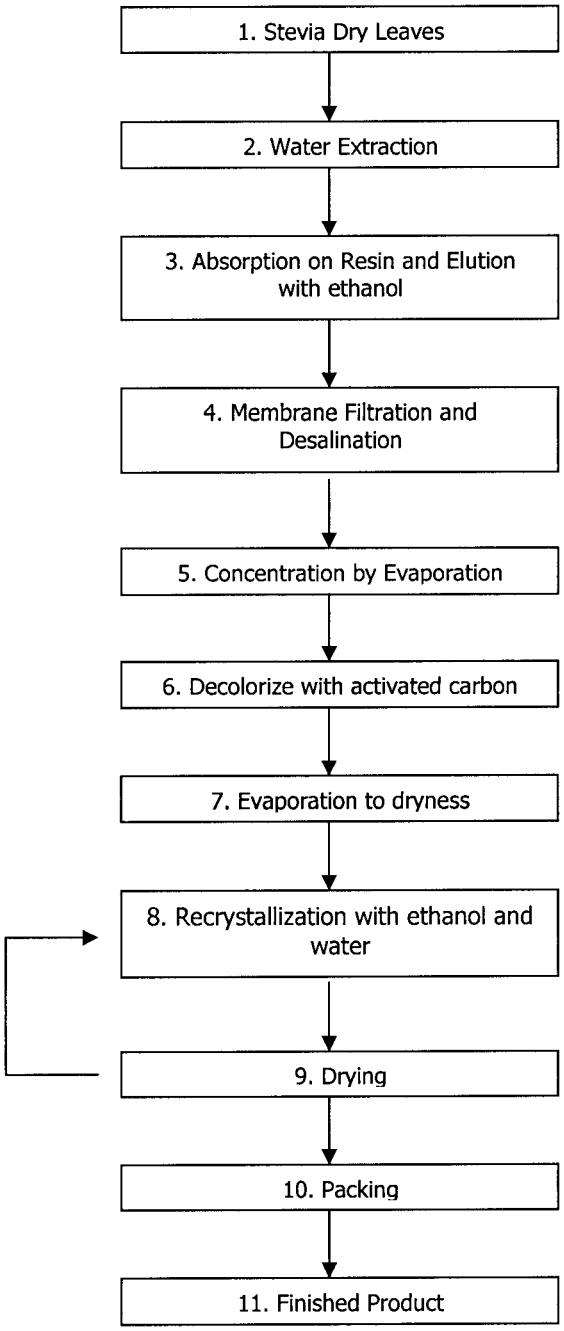
The source of Reb A is the leaves of the *Stevia rebaudiana* (Bertoni) plant.

The steviol glycoside content is slightly influenced by several factors (included but not limited to): the temperature at the particular time of the year of cultivation; latitude; and the timing of harvesting.

High purity Reb A (≥ 95%) is obtained by the extraction of Stevia leaves with water followed by adsorption onto resin where the glycosides are eluted with ethanol. The extract is filtered and desalinated and the concentrated before it is decolorized with activated carbon. The extract is dried and the resulting solid is dissolved in water/ethanol solution for recrystallization. Several recrystallization steps are necessary to obtain high-purity Reb A (98%) before the product is dried for packing.

PRODUCTION PROCESS (cont):

Flow Chart:



PRODUCTION PROCESS (cont):

Equipment Used In Production Process:

Name/Description	Type	Year Purchased	Condition	Employee Responsible
Steeping Tank	4000cm*1500cm*1000cm	2004	Good	Jingwen Li
Setting Tank	2500cm*3500cm	2004	Good	Weiming Wang
Plate and Frame Filter Press	800m2	2004	Good	Fuzan Wang
Resin exchanging column	800cm*2400cm	2004	Good	Fupeng Wang
Concentration Unit	10T	2004	Good	Shuangan Wang
Membrane Filtration	3T/h	2004	Good	Hongwei Li
Spraying and Drying Tower	GLZ-150	2004	Good	Yifei Cao
Boiler	4T	2004	Good	Wenhua Zhuang

DESCRIPTION OF METHOD:

DATA AND MAPS FOR THE 5 BATCHES OF SAMPLES:

To determine that samples meet JECFA specifications, the five different batches were analyzed by and according to JECFA methods.

The five batches of samples are manufactured by Pyure Brands, LLC and are tested by HPLC in the JECFA method.

HPLC SYSTEM: Purchased from Dionex, Inc. (NASDAQ: DNEX) www.dionex.com
Model purchased is type U3000 (UltiMate 3000 Series Pump Systems).

STANDARD PRODUCT OF RA: Purchased from Wako Pure Chemical Industries, Ltd. of Japan; a wholly owned subsidiary of Wako Chemicals USA, Inc. www.wako-chem.co.jp www.wakousa.com

TEST METHOD FOR STANDARD CURVE:
The 0.5mg/ml, 1.0mg/ml and 1.2mg/ml standard solution are separately prepared. Under the same conditions, they are tested by HPLC and get the standard curve.

PURITY CALCULATION:
{RA (standard)/RA(sample)} x {S(sample)/S(standard)} x 100%

LOSS ON DRYING:
Drying under 105°C for 2 hours thermostat drying box, then cooling and finally weighing.

PREPARATION OF SAMPLE INJECTION:

Accurately weigh a 50mg dried sample and then dilute it to 100ml in a volumetric flask. Dissolve with mobile phase and dilute to volume with the mobile phase.

Note: Reason for difference in retention time: the five batches were tested on different dates and there are reasonable errors about the blending proportion of the mobile phase.

The chromatograms of batches 20090906 and 20090930 show the presence of other steviol glycosides; Reb C and stevioside. They are identified as a very small quantity in total content and can be ignored.

Specifications Steviol Glycosides

PARAMETER	SPECIFICATION	090825	090905	090906	090913	090925	METHOD REFERENCE
Chemical Specifications							
Total Glycosides (as St, Reb A, Reb B, Reb C, Dulc A, Rub, and SB) on dry weight basis	> 98%	98□42	98□44	98□50	98□52	98□60	JECFA
Sweetness Intensity	Specify range as time sugar sweetness	400	400	400	400	400	JECFA
Residual methanol	<200 ppm	<10ppm	<10ppm	<10ppm	<10ppm	<10ppm	JECFA
Residual ethanol	<5000 ppm	<3500ppm	<3500ppm	<3500ppm	<3500ppm	<3500ppm	<3500ppm
Moisture (loss on drying)	< 6%	1.65	1.56	1.58	1.62	1.56	JECFA
Ash	<1%	0.03	0.02	0.03	0.02	0.03	JECFA
Solubility	soluble in water and ethanol	>4.8 g/100ml	>4.8 g/100ml	>4.8 g/100ml	>4.8 g/100ml	>4.8 g/100ml	JECFA
pH (1% soln)	4.5-7.0	5	5	5	5	5	JECFA
Lead	< 1 ppm	0.03	0.03	0.03	0.03	0.03	JECFA
Arsenic	< 1 ppm	0.1	0.1	0.1	0.1	0.1	JECFA
Microbiological Specifications							
Aerobic Plate Count	<2000 cfu/g	20	20	20	20	20	
Mold	<100 cfu/g	□10	□10	□10	□10	□10	
Yeast	< 100 cfu/g	□10	□10	□10	□10	□10	
Salmonella	Negative	Not found	Not found	Not found	Not found	Not found	
Total *E. coli*	< 3 npm/g	□3npm/g	□3npm/g	□3npm/g	□3npm/g	□3npm/g	
Fecal *E. coli*	< 3 npm/g	Not found	Not found	Not found	Not found	Not found	

Abbreviations: St=Stevioside; Reb A=Rebaudioside A; Reb B=Rebaudioside B; Reb C=Rebaudioside C; Dulc A=Dulcoside A; Rub= Rubusoside; SB=Steviolbioside

DESCRIPTION OF METHOD (cont): Calibration Curve:

Calibration Curve

Operator:Administrator Timebase:HPLC Sequence:ARA98

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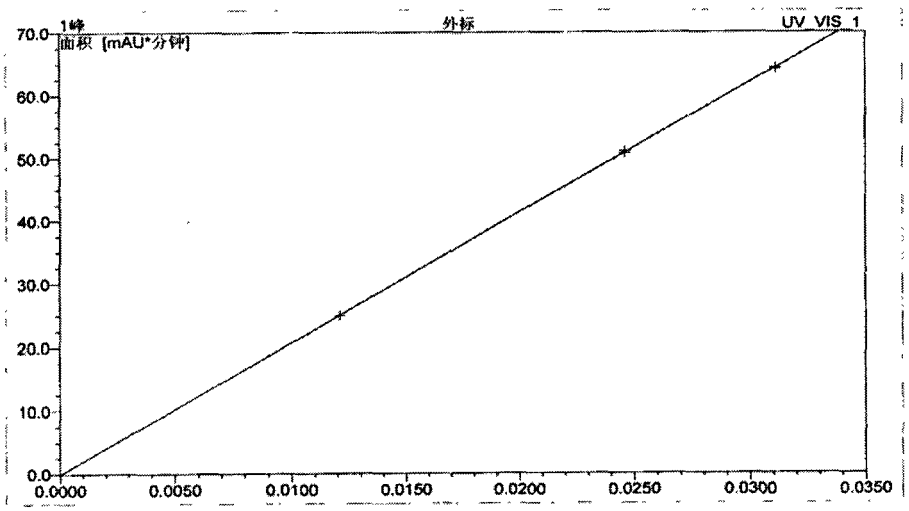
2009-10-24 4:20 下午

15 RA98(Calibration Curve)

Sample Name:	**RA98(Calibration Curve)**	*Injection Volume:*	**20.0**
Vial Number:	**15**	*Channel:*	**UV_VIS_1**
Sample Type:	**standard**	*Wavelength:*	**210**
Control Program:	**2008-12**	*Bandwidth:*	**n.a.**
Quantif Method:	**甜菊糖**	*Dilution Factor:*	**1.0000**
Recording Time:	**2009-9-26 14:27**	*Sample Weight:*	**0.0311**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Cal.Type	Points	Coeff.Det. %	Offset	Slope	Curve
1	19.85	1峰	Lin	5	99.9900	0.0000	2066.6655	0.0000
Average:					99.9900	0.0000	2066.6655	0.0000

defltLCMS/Calibration(Curr.Peak)

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Note: The unit for the x axis of the calibration curve is in minutes (□钟). It is located at the top-left corner of the chromatograph. The sample amount for the calibration curve is 50mg, 100mg and 120mg, separately.

DESCRIPTION OF METHOD (cont): Chromatogram #1:

Batch #20090825

Operator:Administrator Timebase:HPLC Sequence:ARA98

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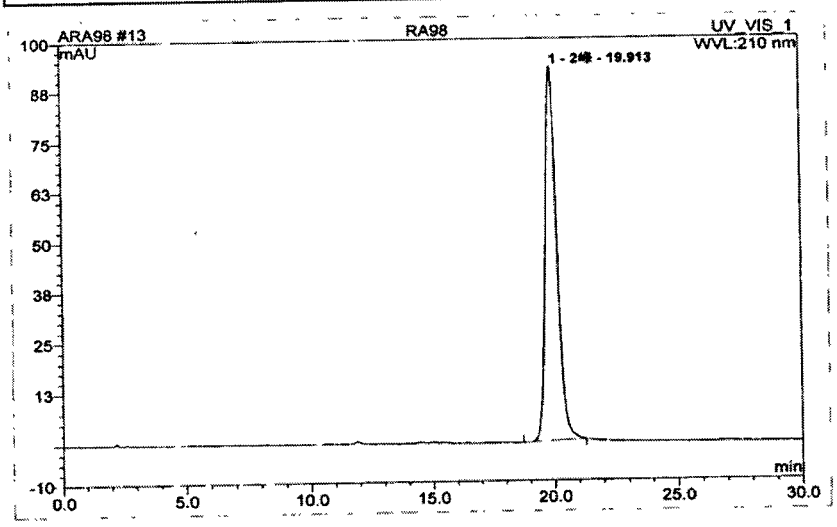
2009-10-24 5:10 下午

36 RA98

Sample Name:	RA98%	*Injection Volume:*	20.0
Vial Number:	36	*Channel:*	UV_VIS_1
Sample Type:	Stevia	*Wavelength:*	210
Control Program:	2008-12	*Bandwidth:*	n.a.
Test Method	JECFA	*Dilution Factor:*	1.0000
Recording Time:	2009-8-26 10:00	*Batch No.*	20090825
Run Time (min):	30.00	*Sample Amount:*	1.0000



No.	Ret.Time min	PeakName	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	19.91	RA98	93.041	3044.904	100.00	0.981	BMB
Total:			93.041	3044.904	100.00	0.000	

defltLCMS/Integration

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Note: a larger sample was weighed out here

DESCRIPTION OF METHOD (cont): Chromatogram #2:

Batch #20090905

Operator:Administrator Timebase:HPLC Sequence:ARA98

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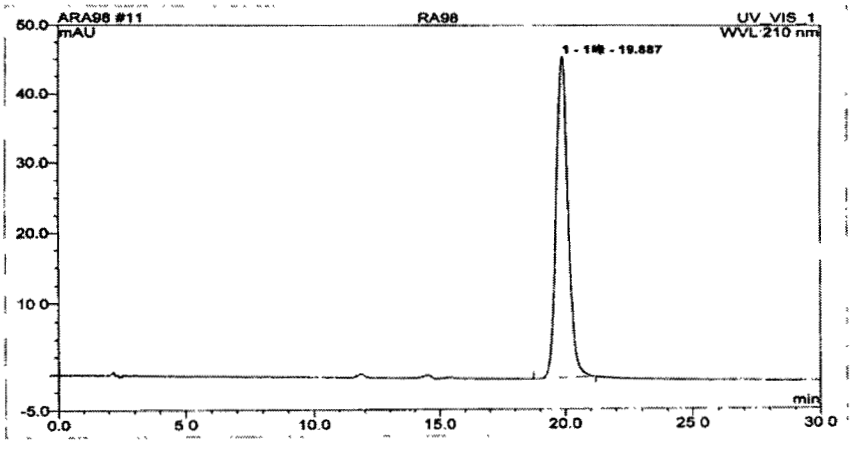
2009-9-6 4:16 下午

11 RA98

Sample Name:	RA 98%	*Injection Volume:*	20.0
Vial Number:	11	*Channel:*	UV_VIS_1
Sample Type:	Stevia	*Wavelength:*	210
Control Program:	2008-12	*Bandwidth:*	n.a.
Test Method	JECFA	*Dilution Factor:*	1.0000
Recording Time:	2009-9-6 10:32	*Batch No*	20090905
Run Time (min):	30.00	*Sample Amount:*	1.0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	19.89	RA	46.081	1494.609	100.00	0.981	BMB
Total:			46.081	1494.609	100.00	0.981	

defitt.CMS/Integration

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Version 6.80 Build 2212

DESCRIPTION OF METHOD (cont): Chromatogram #3:

Batch #20090906

Operator:Administrator Timebase:HPLC Sequence:ARA98

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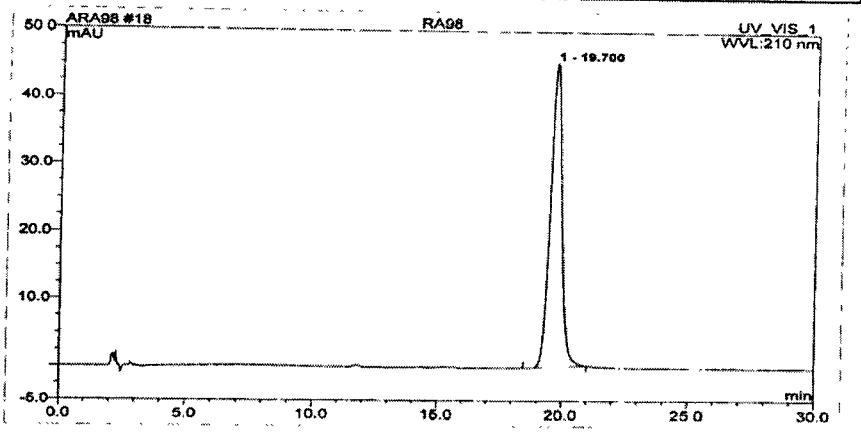
2009-9-9 1:21 下午

18 RA98

Sample Name:	RA 98%	*Injection Volume:*	20.0
Vial Number:	18	*Channel:*	UV_VIS_1
Sample Type:	Stevia	*Wavelength:*	210
Control Program:	2008-12	*Bandwidth:*	n.a.
Test Method	JECFA	*Dilution Factor:*	1.0000
Recording Time:	2009-9-9 0:00	*Batch No.*	20090906
Run Time (min):	30.00	*Sample Amount:*	1.0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	19.70	RA	45.363	1453.585	100.00	n.a.	BMB
Total:			45.363	1453.585	100.00	0.000	

defitLCMS/Integration

Chromeleon (c) Dionex 1996-2001
Version 6.80 Build 2212

DESCRIPTION OF METHOD (cont): Chromatogram #4:

Batch #20090913

Operator:Administrator Timebase:HPLC Sequence:ARA98

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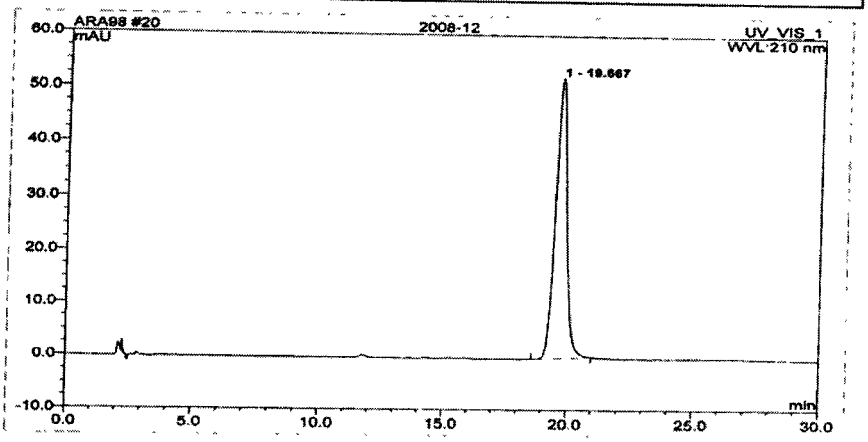
2009-9-13 2:30 PM

20 RA 98%

Sample Name:	RA 98%	*Injection Volume:*	20.0
Vial Number:	20	*Channel.*	UV_VIS_1
Sample Type:	Stevia	*Wavelength:*	210
Control Program:	2008-12	*Bandwidth:*	n.a.
Test Method	JECFA	*Dilution Factor:*	1.0000
Recording Time:	2009-9-13 0:00	*Batch No.*	20090913
Run Time (min):	30.00	*Sample Amount:*	1.0000



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	19.67	RA	52.338	1670.733	100.00	n.a.	BMB
Total:			52.338	1670.733	100.00	0.000	

defittLCMS/Integration

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Version 6.80 Build 2212

DESCRIPTION OF METHOD (cont): Chromatogram #5:

Batch #20090925

Operator:Administrator Timebase:HPLC Sequence:FDARA98

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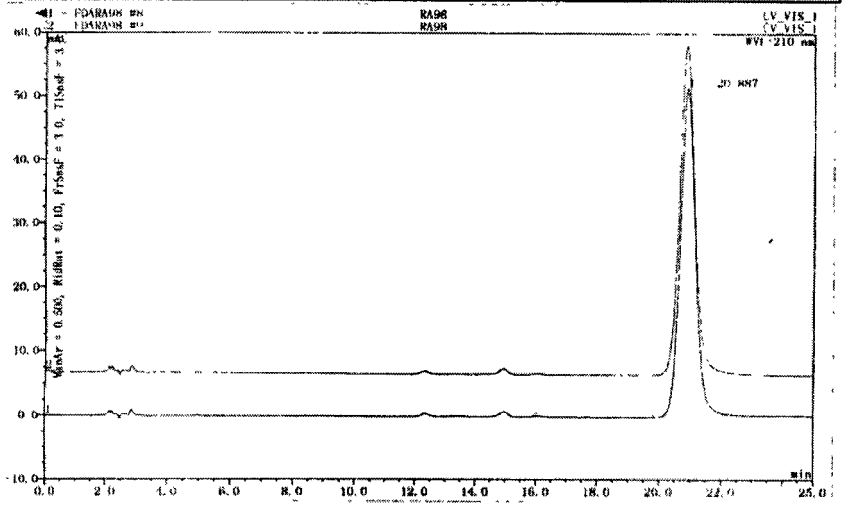
2009-9-30 10:38 上午

8 RA98

样品名称:	80	进样体积:	10.0
样品瓶号:	8	通道:	UV_VIS_1
样品类型:	unknown	波长:	210
控制程序:	2008-12	带宽:	n.a.
定量方法:	甜菊糖	稀释因子:	1.0000
记录时间:	9-25-09 17:00	样品重量:	0.0140
运行时间(min):	25.00	内标量:	1.0000



样品序号	名称	保留时间 min	面积 mAU*分钟	高度 mAU	数量	类型	塔板数 (EP)
		0.34 - 21.0.34 - 21.0.34 - 21.0.34 - 21.0.34 - 21.0.34 - 21.					
		UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
8	RA98	20.89	29.260	51.428	n.a.	BMB	9107
9	RA98	20.86	29.216	51.497	n.a.	BMB	9125
	平均:	20.873	29.233	51.462	n.a.		9116
	RSD:	0.090 %	0.083 %	0.095 %	n.a.		0.140 %

111/峰分析

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Note: Translation in Appendix A

Pyure Brands, LLC
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□□□□□□

INSPECTION REPORT

□□□□□□□□ Product Name: **Stevia**	□□□□□**R-A98%** Product specifications:
□□□20090825 Batch Number:	□□□500 kg BatchQuantity:
□□□□□2009-08-25 Date of production:	□□□□□50g Sample size:
□□□□□JECFA Ref standard:	□□□□□2009-08-26 Analysis Date:

□□□□

Analytical Results

□ □ **Items**	□□ **Standard**	□ □ **Results**
36、 □□□%□(HPLC) Total Content%:	JECFA	98.96
37、 R-A□□%□ R-A Content%□	JECFA	98.42
38、 □□□ Sweetness:	JECFA 200-400	400
39、 □□□□□ Specific optical rotation :	GB8270-1999 -30°□ -38°	-30
40、 □□□□□ Specific absorbance:	GB8270-1999 ≤0.05	0.025
41、 □□□ Ash%:	JECFA ≤0.1%	0.03
42、 □□□ Moisture%:	JECFA ≤6.0%	1.65
8□ □□□□□ Ethanol residual :PPM	JECFA 5000PPM	10
9□ PH□□□□□□%□ PH(%of solution)	JECFA 4.0-7.0	5
10□□□□AS□□□ Arsenic(AS):PPM	JECFA < 1ppm	0.10
11□□□ Lead : PPM	JECFA < 1ppm	0.03
12□□□□□□(□□□□) Microbial Index	<1000/g	<20/g
13□□□□ Appearance:	JECFA WHITE POWDER	□□□□ WHITE POWDER
21、 □□□□□ Storage condition:	GB8270-1999	□□ DRY
22、 □□□ Conclude:	□□□□□□□□□□□□ Qualified	
23、 □□□□□ Storage life:	2□ TWO YEARS	
24、 □□□ Remark:	\	

□□□Analyst□□ □□ □□ (Auditing)□□□□

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INSPECTION REPORT

□□□□□□□□ Product Name: **Stevia**	□□□□□**R-A98%** Product specifications:
□□□20090905 Batch Number:	□□□500 kg BatchQuantity:
□□□□□2009-09-05 Date of production:	□□□□□50g Sample size:
□□□□□JECFA Ref standard:	□□□□□2009-09-06 Analysis Date:

□□□□

Analytical Results

□ □ **Items**	□□ **Standard**	□ □ **Results**
43、 □□□%□(HPLC) Total Content%:	JECFA	98.56
44、 R-A□□%□ R-A Content%□	JECFA	98.44
45、 □□□ Sweetness:	JECFA 200-400	400
46、 □□□□□ Specific optical rotation :	GB8270-1999 -30°□ -38°	-31
47、 □□□□□ Specific absorbance:	GB8270-1999 ≤0.05	0.020
48、 □□□ Ash%:	JECFA ≤0.1%	0.02
49、 □□□ Moisture%:	JECFA ≤6.0%	1.56
8□ □□□□□ Ethanol residual :PPM	JECFA 5000PPM	10
9□ PH□□□□□□□%□ PH(%of solution)	JECFA 4.0-7.0	5
10□□□□AS□□□ Arsenic(AS):PPM	JECFA < 1ppm	0.10
11□□□ Lead : PPM	JECFA < 1ppm	0.03
12□□□□□□(□□□□) Microbial Index	<1000/g	<20/g
13□□□□ Appearance:	JECFA WHITE POWDER	□□□□ WHITE POWDER
25、 □□□□□ Storage condition:	GB8270-1999	□□ DRY
26、 □□□ Conclude:	□□□□□□□□□□□ Qualified	
27、 □□□□□ Storage life:	2□ TWO YEARS	
28、 □□□ Remark:	\	

□□□Analyst□□ □□ □□ (Auditing)□□□□

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INSPECTION REPORT

□□□□□□□□ Product Name: **Stevia**	□□□□□**R-A98%** Product specifications:
□□□20090906 Batch Number:	□□□500 kg BatchQuantity:
□□□□□2009-09-06 Date of production:	□□□□□50g Sample size:
□□□□□JECFA Ref standard:	□□□□□2009-09-09 Analysis Date:

□□□□ **Analytical Results**

□ □ **Items**	□□ **Standard**	□ □ **Results**
50、 □□□%□(HPLC) Total Content%:	JECFA	98.82
51、 R-A□□%□ R-A Content%□	JECFA	98.50
52、 □□□ Sweetness:	JECFA 200-400	400
53、 □□□□□ Specific optical rotation :	GB8270-1999 -30°□ -38°	-31
54、 □□□□□ Specific absorbance:	GB8270-1999 ≤0.05	0.021
55、 □□□ Ash%:	JECFA ≤0.1%	0.03
56、 □□□ Moisture%:	JECFA ≤6.0%	1.58
8□ □□□□□ Ethanol residual :PPM	JECFA 5000PPM	10
9□ PH□□□□□□%□ PH(%of solution)	JECFA 4.0-7.0	5
10□□□□AS□□□ Arsenic(AS):PPM	JECFA < 1ppm	0.10
11□□□ Lead : PPM	JECFA < 1ppm	0.03
12□□□□□□(□□□□) Microbial Index	<1000/g	<20/g
13□□□□ Appearance:	JECFA WHITE POWDER	□□□□ WHITE POWDER
29、 □□□□□ Storage condition:	GB8270-1999	□□ DRY
30、 □□□ Conclude:	□□□□□□□□□□□□ Qualified	
31、 □□□□□ Storage life:	2□ TWO YEARS	
32、 □□□ Remark:	\	

□□□Analyst□□ □□ □□ (Auditing)□□□□

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INSPECTION REPORT

□□□□□□□□ Product Name: **Stevia**	□□□□□**R-A98%** Product specifications:
□□□20090913 Batch Number:	□□□500 kg BatchQuantity:
□□□□□2009-09-13 Date of production:	□□□□□50g Sample size:
□□□□□JECFA Ref standard:	□□□□□2009-09-13 Analysis Date:

□□□□ **Analytical Results**

□ □ **Items**	□□ **Standard**	□ □ **Results**
57、 □□□%□(HPLC) Total Content%:	JECFA	99□10
58、 R-A□□%□ R-A Content%□	JECFA	98.52
59、 □□□ Sweetness:	JECFA 200-400	400
60、 □□□□□ Specific optical rotation :	GB8270-1999 -30°□ -38°	-31
61、 □□□□□ Specific absorbance:	GB8270-1999 ≤0.05	0.023
62、 □□□ Ash%:	JECFA ≤0.1%	0.02
63、 □□□ Moisture%:	JECFA ≤6.0%	1.62
8□ □□□□□ Ethanol residual :PPM	JECFA 5000PPM	10
9□ PH□□□□□□%□ PH(%of solution)	JECFA 4.0-7.0	5
10□□□□AS□□□ Arsenic(AS):PPM	JECFA < 1ppm	0.10
11□□□ Lead : PPM	JECFA < 1ppm	0.03
12□□□□□□(□□□□) Microbial Index	<1000/g	<20/g
13□□□□ Appearance:	JECFA WHITE POWDER	□□□□ WHITE POWDER
33、 □□□□□ Storage condition:	GB8270-1999	□□ DRY
34、 □□□ Conclude:	□□□□□□□□□□□□ Qualified	
35、 □□□□□ Storage life:	2□ TWO YEARS	
36、 □□□ Remark:	\	

检验 (Analyst) ： 姬红 审核 (Auditing) : 王福刚

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□□□□□□

INSPECTION REPORT

□□□□□□□□ Product Name: **Stevia**	□□□□□**R-A98%** Product specifications:
□□□20090924 Batch Number:	□□□500 kg BatchQuantity:
□□□□□2009-09-24 Date of production:	□□□□□50g Sample size:
□□□□□JECFA Ref standard:	□□□□□2009-09-25 Analysis Date:

□□□□ **Analytical Results**

□ □ **Items**	□□ **Standard**	□ □ **Results**
64、 □□□%□(HPLC) Total Content%:	JECFA	99□20
65、 R-A□□%□ R-A Content%□	JECFA	98.60
66、 □□□ Sweetness:	JECFA 200-400	400
67、 □□□□□ Specific optical rotation :	GB8270-1999 -30°□ -38°	-31
68、 □□□□□ Specific absorbance:	GB8270-1999 ≤0.05	0.021
69、 □□□ Ash%:	JECFA ≤0.1%	0.03
70、 □□□ Moisture%:	JECFA ≤6.0%	1.56
8□ □□□□□ Ethanol residual :PPM	JECFA 5000PPM	10
9□ PH□□□□□□%□ PH(%of solution)	JECFA 4.0-7.0	5
10□□□□AS□□□ Arsenic(AS):PPM	JECFA < 1ppm	0.10
11□□□ Lead : PPM	JECFA < 1ppm	0.03
12□□□□□□(□□□□) Microbial Index	<1000/g	<20/g
13□□□□ Appearance:	JECFA WHITE POWDER	□□□□ WHITE POWDER
37、 □□□□□ Storage condition:	GB8270-1999	□□ DRY
38、 □□□ Conclude:	□□□□□□□□□□□□ Qualified	
39、 □□□□□ Storage life:	2□ TWO YEARS	
40、 □□□ Remark:	\	

□□Analyst□□ □□ □□ (Auditing)□□□□

CERTIFICATE OF ANALYSIS:

ETHANOL

Jining Sanjiu Commercial and Trading Co., Ltd.

Appearance	colorless and clear
Hazen	7
Odor	no strange smell
Taste	Purity
Ethanol(20°C),%(V/V)	96.2
Sulfuric acid test, No,	42
Oxidation test□min	26
Aldehyde (calculate with acetaldehyde) ,mg/L	12
N-propyl alcohol, mg/L	88
Fusel oil, mg/L	18
Methanol, mg/L	98
Acid (calculate with acetic acid	12
Non-volatile matter, mg/L	18
Heavy metal (calculate with Pb), mg/L	---

FAX

济宁三九商贸有限公司	食用酒精出厂质量证明书				Q/LJJ-JL-8.2.4-15-03-01 NO.
产品原料	玉米	执行标准	GB10343-2002	产品等级	数量（T）
需方名称	济宁		车号		出厂时间

产品质量检验结果

外观	无色透明
色度，号	7
气味	无异味
口味	纯正
乙醇（20℃），%（V/V）	96.2
硫酸试验，号	42
氧化试验，min	26
醛（以乙醛计），mg/L	12
正丙醇，mg/L	88
杂醇油（以异丁醇，异戊醇计），mg/L	18
甲醇，mg/L	98
酸（以乙酸计），mg/L	12
不挥发物，mg/L	18
重金属（以Pb计），mg/L	/

备注：

报告日期：　　审核：　　检验：

PESTICIDE RESIDUE REPORT: PART 1 of 7

SGS Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

The following sample(s) was/were submitted by/ on behalf of the client as:
Sample information (sample name, batch No., manufacture) stated by the client to be:

code	SGS job No.	Sample name	Batch No.
#1	QDFDO090401282FD.1	Stevia Sugar	2009.3.10

SGS reference No.: SH9048816/CHEM
Date of receipt: Mar 23, 2009
Testing period: Mar 24, 2009 ~ Apr 03, 2009

TEST (S) REQUESTED:
Selected test(s) as requested by applicant:
--To determine the Pesticide Residue Content in the submitted samples.

TEST RESULT(S):

No.	Test Items		Test method	Unit	Test limit	Test result
1	Alachlor	甲草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
2	Aldrin	艾氏剂	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
3	Benalaxyl	苯霜灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
4	Benfluralin	乙丁氟灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
5	Benfuresate	呋草磺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
6	a-BHC	a-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
7	β-BHC	β-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
8	γ-BHC	林丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
9	δ-BHC	δ-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND

Remark:
1. The results apply only to the samples as supplied.
2. The test was carried out by a SGS laboratory.
3. ND=Not detected.
4. NR= Not recoverled

Signed for and on behalf of SGS
(b) (6)
[redacted]
Authorized Signatory

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PESTICIDE RESIDUE REPORT: PART 2 of 7

SGS

Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

No.	Test Items		Test method	Unit	Test limit	Test result
10	Bifenthrin	联苯菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
11	Bromophos-ethyl	乙基溴硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
12	Bromopropylate	溴螨酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
13	Butachlor	丁草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
14	Chlorbenside	氯杀螨	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
15	cis-Chlordane	顺式-氯丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
16	trans-Chlordane	反式-氯丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
17	Chlorfenapyr	虫螨腈	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
18	Chlorfenson	杀螨酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
19	Chlorfenvinphos	毒虫畏	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
20	Chlorobenzilate	乙酯杀螨醇	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
21	Chlorpyrifos	毒死蜱	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
22	Chlorpyrifosmethyl	甲基毒死蜱	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
23	Chlozolinate	克氯得	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
24	λ-Cyhalothrin	λ-氯氟氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
25	o,p'-DDD	o,p'-滴滴滴	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
26	p,p'-DDD	p,p'-滴滴滴	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
27	o,p'-DDE	o,p'-滴滴伊	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
28	p,p'-DDE	p,p'-滴滴伊	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
29	o,p'-DDT	o,p'-滴滴涕	With reference to US FDA PAM, GB/T 19648-2005	mg/kg	0.01	ND
30	p,p'-DDT	p,p'-滴滴涕	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
31	Diazinon	二嗪磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
32	Dicofol	三氯杀螨醇	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
33	Dieldrin	狄氏剂	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND

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PESTICIDE RESIDUE REPORT: PART 3 of 7

SGS

Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

No.	Test Items		Test method	Unit	Test limit	Test result
34	Dimethylvinphos	甲基毒虫畏	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
35	Disulfoton	乙拌磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
36	a-Endosulfan	a-硫丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
37	β-Endosulfan	β-硫丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
38	EPN	苯硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
39	Ethion	乙硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
40	Ethofumesate	乙氧呋草黄	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
41	Ethoprophos	灭线磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
42	Fenitrothion	杀螟硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
43	Fenpropathrin	甲氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
44	Fenthion	倍硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
45	Flusilazole	氟硅唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
46	Fonofos	地虫硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
47	Furalaxyl	呋霜灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
48	Heptachlor	七氯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
49	Heptenophos	庚烯磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
50	Hexachlorobenzene	六氯苯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
51	Isofenphos	异柳磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
52	Nitrothal-isopropyl	酞菌酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
53	Paclobutrazol	多效唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
54	Penconazole	戊菌唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
55	Pendimethalin	二甲戊乐灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
56	Pentachloroanisole	五氯间香醚	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
57	Phenthoate	稻丰散	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND

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PESTICIDE RESIDUE REPORT: PART 4 of 7

SGS

Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

No.	Test Items		Test method	Unit	Test limit	Test result
58	Phorate	甲拌磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
59	Pirimiphos-ethyl	乙基嘧啶磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
60	Pirimiphos-methyl	甲基嘧啶磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
61	Profenophos	丙溴磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
62	Prometryn	扑草净	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
63	Propiconazole	丙环唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
64	Propyzamide	炔苯酰草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
65	Quinalphos	喹硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
66	Safrotin	巴胺磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
67	Salithion	蔬果磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
68	Tecnazene	四氯硝基苯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
69	Terbufos	特丁硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
70	Tetrachloryinphos	杀虫畏	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
71	Tolclofos-methyl	甲基立枯磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
72	Trifluralin	氟乐灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
73	Fensulfothion	丰索磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
74	Triazophos	三唑磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
75	Acephate	乙酰甲胺磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
76	Acetamiprid	啶虫脒	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
77	Acetochlor	乙草胺	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
78	Anilofos	莎稗磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
79	Atrazine	莠去津	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
80	Azinophos-methyl	保棉磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND

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PESTICIDE RESIDUE REPORT: PART 5 of 7

SGS

Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

No.	Test Items		Test method	Unit	Test limit	Test result
81	Bendiocarb	恶虫威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
82	Bitertanol	联苯三唑醇	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
83	Buprofezin	噻嗪酮	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
84	Carbaryl	甲萘威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
85	Carbendazim	多菌灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	Trace
86	Carbofuran	虫螨威(克百威)	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
87	Coumaphos	蝇毒磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
88	Deltamethrin	溴氰菊酯	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
89	Dichlorvos	敌敌畏	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
90	Diethofencarb	乙霉威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
91	Dimethoate	乐果	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
92	Endosulfan sulfate	硫丹硫酸酯	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
93	Esprocarb	戎草丹	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
94	Ethiofencarb	乙硫苯威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
95	Fipronil	氟虫腈	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
96	Haloxyfop-ethoxyethyl	氟吡乙禾灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
97	Haloxyfop-methyl	氟吡甲禾灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
98	Iprovalicarb	丙森锌	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.10	ND
99	Isoprocarb	异丙威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
100	Isoprothiolane	稻瘟灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
101	Lenacil	环草啶	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
102	Linuron	利谷隆	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
103	Malathion	马拉硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
104	Mefenacet	苯噻酰草胺	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND

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PESTICIDE RESIDUE REPORT: PART 6 of 7

SGS

Test Report No: QDFDO090401282FD-1 Date: Apr 13, 2009

No.	Test Items		Test method	Unit	Test limit	Test result
105	Metalaxyl	甲霜灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
106	Metamitron	灭它通	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
107	Methamidophos	甲胺磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
108	Methidathion	杀扑磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
109	Mevinphos	速灭磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
110	Monocrotophos	久效磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
111	Omethoate	氧化乐果	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
112	Oxadixyl	恶霜灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
113	Oxamyl	杀线威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
114	Oxydemeton-methyl	砜吸磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
115	Parathion	对硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
116	Phosalone	伏杀硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
117	Phosmet	亚胺硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
118	Phoxim	辛硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
119	Pirimicarb	抗蚜威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
120	Promecarb	猛杀威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
121	Propargite	炔螨特	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
122	Propoxur	残杀威	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
123	Pyrazophos	吡菌磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
124	Pyridaphenthion	哒嗪硫磷	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
125	Simazine	西玛津	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
126	Tebuconazole	戊唑醇	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND

(b) (6)

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SGS-CSTC QINGDAO BRANCH

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SGS

QDFD 017287

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PESTICIDE RESIDUE REPORT: PART 7 of 7

SGS

Test Report **No: QDFDO090401282FD-1** **Date: Apr 13, 2009**

No.	Test Items		Test method	Unit	Test limit	Test result
127	Tebufenozide	虫酰肼	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.10	ND
128	Tebufenpyrad	吡螨胺	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND
129	Thiophanate-methyl	甲基硫菌灵	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	Trace
130	Tolfenpyrad	唑虫酰胺	With reference to US FDA PAM, GB/T 20769-2006	mg/kg	0.01	ND

(b) (6)

Sample description: sample in the plastic bag

SGS-CSTC QINGDAO BRANCH STANDARDS TECHNICAL SERVICES CO., LTD.

Page 7 of 7
*** End of Report***

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JECFA METHOD:

JECFA Standard

STEVIOL GLYCOSIDES

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding specifications prepared at the 68th JECFA (2007), published in FAO JECFA Monographs 5 (2008). An
ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the
69th JECFA (2008).

SYNONYMS INS no. 960

DEFINITION The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B generally present in preparations of steviol glycosides
at levels lower than stevioside or rebaudioside A.

Chemical name

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D- glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-6-en-8-oic acid, β-D- glucopyranosyl ester

C.A.S. number

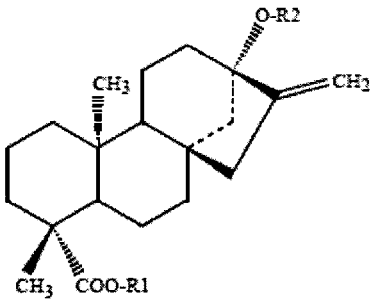
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: C38H60O18
Rebaudioside A: C44H70O23

Structural Formula

The seven named steviol glycosides:



Compound name	*R1*	*R2*
Stevioside	□-Glc	□-Glc-□-Glc(2□1)
Rebaudioside A	□-Glc	□-Glc-□-Glc(2□1) □-Glc(3□1)
Rebaudioside C	□-Glc	□-Glc-*a*-Rha(2□1) □-Glc(3□1)
Dulcoside A	□-Glc	□-Glc-*a*-Rha(2□1)
Rubusoside	□-Glc	□-Glc
Steviolbioside	H	□-Glc-□-Glc(2□1)
Rebaudioside B	H	□-Glc-□-Glc(2□1) □-Glc(3□1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides.
Glc and Rha represent, respectively, glucose and rhamnose sugar moieties.

Formula weight

Stevioside: 804.88
Rebaudioside A: 967.03

Assay

Not less than 95% of the total of the seven named steviol glycosides, on the dried basis.

DESCRIPTION

White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.

FUNCTIONAL USES

Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Freely soluble in water

Stevioside and rebaudioside A

The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A

pH (Vol. 4)

Between 4.5 and 7.0 (1 in 100 solution)

PURITY

Total ash (Vol. 4)

Not more than 1%

Loss on drying (Vol. 4)

Not more than 6% (105°, 2h)

Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000mg/kg ethanol (Method I in Volume 4, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

METHOD OF ASSAY

Determine the percentages of the individual steviol glycosides by high pressure liquid chromatography (Volume 4).

Standards
Stevioside, >99.0% purity and rebaudioside A, >97% purity (available from Wako pure Chemical Industries, Ltd. Japan).

Mobile phase
Mix HPLC-grade acetonitrile and water (80:20). Adjust the pH to 3.0 with phosphoric acid (85% reagent grade). Filter through 0.22 µm Millipore filter or equivalent.

Standard solutions
(a) Accurately weigh 50 mg of dried (105°, 2 h) stevioside standard into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
(b) Repeat with previously dried rebaudioside A standard.

Sample solution
Accurately weigh 60-120 mg of dried (105°, 2 h) sample into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with the mobile phase.

Chromatography Conditions
Column: Supelcosil LC-NH_2 or equivalent (length: 15-30 cm; inner diameter: 3.9-4.6 mm)
Mobile phase: A 80:20 mixture of acetonitrile and water (see above) Flow rate: Adjust so that the retention time of rebaudioside A is
about 21 min.
Injection volume: 5-10 µl Detector:
UV at 210 nm Column temperature:
40°

Procedure
Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample solution and of the standard solutions.

The retention times relative to rebaudioside A (1.00) are:

0.45-0.48 for stevioside 0.12-0.16 for rubusoside
0.25-0.30 for dulcoside A 0.35-0.41 for steviolbioside
0.63-0.69 for rebaudioside C 0.73-0.79 for rebaudioside B

Measure the peak areas for the seven steviol glycosides from the sample solution (the minor components might not be detected). Measure the peak area for stevioside for the standard solution.

Calculate the percentage of each of the seven steviol glycosides, *X*, in the sample from the formula:

$$\%X = [WS/W] \times [fXAX/AS] \times 100$$

where
WS is the amount (mg) of stevioside in the standard solution
W is the amount (mg) of sample in the sample solution
AS is the peak area for stevioside from the standard solution
A*X* is the peak area of *X* for the sample solution
f*X* is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 (stevioside), 0.98 (dulcoside A), 1.20 (rebaudioside A), 1.18 (rebaudioside C), 0.80 (rubusoside), 0.80 (steviolbioside), and 1.00 (rebaudioside B).

Calculate the percentage of total steviol glycosides (sum the seven percentages)

APPENDIX A:

APPENDIX A

1. □□□□□ Name of the sample
2. □□□□□ Bottle No. of the sample
3. □□□□□ Type of the sample
4. □□□□□ Control Procedure
5. □□□□□ Quantitative methods
6. □□□□□ Record time
7. □□□□□ Run time
8. □□□□□ Volume
9. □□□ Path
10. □□□ Wave length
11. □□□ Band width
12. □□□□□ Diluting factor
13. □□□□□ Weight of the sample
14. □□□□ Inside scalar

15. □□□ No.

16. □□□□□ Retention time

17. □□□□ Name of peak

18. □□□ Height

19. □□□ Area

20. □□□□□ Relative area

21. □□□□□ Relative quantity

-END OF REPORT-

APPENDIX C

SUMMARY OF STEVIOL GLYCOSIDES SAFETY STUDIES REVIEWED BY JECFA

The literature on steviol glycosides (other than on purified rebaudioside A) and on steviol that was relied upon in the JECFA reviews are summarized below. The JECFA summaries for these studies appeared in the first draft of a monograph (WHO, 2006) and in an addendum (WHO, 2009).

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

Many studies in rats (Wingard et al., 1980; Nakayama et al., 1986; Koyama et al., 2003a) and other animal models, including chickens (Geuns et al. 2003c), hamsters (Hutapea et al., 1999), and pigs (Geuns et al., 2003b) indicate that stevioside is not readily absorbed from the GI tract. Transport of steviol was more than an order of magnitude faster than stevioside or Rebaudioside A in an *in vitro* system using human colon carcinoma cell line (Geuns, 2003b).

There is evidence from *in vitro* metabolism studies that bacteria in the colon of rats and humans can transform various stevia glycosides into steviol (Gardana et al., 2003). Steviol was shown to be more readily transported with *in vitro* intestinal preparations than various steviosides (Geuns et al. 2003b, Koyama et al., 2003b). Slow absorption of steviol was indicated by detection in the plasma of rats given oral stevioside (Wang et al., 2004). However, Sung (2002) did not detect plasma steviol after oral doses of steviosides when administered to rats. In studies with human and rat liver extracts, it was demonstrated that steviol can be converted to various glucuronides (Koyama et al., 2003b).

Excretion of metabolites of stevioside after oral doses has been found 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., 2003b).

In a study using 10 healthy human subjects, blood, urine and fecal metabolites were measured after subjects received 3 doses of 250 mg of purified stevioside (>97%) 3 times a day for 3 days. Urine was collected for 24 hours on day 3 and blood and fecal samples were also taken on day 3. Free steviol was detected in feces but not in blood or urine. Steviol glucuronide was detected in blood, urine and feces. 76% of the total steviol equivalents dosed were recovered in urine and feces. Based on the measurements, the author concluded that there was complete conversion in the colon to steviol, which was absorbed and rapidly converted to the glucuronide (Geuns, et al., 2006).

B. Subchronic Toxicity Studies

Several subchronic studies with oral administration of steviol glycosides have been conducted in rats (Aze et al., 1991, Mitsuhashi 1976, Akashi and Yokoyama, 1975).

The most recent and the most well documented subchronic study was a 13-week toxicity study was carried out in Fischer 344 rats given doses of 0, 0.31, 0.62, 1.25, 2.5, or 5% in the diet (equivalent to 160, 310, 630, 1300, and 2500 mg/kg bw/day) to determine the appropriate doses for a two-year study of carcinogenicity. The rats were randomly allocated to six groups, each

consisting of 10 males and 10 females. None of the animals died during the administration period, and there was no difference in body-weight gain between the control and treated groups during administration or in food consumption in the latter part of the study. The activity of lactic dehydrogenase and the incidence of single-cell necrosis in the liver were increased in all groups of treated males. The authors considered these effects to be nonspecific because of the lack of a clear dose-response relationship, the relatively low severity, and their limitation to males. Other statistically significant differences in hematological and biochemical parameters were also considered to be of minor toxicological significance. The authors concluded that a concentration of 5% in the diet was a suitable maximum tolerable dose of stevioside for a two-year study in rats (Aze et al., 1991).

In earlier 3-month rat studies reviewed by Geuns (2003a)---the sample purity, doses, strain of rat were not reported---a no effect level was determined to be in excess of 2500 mg/kg bw/day and 7% of the diet, apparently due to lack of effects at highest dose tested in both studies (Akashi and Yokoyama, 1975).

C. Reproductive and Developmental Studies[17]

S. rebaudiana has been used by Indians in Paraguay as an oral contraceptive (Mazzei-Planas and Kuc, 1968; Schvartzman et al., 1977). Crude stevia leaf extract has been shown to inhibit fertility in rats (Mazzei-Planas and Kuc, 1968). Several reproductive studies have been done with orally administered purified steviol glycosides. No effect on fertility or reproductive parameters was seen in a three-generation study in hamsters at doses up to 2500 mg/kg (sample purity 90% stevioside; Yodyingyuad et al., 1991). There was an absence of statistically significant effects at doses up to 3% (equivalent to 3000 mg/kg bw/day; sample purity 96% stevioside; Mori et al., 1981). Similar results were observed in an additional rat study that was reviewed by Geuns (2003a) where limited information is available in English (sample purity 95.6% stevioside[1] Usami et al., 1995).

D. Mutagenicity and Genotoxicity on Steviol Glycosides

Many mutagenicity and genotoxicity studies on stevioside and they are summarized in Table C-1. All showed an absence of adverse genetic activity with the exception of the comet assay performed by Nunes et al. (2007).

[17] Since the last JECFA review, an additional developmental study on steviol glycosides has been published in the scientific literature. No effects on pregnancy or developmental parameters were observed in Swiss albino mice with stevioside or aqueous stevia extract at doses up to 800 mg/kg bw/day in female mice (Kumar and Oommen, 2008).

Table C-1. Mutagenicity & Genotoxicity Studies on Stevia Extracts & Various Steviol Glycosides

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCEN-TRATION / DOSE	RESULT	REFERENCE
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	99	50 mg/plate	Negative[a]	Suttajit et al. (1993)
Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Stevioside	83	5 mg/plate[e] 1 mg/plate[f]	Negative	Matsui et al. (1996)
Forward mutation	*S. typhimurium* TM677	Stevioside	83	10 mg/plate	Negative[a]	Matsui et al. (1996)
Forward mutation	*S. typhimurium* TM677	Stevioside	NS	Not specified	Negative[a]	Medon et al. (1982)
Forward mutation	*S .typhimurium* TM677	Stevioside	NS	10 mg/plate	Negative[a]	Pezzuto et al. (1985)
Gene mutation (umu)	*S. typhimurium* A1535/pSK1002	Stevioside	83	5 mg/plate	Negative[a]	Matsui et al. (1996)
Gene mutation	*B. subtilis* H17 rec+, M45 rec-	Stevioside	83	10 mg/disk	Negative[a]	Matsui et al. (1996)
Gene mutation	Mouse lymphoma L5178Y cells, TK- locus	Stevioside	NS	5 mg/mL	Negative[a,b]	Oh et al. (1999)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	83	8 mg/mL 12 mg/mL	Negative	Matsui et al. (1996)
Chromosomal aberration	Human lymphocytes	Stevioside	NS	10 mg/mL	Negative	Suttajit et al. (1993)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	85	12 mg/mL	Negative[e]	Ishidate et al. (1984)
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Rebaudio-side A	NS	1.2—55 mg/mL	Negative[a]	Nakajima (2000a)
Mutation	*D. melanogaster* Muller 5 strain	Stevioside	NS	2% in feed	Negative[b]	Kerr et al. (1983)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevioside, 52; rebaudio-side A, 22	250—2000 mg/kg	Negative[c]	Sekihashi et al. (2002)
DNA damage (comet assay)	Male ddY mouse stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	Stevia	NS	2000 mg/kg	Negative[c]	Sasaki et al. (2002)
Micronucleus formation	ddY mouse bone marrow and regenerating liver	Stevioside	NS	62.5—250 mg/kg	Negative[b]	Oh et al. (1999)
Micronucleus formation	BDF1 mouse bone marrow	Rebaudio-side A	NS	500-2000 mg/kg bw per day for 2 days	Negative[d]	Nakajima (2000b)
Comet Assay	Wistar rats (Blood, liver and brain cells examined)	Stevioside	88.62%	Wistar rats treated w/ 4 mg/ml stev-ioside sol-lution via oral admin-istration for 45 days.	Positive Stevioside generated DNA lesions in the blood, liver (36 x higher than control), brain (2.5 x higher than control) and spleen (3.4 x higher than control).	Nunes et al., 2007

NS = Not specified. [a] With and without metabolic activation (source not specified in original monograph). [b] Inadequate detail available. [c] Sacrificed at 3 hours and 24 hours. [d] Sacrificed at 30 hours after 2nd administration. [e] Without metabolic activation.

E. Chronic Toxicity Studies on Steviol Glycosides

There have been three chronic rat studies conducted on steviol glycosides. No treatment related increase in tumor incidence was seen in any of these studies. In the most recent and best documented study (additional study details were presented to JECFA in 2006), the apparent no adverse effect level (NOAEL) in F344 rats was the dietary level of 2.5% (test sample purity 96%, Toyoda et al.,1997). At 5% of the diet, statistically significant decreases in body weight, percent survival and kidney weight were seen. The author attributed these effects to various factors. The decrease in body weight was attributed to an inhibition of glucose utilization. The decrease in survival seemed to have been caused by an unusual late onset of large granular lymphocyte leukemia in high dose males. The author reported that this tumor is rather common in F344 rats and that the overall incidence in male rats was within the historical control range experienced in the particular laboratory. The decrease in kidney weight may have been due to a decrease in chronic inflammation found in the histopathological examination. JECFA agreed that 2.5% level is the NOAEL and calculated this dose to be equivalent to 970 in males (JECFA, 2006).

F. Clinical Studies and Other Reports in Humans

Several pharmacological and biochemical effects have been reported for crude extracts of stevia leaves and purified steviol glycosides. These include effects on glucose uptake, insulin secretion and blood pressure (Geuns, 2003a). Stevioside is used in South America as a treatment for Type II diabetes. These were key concerns for JECFA. The available clinical studies were summarized in 2006, and further studies were recommended (WHO, 2006). Several studies were subsequently conducted, and JECFA reviewed the findings in 2009 (WHO, 2009). JECFA's summaries of the key studies are provided below.

1. Studies Summarized in 2006.

Aqueous extracts of 5 g of *S. rebaudiana* leaves were administered to 16 volunteers at 6-h intervals for three days, and glucose tolerance tests were performed before and after administration. Another six volunteers were given an aqueous solution of arabinose in order to eliminate possible effects of stress. The extract increased glucose tolerance and significantly decreased plasma glucose concentrations during the test and after overnight fasting in all volunteers (Curi et al., 1986).

In a multi-center randomized, double-blind, placebo-controlled trial of hypertensive Chinese men and women (aged 28–75 years), 60 patients were given capsules containing 250 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 750 mg of stevioside per day (equivalent to 11 mg/kg bw/day as calculated by FSANZ, 2008) and followed up at monthly intervals for one year. Forty-six patients were given a placebo. After 3 months, systolic and diastolic blood pressure in men and women receiving stevioside decreased significantly and the effect persisted over the year. Blood biochemistry parameters, including lipids and glucose, showed no significant changes. Three patients receiving stevioside and one receiving the placebo withdrew from the study as a result of side-effects (nausea, abdominal fullness, dizziness). In addition, four patients receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment. These effects subsequently resolved and the patients remained in the study (Chan et al., 2000).

A follow-up multi-center randomized, double-blind, placebo-controlled trial was conducted in hypertensive Chinese men and women (aged 20–75 years). Eighty-five patients were given capsules containing 500 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 1500 mg of stevioside per day (equivalent to 21 mg/kg bw /day, as calculated by FSANZ, 2008). Eighty-nine patients were given a placebo. Three patients in each group withdrew during the course of the study. There were no significant changes in body mass index or blood biochemistry parameters throughout the study. In the group receiving stevioside, mean systolic and diastolic blood pressure was significantly decreased compared with the baseline, commencing from about 1 week after the start of treatment. After 2 years, 6 out of 52 patients (11.5%) in the group receiving stevioside had left ventricular hypertrophy compared with 17 of 50 patients (34%) in the group receiving the placebo ($p < 0.001$). Eight patients in each group reported minor side-effects (nausea, dizziness and asthenia), which led two patients in each group to withdraw from the study. Four patients in the group receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment. These effects subsequently resolved and the patients remained in the study (Hsieh et al., 2003).

In a paired cross-over study, 12 patients with Type II diabetes were given either 1 g of stevioside (stevioside, 91%; other stevia glycosides, 9%) or 1 g of maize starch (control group), which was taken with a standard carbohydrate-rich test meal. Blood samples were drawn at 30 minutes before and for 240 minutes after ingestion of the test meal. Stevioside reduced postprandial blood glucose concentrations by an average of 18% and increased the insulinogenic index by an average of 40%, indicating beneficial effects on glucose metabolism. Insulin secretion was not significantly increased. No hypoglycemic or adverse effects were reported by the patients or observed by the investigators. Systolic and diastolic blood pressure was not altered by stevioside administration (Gregersen et al., 2004).

Forty-eight hyperlipidemic volunteers were recruited to a randomized, double-blind trial designed to investigate the hypolipidemic and hepatotoxic potential of steviol glycoside extract. The extract used in this study was a product containing stevioside (73 ± 2%), rebaudioside A (24 ± 2%) and other plant polysaccharides (3%). The subjects were given two capsules, each containing 50 mg of steviol glycoside extract or placebo, twice daily (i.e., 200 mg/day, equivalent to 3.3 mg/kg bw/day assuming an average body weight of 60 kg), for 3 months. One volunteer receiving placebo and three volunteers receiving steviol glycoside failed to complete the study for personal reasons, not related to adverse reactions. At the end of the study, both groups showed decreased serum concentrations of total cholesterol and of low-density lipoproteins. Analyses of serum concentrations of triglycerides, liver-derived enzymes and glucose indicated no adverse effects. The authors questioned the subjects' compliance with the dosing regime, in view of the similarity of effect between treatment and placebo (Anonymous, 2004a). In a follow-up study, 12 patients were given steviol glycoside extract in incremental doses of 3.25, 7.5 and 15 mg/kg bw/day, for 30 days per dose. Preliminary results indicated no adverse responses in blood and urine biochemical parameters (Anonymous, 2004b).

2. Studies Summarized in 2009.

Four male and five female healthy volunteers (aged 21–29 years) were provided with capsules containing 250 mg stevioside (97% purity) to be taken 3 times per day for 3 days. Doses, expressed as steviol, were 288 mg/day or 4.4 mg/kg bw/day for females and 3.9 mg/kg bw/day

for males. Twenty-four hour urine samples were taken before dosing on day 1 and after dosing on day 3. Fasting blood samples were taken before dosing on day 1, and six samples were taken at different time points on day 3 after dosing. Fasting blood pressure measurements were taken before the first capsule and at six different time intervals after the first dose. Urine was analysed for creatinine, sodium, potassium, calcium, and urea. Blood was analysed for plasma glucose, plasma insulin, alkaline phosphatase, ALT, GPT, creatine kinase, and lactate dehydrogenase. The clinical analyses of blood, blood pressure, and urine showed no differences between samples taken before or after dosing. This study was approved by the local ethics committee (Temme et al., 2004).

In a study unpublished at the time of the sixty-eighth meeting, 250 mg of a product containing 91.7% total steviol glycosides, including 64.5% stevioside and 18.9% rebaudioside A, was administered to groups of type 1 (n = 8) and type 2 diabetics (n = 15) and non-diabetics (n = 15) 3 times daily for 3 months in a double blind, placebo-controlled trial. Control groups with the same number of subjects received a placebo. After 3 months, there were no significant changes in systolic or diastolic blood pressure, glycated hemoglobin (HbA1c), blood lipids, or renal or hepatic function. No side effects were reported. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Barriocanal et al., 2006, 2008). The Committee previously noted that this product did not meet the proposed specification of "not less than 95% steviol glycosides" and that the study was conducted in a small number of subjects. A study of antihypertensive effects was conducted in previously untreated mild hypertensive patients with crude stevioside obtained from the leaves of *S. rebaudiana*. Patients with essential hypertension were subjected to a placebo phase for 4 weeks and then received either capsules containing placebo for 24 weeks or crude stevioside at consecutive doses of 3.75 mg/kg bw/day (7 weeks), 7.5 mg/kg bw/day (11 weeks) and 15 mg/kg bw/day (6 weeks). Comparison of patients receiving stevioside with those on placebo showed neither antihypertensive nor adverse effects of stevioside. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Ferri et al., 2006). The product in this study also did not meet the proposed specification. According to a study available in abstract form only, a randomized placebo-controlled double-blind control study was conducted in subjects with type 2 diabetes. Fifty-five subjects received 500 mg stevioside (purity unspecified) or placebo (maize starch) 3 times daily for 3 months. Compared with the placebo, stevioside did not reduce the incremental area under the glucose response curve and maintained the insulin response and HbA1c and fasting blood glucose levels. HbA1c is an indicator of mean glucose levels and is used in identifying effects on the control of diabetes. No difference in lipids or blood pressure was observed. It is not clear whether this study was approved by the local ethics committee or met the requirements of the Declaration of Helsinki (Jeppesen et al., 2006).

A placebo-controlled double-blind trial was carried out in 49 hyperlipidaemic patients (aged 20–70 years, number of males and females not supplied) not undergoing treatment. The study was approved by the local ethics committee and complied with the principles of the Declaration of Helsinki. Individuals were divided into two groups, with 24 subjects receiving placebo capsules and 25 receiving capsules containing a dose of 50 mg steviol glycosides (70% stevioside, 20% rebaudioside A), equivalent to 1.04 mg steviol/kg bw/day, using the mean body weight of the treatment group, 72.7 kg. Two capsules were taken before lunch and two before dinner each day for 90 days. Six subjects withdrew from the study, four in the placebo group and two in the test group. Self-reported adverse reactions were recorded, and fasting blood samples were taken at

the end of the study and analysed for ALT, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), and triglycerides. No effects of treatment on ALT, AST or GGT were found. Decreases in the total cholesterol and LDL were observed in both the stevioside group and the placebo group, which were not treatment related. No adverse effects were observed (Cavalcante da Silva et al., 2006). The Committee noted at its sixty-eighth meeting that the product used in this study did not meet the proposed specification.

G. Studies On Metabolites: Steviol

There have been a number of studies conducted on steviol, and the results are provided below.

1. Acute Toxicity

In male and female mice and rats given steviol (purity, 90%) orally, the LD_{50} was > 15 g/kg bw, and 1/15 animals died within 14 days of administration. The LD_{50} values in hamsters given steviol orally were 5.2 g/kg bw in males and 6.1 g/kg bw in females. Histopathological examination of the kidneys revealed severe degeneration of the proximal tubular cells, and these structural alterations were correlated with increased serum blood urea nitrogen and creatinine. The authors concluded that the cause of death was acute renal failure (Toskulkao et al., 1997).

2. Mutagenicity and Genotoxicity

Several mutagenicity and genotoxicity studies have been conducted on steviol. The studies reviewed by JECFA are summarized in Table C-2.

3. Developmental Toxicity Studies: Steviol

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1000 mg/kg bw/day (only 12 animals at the highest dose) 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, and the number of live fetuses per litter and mean fetal weight decreased in parallel. Histopathological examination of the maternal kidneys showed a dose-dependent increase in the severity of effects on the convoluted tubules (dilatation, hyaline droplets). No dose-dependent teratogenic effects were seen. The NOEL was 250 mg/kg bw/day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

Table C-2. Mutagenicity & Genotoxicity Studies on Steviol

STUDY	*IN VIVO/IN VITRO*	SYSTEM	TEST SAMPLE PURITY	AUTHOR CONCLUSION	RESULTS AND REMARKS
Sekihashi et al., 2002[a]	*in vivo/in vitro*	Comet Assay	Not reported	Negative	In *in vitro* study, steviol at 62.5, 125, 250 and 500 µg/ml did not damage DNA of TK6 and WTK1 cells in presence or absence of S9 mix. In *in vivo* study, mice sacrificed 3 or 24 hours after one-time oral administration of 250, 500, 1000 or 2000 mg/kg of steviol. Stomach, colon, kidneys, testis and liver DNA not damaged as shown by comet assay. An identical *in vivo* experiment with stevia extract performed, which also gave negative results via comet assay.
Oh et al., 1999[b]	*in vitro*	Cell Mutation and DNA damage	Not reported	Negative	Steviol gave negative results for cell mutation and DNA damage in cultured cells.
Terai, et al, 2002[a]	*in vitro*	Bacterial Mutagenicity	Not Reported	Positive	Steviol found to be mutagenic in Aroclor induced rat liver S9 fraction. 15-oxo-steviol found to be mutagenic at 10% level of steviol. Specific mutagenicity of lactone derivative in presence of S9 mixture 10x lower than that of derivative without S9 mixture.
Temcharoen et al., 1998[c]	*in vitro*	Bacterial Mutagenicity	Not Reported	Positive	Mutagenic effects of steviol and/or metabolites found in *Samonella Typhimurium* TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene. Magnitude of increase of these mutations over the control not reported.
Klongpanich-pak et al., 1997[c]	*in vitro*	Bacterial Mutagenicity	Not Reported	Negative	Steviol and stevioside inactive in TA strains of *Salmonella Typhimurium, e. coli WP2, uvrA/PKM101* and rec assay using *Bacillius subtilis* even when microsomal activated fraction present. Magnitude of increase of these mutations over the control not reported
Matsui et al., 1989[a]	*In vitro*	Bacterial Mutagenicity	Not Reported	Negative	Testing of Southern Blot technique with probe for gpt gene DNA of *E. coli.* The chromosomal DNA of TM677 and steviol-induced TM677 mutants digested by restriction enzymes and probed. No significant differences found in fragment length between wild-type and mutant DNA.
Matsui et al., 1996[a]	*In vitro*	Bacterial Mutagenicity, Mammalian Cells	Not Reported	Both	Steviol weakly positive in umu test, either with or without metabolic activation. Steviol negative in reverse mutation and other bacterial assays even in presence of S9 activation. The magnitude of increase over control in umu test not discussed.
Matsui et al., 1996[a]	*In vivo*	Mouse micronucleus	Not Reported	Negative	Steviol did not increase number of micronuclei observed in this study.
Procinska et al., 1991[c]	*In vitro*	Bacterial Mutagenicity	Not Reported	Negative	The direct mutagenic activity of 15-oxo-steviol was refuted.
Compadre et al., 1988[a]	*In vitro*	Bacterial Mutagenicity, Mass Spec	Not Reported	Positive	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 direct-acting mutagen. Magnitude of increase over control in assay not discussed.

STUDY	*IN VIVO/IN VITRO*	SYSTEM	TEST SAMPLE PURITY	AUTHOR CONCLUSION	RESULTS AND REMARKS
Pezzuto et al., 1985[d]	*In vitro*	Bacterial Mutagenicity	Not Reported	Positive	Using *Salmonella Typhimurium* TM677 strain, steviol found to be highly mutagenic in presence of 9000 x g supernatant from livers of Aroclor 1254-pretreated rats. This mutagenicty dependent on pretreatment of rats with Aroclor and NADPH addition, as unmetabolized steviol was inactive. None of other metabolites tested was mutagenic. Authors conclude that structural features of requisite importance for the expression of mutagenic activity may include a hydroxy group at position 13 and an unsaturated bond joining the carbon atoms at positions 16 and 17.
Matsui et al, 1996[c]	*In vitro*	Mutagenicity and Chromosome aberration (Chinese hamster lung fibroblasts)	Not reported	Positive	Gene mutation and chromosomal aberration 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.
Temacharoen et al., 2000[c]	*In vivo*	Micronucleus (rat)	90%	Negative (see remarks)	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals. However, some cytotoxic effects were seen in females, but these not discussed further.
Temacharoen et al., 2000[c]	*In vivo*	Micronucleus (mouse)	90%	Negative (see remarks)	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals. However, some cytotoxic effects seen in the females, but were not discussed further.
Temacharoen et al., 2000[c]	*In vivo*	Micronucleus (hamster)	90%	Negative (see remarks)	Very high doses (4 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals. However, some cytotoxic effects were seen in the females, but were not discussed further.

[a] Abstract Only; [b] As Reported in JECFA 2006; [c] As Reviewed by Geuns 2003a; [d] Full Article.

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