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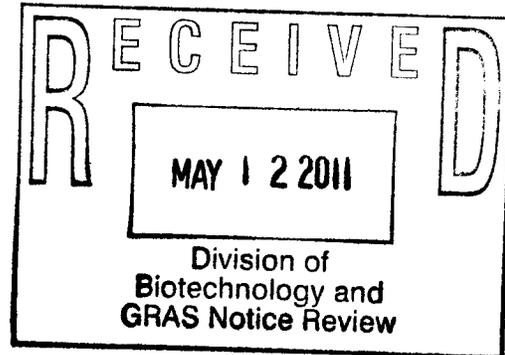
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May 5, 2011

Food and Drug Administration  
Center for Food Safety & Applied Nutrition  
Office of Food Additive Safety (HFS-255)  
5100 Paint Branch Parkway  
College Park, MD 20740-3835



Attention: Dr. Mary D. Ditto

Re: GRAS Notification – Rebaudioside A ( $\geq 95\%$ )

Dear Dr. Ditto:

On behalf of GLG Life Tech Corporation of Vancouver, British Columbia, Canada, we are submitting for FDA review a GRAS notification for Rebaudioside A ( $\geq 95\%$ ). 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 – Rebaudioside A ( $\geq 95\%$ ) (in triplicate)

000002



**GRAS ASSESSMENT**

**REBAUDIOSIDE A ( $\geq 95\%$ )**

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

**For**

**GLG Life Tech Corporation**  
Vancouver, British Columbia  
CANADA

Evaluation by

Richard C. Kraska, Ph.D., DABT  
Robert S. McQuate, Ph.D.  
Madhusudan G. Soni, Ph.D., FACN

May 4, 2011



000003

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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)<sup>1</sup>**

GLG Life Tech Corporation has determined that its high purity rebaudioside A (≥ 95%) product, which is referred to as Rebpure™ RA95, and which meets the specifications as described below, is Generally Recognized As Safe (GRAS) in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the following sections. The evaluation accurately reflects the conditions of the stevia-derived sweetener's intended uses in foods.

Signed:

(b) (6)

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

*May 4, 2011*

Date

**B. Name & Address of Notifier**

GLG Life Tech Corporation  
World Trade Centre, 999 Canada Place, Suite 519  
Vancouver, British Columbia, V6C3E1  
CANADA

As the notifier, GLG Life Tech Corporation ("GLG") accepts responsibility for the GRAS determination that has been made for its purified rebaudioside A product<sup>2</sup> as described in the subject notification; consequently, these rebaudioside A preparations, i.e., having purities of no less than 95% rebaudioside A, meeting the conditions described herein are exempt from pre-market approval requirements for food ingredients.

<sup>1</sup> See 62 FR 18938 (17 April 1997) which is accessible at <http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedAsSafeGRAS/ucm083058.htm>.

<sup>2</sup> GLG Life Tech Corporation refers to its high purity rebaudioside A product from leaves of *Stevia rebaudiana* Bertoni with the tradename of Rebpure™.

### **C. Common Name & Identity of Notified Substance**

High purity rebaudioside A, commonly abbreviated as 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 high purity rebaudioside A preparations are intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into foods in general, other than in 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, GLG's standardized rebaudioside A preparation from the leaves of *Stevia rebaudiana* Bertoni has 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 GLG, GRAS Associates, LLC (“GA”) has undertaken an independent safety evaluation of GLG’s Rebpure™ RA95 preparation. The preparation is composed of high purity rebaudioside A, which is extracted from the leaves of *Stevia rebaudiana* Bertoni and purified to yield rebaudioside A with a purity of ≥ 95%. The purpose of the evaluation is to ascertain whether or not the intended food uses of rebaudioside A as a non-nutritive general purpose sweetener as described in Section IV.A are generally recognized as safe, i.e., GRAS.

### B. Foreword

GLG provided GA with background information needed to enable the GRAS assessment to be undertaken. In particular, the information that was 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 purified rebaudioside A. GLG 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. GLG was also asked to supply past and present human food use information. Knowing how much steviol glycosides has been safely consumed, i.e., the 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.

In addition to the product specifications, chemical properties, manufacturing, and safety related information, GLG also provided some consumption/exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through May 4, 2011. A GRAS assessment based on the composite safety information, i.e., 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

Sweeteners derived from stevia are permitted as food additives in South America and in several countries in Asia, including China, Japan, and Korea. In recent years, the subject sweeteners have received food usage approvals in Mexico, Australia, New Zealand, Switzerland, France and Hong Kong. Steviol glycosides have been used as a dietary supplement in the US, since 1995 (Geuns, 2003). Based on the available information, no New Dietary Ingredient Notification for dietary supplement use of purified rebaudioside A has been made to the US FDA. Since 1989 and prior to 2008, at least two GRAS petitions seeking authorization for the addition of stevioside or steviol glycosides to foods had been submitted to FDA. However, no authorizations had been

issued by FDA in response to these filings, and these petitions were withdrawn. It appears that the previously available safety data—including purity considerations—for stevia, stevioside, or steviol glycosides were inadequate.

Based on information from FDA's GRAS Notice Inventory<sup>3</sup> website as of May 4, 2011, the agency has received 19 notices on rebaudioside A or steviol glycosides. Eleven of these notices have received "no question" letters from the FDA, while eight notices are under the agency review. In May 2008, Merisant and Cargill independently submitted GRAS notifications for rebaudioside A, highly purified forms of the steviol glycosides, to FDA. On December 17, 2008, FDA issued "no question" letters for each of these GRAS notices. Since December 2008, a series of GRAS notifications were submitted to FDA for stevia-derived sweetener products by the following companies: McNeil Nutritionals, LLC; Blue California; Sweet Green Fields, LLC; Wisdom Natural Brands; Sunwin and Wild Flavors (two notifications); Pyure Brands, LLC, PureCircle USA, Inc, and GLG Life Tech, Ltd. Each of these firms received a "no question" letter from FDA.<sup>4</sup> Additionally, eight notifications submitted to FDA by different manufacturers are pending with the agency.

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

The Joint Expert Committee on Food Additives (JECFA) has reviewed steviol glycosides at its 51<sup>st</sup>, 63<sup>rd</sup>, 68<sup>th</sup> and 73<sup>rd</sup> meetings. In 2000, JECFA published the original review on steviol glycosides (WHO, 2000). JECFA established a temporary ADI (acceptable daily intake) of 0-2 mg/kg (on a steviol basis) at its 63<sup>rd</sup> meeting (WHO, 2006). Additionally, JECFA finalized food grade specifications (FAO, 2007a), although they were subsequently updated in 2008 (FAO, 2008) and 2010 (FAO, 2010) (see below). At the 69<sup>th</sup> 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). In 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009).

In early 2009, a number of parties, including the government of Australia and the Calorie Control Council, submitted a request to the Codex Committee on Food Additives in which it was proposed that the JECFA specifications for steviol glycosides should be modified to allow inclusion of Rebaudioside D and Rebaudioside F as specifically named acceptable glycosides that would be considered as part of the minimum 95% steviol glycosides composition (CCFA, 2009). This

<sup>3</sup> Accessible at: <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true>.

<sup>4</sup> GRAS notification 252 was submitted by Merisant, GRAS notification 253 was submitted by Cargill, GRAS notification 275 was submitted by McNeil Nutritionals, GRAS notification 278 was submitted by Blue California, GRAS notification 282 was submitted by Sweet Green Fields, GRAS notification 287 was submitted by Wisdom Natural Brands, GRAS notifications 303 and 304 were submitted by Sunwin and Wild Flavors, GRAS notification 318 was submitted by Pyure Brands, GRAS notification 323 was submitted by PureCircle USA, and GRAS notification 329 was submitted by GLG Life Tech; information pertaining to these notifications are listed on FDA's website at <http://www.accessdata.fda.gov/scripts/fc/fcnNavigation.cfm?rpt=grasListing>, along with their respective "no question" letters.

proposed modification was endorsed by the Codex Alimentarius Committee in July 2009; it was on the agenda for discussion at the JECFA Meeting in June, 2010 (WHO, 2009), and JECFA recently took final action in approving the modified steviol glycosides specifications to include Rebaudioside D and Rebaudioside F (FAO, 2010; Appendix A).

In 2008, Switzerland's Federal Office for Public Health (2008) approved the use of stevia as a sweetener citing the favorable actions of JECFA. Subsequently, France published its approval for the food uses of rebaudioside A with a purity of 97% (AFSSA, 2009).

As of May 2010, the government of Hong Kong amended its food regulations to allow the use of steviol glycosides as a permitted sweetener in foods (Hong Kong Centre for Food Safety, 2010). This action followed in the aftermath of the detailed safety evaluation and favorable findings as reported by JECFA.

On September 18, 2009, based on a review of the international regulation of *Stevia rebaudiana* and the clinical evidence for safety and efficacy, the Natural Health Products Directorate, Health Canada (2009), has adopted the following guidelines for the use of Stevia and steviol glycosides in Natural Health Products (NHPs). The revised recommendation for the maximum limit for steviol glycosides in NHPs is in accordance with the full ADI (acceptable daily intake) of 4 mg steviol/kg bw established by WHO (2008).

In light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission for European Food Safety Authority (EFSA) to deliver a scientific opinion on the safety of steviol glycosides as a sweetener for use in the food categories specified in the dossiers from the three petitioners, EFSA reexamined the safety of steviol glycosides (EFSA, 2010). After considering all the data on stability, degradation products, metabolism and toxicology, the EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day, which is similar to JECFA's determination.<sup>5</sup>

#### **D. FDA Regulatory Framework**

Since 1995, steviol glycosides (or stevioside) have been used in dietary supplements in the US (Geuns, 2003). These supplements are widely available to consumers in the US through retail outlets and Internet purchases (Al-Achi and Greenwood, 2000). According to FDA regulation of foods, dietary supplements cannot legally be added to conventional foods. In order for their uses in conventional foods, dietary supplements must undergo premarket approval by FDA as food

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<sup>5</sup> From a historical perspective, it is noted that the UK's Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food on September 24, 1998 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." (See <http://www.maff.gov.uk/food/novel/980924.html>.) In 1999, 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" (European Commission, 1999a). In another opinion also dated June 17, 1999, the Committee also reiterated "its earlier opinion that stevioside is not acceptable as a sweetener on the presently available data" (European Commission, 1999b).

additives or, alternatively, the ingredients 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.<sup>6</sup>

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.<sup>7</sup> 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.

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<sup>6</sup> See 21 CFR 170.3(i)(3).

<sup>7</sup> See 62 FR 18938 (17 April 1997) which is accessible at <http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm>.

### III. CHEMISTRY & MANUFACTURE OF REBAUDIOSIDE A (≥ 95%)

#### A. Common or Usual Name

The common or usual name for the products that are the subject of this notification is high purity rebaudioside A, which is derived from the leaves of *Stevia rebaudiana* Bertoni. Reb A is one of the common steviol glycosides found in nature. The rebaudioside A content of the commercial product is equal to or higher than 95%. Rebpure™ RA95 is the commercial name used by GLG in referring to the notified substance. In the scientific literature, steviol glycosides have been referred to as stevia, stevioside, steviol glycosides, and stevia glycoside. 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 reb A---like stevioside---is the common name for another one of the specific glycosides that is extracted from stevia leaves.

#### B. Description

In 2009, Food Chemical Codex (FCC) prepared a monograph with a description and specifications for rebaudioside A. In this monograph, rebaudioside A is described as a white to off-white, hygroscopic fine crystal, granule, or powder having a sweet taste (FCC, 2009). It is freely soluble in ethanol:water 50/50 (v/v) and is sparingly soluble in water and in ethanol. Rebaudioside A is obtained from the leaves of the *Stevia rebaudiana* Bertoni plant in a multistep separation and purification process. The principal steps of manufacturing include extraction of steviol glycosides from the leaves using an aqueous or aqueous alcoholic (ethanol or methanol) solvent, and purification of rebaudioside A from the resulting mixture of steviol glycosides by resin absorption followed by recrystallization from an aqueous or aqueous alcoholic (ethanol or methanol) solvent. It is primarily composed of rebaudioside A, a glycoside of the *ent*-kaurenoid diterpenoid aglycone known as steviol (FCC, 2009).

#### C. Chemistry of Rebaudioside A

At its 51<sup>st</sup> meeting, JECFA reviewed the safety related information on steviol glycosides, including the identity and chemistry of these compounds. The following description is taken from the original JECFA monograph (WHO, 2000).

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).

Of the eight different steviol glycosides, the two principal sweeteners of stevia extracts have been identified as rebaudioside A and stevioside. The chemical identities and key chemical identifiers for the two major components are presented in Table 1.

**Table 1. Chemical Identity of Rebaudioside A & Stevioside**

<b>REBAUDIOSIDE A</b>	
<b>Common name</b>	Rebaudioside A
<b>Chemical name</b>	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
<b>Chemical formula</b>	C <sub>44</sub> H <sub>70</sub> O <sub>23</sub>
<b>Formula weight</b>	967.03
<b>CAS Number</b>	58543-16-1
<b>STEVIOSIDE</b>	
<b>Common Name</b>	Stevioside
<b>Chemical name</b>	13-[2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
<b>Chemical formula</b>	C <sub>38</sub> H <sub>60</sub> O <sub>18</sub>
<b>Formula weight</b>	804.88
<b>CAS Number</b>	57817-89-7

The chemical structure of rebaudioside A is presented in Figure 1. JECFA (FAO, 2007b) identified the sweetener components of stevia and updated the list of common glycosides and their chemical structures (Figure 2) that are slightly different than compounds shown in other older publications (Nanayakkara et al., 1987; Suttajit et al., 1993). The structures of the components of stevia glycosides were also described in reviews by Kinghorn and Soejarto (1985), Kennelly (2002), and Geuns (2003). Other substances that lack sweetness include the labdane diterpenes, triterpenes, sterols and flavonoid glycosides.

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

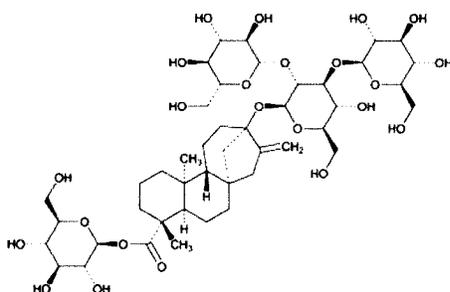
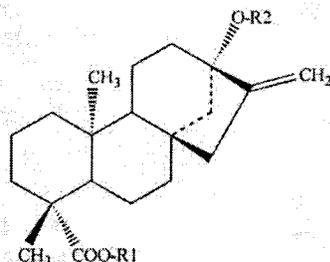


Figure 2. Chemical Structures of Various Steviol Glycosides Reproduced from FAO<sup>a, b</sup>



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

<sup>a</sup> From FAO, 2007b.

<sup>b</sup> The indicated C.A.S. No. for Rubusoside as reported in the cited reference is incorrect and should be 64849-39-4.

#### D. Manufacturing Processes

Based on available scientific and patent literature, several manufacturing processes for steviol glycosides have been reported. These processes are summarized below, along with GLG's manufacturing process for its rebaudioside A ( $\geq 95\%$ ).

## 1. Scientific & Patent Literature

In general, steviol glycosides are obtained by extracting leaves of *Stevia rebaudiana* Bertoni with hot water or alcohols (ethanol or methanol). The extract is a dark particulate solution containing all the active principles along with 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 several 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. In recent patents, methods using ultrafiltration, metallic ions, supercritical fluid extraction with CO<sub>2</sub> and extract clarification with zeolite are employed.

At the 68<sup>th</sup> JECFA meeting, steviol glycosides were defined as the products obtained from the leaves of *Stevia rebaudiana* Bertoni. As described 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. GLG’s Manufacturing Process for Purified Rebaudioside A

The source of GLG’s rebaudioside A is the leaves of the *Stevia rebaudiana* Bertoni plant. The manufacturing process employed by GLG is fairly typical and similar to that yielding other related stevia-derived sweetener products on the market. The ethanol and methanol used in the purification process comply with FCC’s 5<sup>th</sup> Edition specifications for these solvents. The ion exchange resins used in the manufacturing comply with 21 CFR 173.65. The GLG rebaudioside A is prepared in accordance with current Good Manufacturing Practices (cGMP) at Qingdao Runhao Rabiana High Tech Co., Ltd, North Chenggang Road, Qangdao Export Processing Zone, Hetao, Chengyang District, Quangdao City, Shangdong Province, P.R. China.

GLG has developed a state-of-the-art process for extracting steviol glycosides from the stevia leaf. The primary stevia extract preparation process is identical to that described in GLG’s GRAS notification, GRN 329. The process is summarized by flow diagrams in Appendix B-1. In brief, steviol glycosides are obtained by the extraction of stevia leaves with water. Leaves from selected varieties of stevia plants are used for rebaudioside A production. Ferric chloride and calcium hydroxide are added to the extract solution to facilitate precipitation. The extraction solution is passed through plate filtration followed by adsorption onto resin; the glycosides are subsequently eluted with ethanol. The desorpted solution is decolorized with active carbon and concentrated with film evaporators. It is again decolorized with active carbon and filtered. The concentrate is spray dried to obtain the primary stevia extracts rich in rebaudioside A. The extract thus obtained is further processed with additional purification steps to obtain the high purity rebaudioside A. These additional processing steps are summarized by the flow diagram in Appendix B-2. The stevia extract is dissolved in ethanol and/or methanol, crystallized and

filtered. The crystallization and drying process is repeated two more times using ethanol to obtain high purity rebaudioside A. The content of rebaudioside A in the final Rebpure™ products is  $\geq 95\%$ , while the total steviol glycosides content is  $\geq 97\%$ .

## **E. 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, and JECFA finalized food grade specifications at the 68<sup>th</sup> JECFA meeting with publication in the FAO JECFA Monograph 4 (FAO, 2007a). 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 specifications with 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 updated the specifications for steviol glycosides in 2008 (FAO, 2008), and then again in 2010 when the specifications were expanded to include the original seven specific steviol glycosides plus Reb D and Reb F (FAO, 2010); also see Appendix A.

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 9 specific steviol glycosides. 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. Any residual methanol levels should not exceed 200 ppm, and ethanol residues should not exceed 5000 ppm. Arsenic levels should not exceed 1 ppm as determined by the atomic absorption hydride technique. Lead levels should not exceed 1 ppm.

### **2. Specifications for GLG's Purified Rebaudioside A ( $\geq 95\%$ )**

GLG has adopted product specifications for its purified rebaudioside A product that meet or exceed JECFA recommendations while also complying with Food Chemicals Codex (FCC, 2009) specifications for rebaudioside A. A comparison of the specifications provided by GLG and those from JECFA and FCC is presented in Table 2. Results of analyses performed by GLG quality control laboratories at the manufacturing site demonstrating that 5 production batches of Rebpure™ RA95 meet the required specifications are provided in Appendix C-1. An analytical report from an independent laboratory pertaining to the identity of the components from the same five lots of Rebpure™ RA95, along with details of the methodology, are included as Appendix C-2. These data and reports demonstrate that the GLG product meets the purity criteria. A test report for analyses of pesticide residues in one production lot is included in Appendix C-3.

**Table 2. Specifications for GLG's Rebaudioside A (≥ 95%) Product**

PARAMETER	JECFA <sup>a</sup> SPECIFICATIONS STEVOL GLYCOSIDES	FCC SPECIFICATIONS REBAUDIOSIDE A	GLG SPECIFICATIONS REBAUDIOSIDE A (≥ 95%)	METHODS
Appearance	White to light yellow powder	White to off-white, hygroscopic fine crystal, granule, or powder	White powder	Visual
Sweetness	200-300 times sweeter than sucrose	NA	300-400 times sweeter than sugar	Gustatory
Rebaudioside A	NA	NLT 95%	≥ 95%	JECFA HPLC
Total Steviol Glycosides	NLT 95%	NA	≥ 97%	JECFA HPLC
Other Related Steviol Glycosides (as Stev, Reb A, B, C, Dulc A, Rubu, and SB) on dry weight basis	NLT 95%	NMT 5% <sup>b</sup>	NS	JECFA, 2007
Residue on Ignition	NS	NS	≤ 1%	USP
Moisture (loss on drying)	NMT 6%	NMT 6%	≤ 4%	USP
Ash	NMT 1%	NMT 1%	< 1%	USP
Optical rotation	NS	NS	-29° to -31°C	USP
Solubility	Freely soluble in water and ethanol	Freely soluble in water:ethanol (50:50)	Freely soluble in water	USP
pH (1% solution)	4.5 - 7.0	4.5 - 7.0	4.5-7.0	USP
<b>Residual solvent levels</b>				
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	NMT 0.02%	USP
Residual Ethanol	NMT 5000 mg/kg	NMT 0.5%	NMT 0.5%	USP
<b>Heavy metals</b>				
Lead	NMT 1 mg/kg	NMT 1 mg/kg	< 1 ppm	AFS
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	< 1 ppm	AFS
<b>Microbiological</b>				
Total Plate Count	NA	NA	< 1000 cfu/g	FDA BAM
Yeast and Mold	NA	NA	≤ 100 cfu/g	FDA BAM
Total coliform	NA	NA	≤ 100 cfu/g	FDA BAM
<i>Salmonella</i>	NA	NA	Negative	FDA BAM
<i>Escherichia coli</i>	NA	NA	Negative	FDA BAM
<i>Staphylococcus aureus</i>	NA	NA	Negative	FDA BAM

<sup>a</sup> Prepared at 69<sup>th</sup> JECFA (WHO, 2008).

<sup>b</sup> Excludes Reb A but includes additional two glycosides Reb D and Reb F; 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; NLT = not less than; NMT = not more than.

## F. Stability Data

Stevioside has been reported to be stable over the pH range 3-9 and can be heated at 100°C for 1 hour, but at pH levels greater than 9 under these conditions it rapidly decomposes (Kinghorn and Soejarto, 1985). These investigators also speculated that at pH 10 steviolbioside would be the major decomposition product produced from stevioside by alkaline hydrolysis. In another study, Chang and Cook (1983) investigated the stability of pure stevioside and rebaudioside A in carbonated phosphoric and citric acidified beverages. Some degradation of each sweetening component after 2 months of storage at 37°C was noted. However, no significant change at room temperature or below following 5 months of storage of stevioside and 3 months of storage of rebaudioside A was noted. 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.

Merisant (2008) conducted stability testing on rebaudioside A (1) as a powder, (2) as a pure sweetener in solution, and (3) on both cola-type and citrus carbonated beverages. In these investigations no degradation was detected when the powder was stored at 105°C for 96 hours. It was concluded that the powder was stable when stored for 26 weeks at 40±2°C with relative humidity of 75±5%. Both published and unpublished testing results from Merisant revealed that rebaudioside A in carbonated citric acid beverages and phosphoric acid beverages did not significantly degrade during prolonged storage at refrigeration, normal ambient, or elevated ambient temperatures. Minimal loss of rebaudioside 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 (2008) also conducted extensive stability testing on rebaudioside A as a powder under various storage conditions and under a range of pH and temperatures. Additionally, Cargill also investigated rebaudioside 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, root beer and lemon-lime, thermally processed beverages, yogurt, and white cake. The results of stability testing revealed some degradation products that had not been detected in bulk rebaudioside A. These degradation products were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana* Bertoni. All the degradation products were found to share the same steviol aglycone backbone structure as found in stevioside and rebaudioside A, but they differ by virtue of the glucose moieties present. The results of stability testing revealed that rebaudioside 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, rebaudioside A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

In photostability studies of the dry powder and mock beverages to ascertain rebaudioside A behavior under defined conditions of fluorescent and near UV light exposure, rebaudioside A was found to be photostable under the defined conditions of analysis (Clos et al., 2008).

In addition to the above described stability reports for purified rebaudioside A, in a GRAS notification by Sunwin and WILD Flavors (2010) on purified steviol glycosides with rebaudioside A and stevioside as the principal components, stability was investigated using a 0.04% solution of Reb A 80% in acidic solutions between pH 2.81 and 4.18. In this study, the solutions were stored at 32°C for 4 weeks, and the Reb A content was determined at 1, 2 and 4 weeks. Reb A 80% was found to be very stable at pH 3.17 and above. At pH 2.81, after 4 weeks of storage under accelerated conditions only a 7% loss of Reb A was noted. Sunwin and WILD Flavors also studied the stability of Reb A 80% in simulated beverages using 0.1 % citric acid (pH 3.2). The solutions were pasteurized and stored for 8 weeks at 4° and 32°C, and little difference in sweetness perception was found under these conditions.

The stability data in the scientific literature for stevioside, the JECFA report, and the extensive stability testing presented by Merisant, Cargill and Sunwin and WILD Flavors support the position that high purity rebaudioside A products are well-suited for the intended food uses as reported by GLG.

## IV. INTENDED DIETARY USES

### A. Intended Uses

The subject GLG Rebpure™ RA95 preparation with rebaudioside A (≥ 95%) as the principal component is intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener in various foods other than meat and poultry products. The intended use will be as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19).<sup>8</sup> The intended use levels will vary by actual food category, but the actual levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts of purified rebaudioside A (≥ 95%) 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.<sup>9</sup>

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

As part of its safety deliberations, JECFA reviewed various estimates of possible daily intake of steviol glycosides (WHO, 2006). These estimates are presented in Table 3. Merisant also listed intended use levels of rebaudioside A for various food applications in their GRAS Notification (Table 4). Merisant utilized food consumption survey data from 2003-2004 NHANES to determine the estimated daily intake from the proposed uses of rebaudioside A. On a per user basis, the mean and 90<sup>th</sup> percentile daily consumption of rebaudioside A was estimated as 2.0 and 4.7 mg/kg bw/day, respectively. In its notification, Cargill (2008) utilized a different approach in estimating dietary intake figures for rebaudioside A when incorporated as a general sweetener in a broad cross-section of processed foods. Cargill considered that with a few minor exceptions rebaudioside A uses and use levels would be comparable to those of aspartame uses in the US. Using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008), Cargill performed a side-by-side consumption analysis for rebaudioside A versus aspartame. Findings from the above-described different sources along with FSANZ estimates are further discussed in Section IV.C, and the intake estimates are presented in Table 5.

### C. Estimated Daily Intake

The very conservative consumer intake estimates provided by JECFA as shown in Table 3 were utilized to gauge the potential human exposures of steviol glycosides and rebaudioside A in foods as reported in the US and in other countries. As rebaudioside A is about twice as sweet as the mixed glycosides, these levels can be adjusted accordingly. GLG intends to use rebaudioside A in a number of food categories at levels that comply with GMP uses. The application of rebaudioside A to the same foods and at the same levels as those described in earlier FDA

<sup>8</sup> Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.

<sup>9</sup> See 21 CFR 182.1(b)(1).

notices by Merisant and Cargill is unlikely to affect the dietary intake of rebaudioside A from introduction into the market by another supplier who will have to compete in essentially the same markets and foods. This also negates the need for cumulative intake analysis.

**Table 3. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents**

FOOD TYPE	MAXIMUM USE LEVEL REPORTED <sup>a</sup> (MG STEVIOL GLYCOSIDES /KG OF FOOD)	MAXIMUM USE LEVEL CALCULATED FOR REBAUDIOSIDE A <sup>b</sup> MG REBAUDIOSIDE A /KG OF FOOD	MAXIMUM USE LEVEL CALCULATED FOR REBAUDIOSIDE A <sup>b</sup> 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

<sup>a</sup> Reproduced from WHO, 2006.

<sup>b</sup> 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 4. Proposed Uses & Levels of Rebaudioside A by Merisant<sup>a</sup>**

FOOD GROUP	REBAUDIOSIDE A (PPM)
Tabletop sweeteners	30,000 <sup>b</sup>
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

<sup>a</sup> Merisant, 2008.

<sup>b</sup> Reb A content of sachet prior to dilution and not representative of "as consumed."

**Table 5. Summary of Estimated Daily Intake Assessments for Rebaudioside A & Calculation of Rebaudioside A Values from JECFA & FSANZ Estimates of the EDI**

Scenarios	EDI		
	As Steviol <sup>a</sup> (mg/kg bw/day)	As Rebaudioside A <sup>b</sup> (mg/kg bw/day)	Total Daily Intake <sup>c</sup> (mg/day)
<b>JECFA</b>			
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
<b>FSANZ</b>			
100% Reb A replacement of sugars	0.3 - 1.0	0.5 - 1.5	30 - 90
<b>MERISANT</b>			
		2.0 - 4.7 <sup>d</sup>	120 - 282
<b>CARGILL</b>			
		1.3 - 3.4 <sup>d</sup>	78 - 204

- <sup>a</sup> 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.
- <sup>b</sup> 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.
- <sup>c</sup> Total daily intake figures were calculated for a 60 kg adult.
- <sup>d</sup> Published values are shown for comparison purposes.

Further consideration was given to anticipated human exposures as projected independently and with different approaches by JECFA (WHO, 2006), Merisant (2008), and Cargill (2008). As described 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.

JECFA 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. Additionally, 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. The exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee are summarized in Table 6.

**Table 6. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)**

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

<sup>a</sup> WHO Global Environment Monitoring System—Food Contamination Monitoring and Assessment Programme.

<sup>b</sup> 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.

In its assessment, JECFA concluded that the replacement estimates were highly conservative as 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 rebaudioside A based on the molecular weight adjustment. Furthermore, by adjusting for the 400-fold increased sweetness of rebaudioside 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 rebaudioside A would likely be about 1.5 to ~ 2.3 mg/kg bw/day.

Similar to JECFA, FSANZ (2008) also 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 rebaudioside 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 4 and 4.7 mg/kg bw/day for a 90<sup>th</sup> percentile consumer. In another review conducted on behalf of Cargill and included in their GRAS notification, the intake of rebaudioside A when used as a complete sugar replacement was estimated at 1.3 – 3.4 mg/kg bw/day when calculated as rebaudioside A (Renwick, 2008). The estimated daily intake assessments have been compiled in Table 5. These different assessments suggest that total daily consumption of rebaudioside A for specified food categories and as a general purpose sweetener is unlikely to exceed 5 mg/kg bw/day, for a total daily dietary exposure of up to 300 mg rebaudioside Reb for an adult weighing 60 kg.

In October 2009, Cargill applied to FSANZ to increase the maximum usage levels of high purity steviol glycosides in the high volume food categories of ice cream and various beverages. Cargill supported its application with increased usage levels by presenting market share analyses which overestimate actual intake while remaining well below the generally accepted ADI. In December

2010, FSANZ recommended accepting the increased usage levels as requested since no public health and safety issues were identified. Final action is expected to materialize in 2011 (FSANZ, 2010).

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

There have been many scholarly estimates of potential dietary intake of replacement sweeteners-- including steviol glycosides---that have been published (FSANZ, 2008; Renwick, 2008; WHO, 2003) or submitted to FDA (Merisant, 2008). In GRAS notification 301, a simplified estimate was proposed to and accepted by FDA, based on the estimates of exposure in “sucrose equivalents” (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90<sup>th</sup> percentile consumer of a sweetener which is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg/kg bw/day for any population subgroup. As noted in Table 2, the minimum sweetness intensity for GLG’s reb A preparation is 300-fold that of sucrose. Therefore, the 90<sup>th</sup> percentile consumer of this reb A preparation would consume no more than half this level or less than 5 mg/kg bw/day. Based on an estimate that steviol glycosides preparations consist of 40% steviol equivalents,<sup>10</sup> the consumption of steviol glycosides would be less than 2 mg/kg bw/day.

The extent that stevia-based sweeteners will penetrate the US food supply and the extent the market will select mixed steviol glycoside products versus reb A products remains uncertain. Furthermore, many competing non-caloric sweeteners are currently available to consumers, which have been successful in the marketplace, most notably aspartame and sucralose.

Based on the totality of dietary intake considerations presented above, the intake estimates are viewed as being conservative. When comparing these EDI assessments for steviol glycosides, we see that total daily consumption of the steviol glycosides and reb A for defined food uses and as a general purpose sweetener is expected to be substantially less than the acceptable daily intake values discussed at length in Section VI.C.

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

For about 20 years, consumers in Japan and Brazil, where stevia has long been approved as a food additive, have been using stevia extracts as non-caloric sweeteners.<sup>11</sup> It was reported that 40% of the artificial sweetener market in Japan is stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999). Although there are no reported uses of rebaudioside A as a dietary supplement, use of steviol glycosides as a dietary supplement is presently permitted in the US, Australia, and New Zealand and as a natural health product in Canada. It has wide use in China and Japan in food and in dietary supplements. In 2005, it was estimated

<sup>10</sup> Calculated by Expert Panel by multiplying by the ratio of molecular weight of steviol to molecular weight of stevioside.

<sup>11</sup> See Raintree Nutrition Tropical Plant Database ([www.rain-tree.com/stevia.htm](http://www.rain-tree.com/stevia.htm)).

that sales of stevia in the US reached \$45 million (The Food Institute Report, 2006). More recent reports of consumption figures for stevia reveal pronounced increases in global consumption. Worldwide, Zenith International estimates stevia sales of 3500 metric tons in 2010 which represents a 27% increase over 2009 figures. The market value is estimated to have increased to \$285 million (Zenith, 2011).

Hawke (2003) reported that stevia is commonly used as a treatment for Type 2 diabetes in South America. However, for its therapeutic effects elevated doses in the range of 1 g/person/day or more were reported to be necessary (Gregersen et al., 2004).

## V. SAFETY DATA FOR REBAUDIOSIDE A

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

Stevia and steviol glycosides have been extensively investigated for their biological, toxicological, and clinical effects (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002). Additionally, the national and international regulatory agencies have thoroughly reviewed the safety of stevia and its glycosides. Most notably, over the years JECFA has evaluated stevia and steviol glycoside multiple times (WHO, 2000, 2006, 2007, 2008). Recently FSANZ (2008) also evaluated steviol glycosides for use in food. The JECFA reviews, as well as the other reviews completed before 2008, primarily focused on mixtures of steviol glycosides typically and were not specific for purified rebaudioside A.

From the safety perspective, some of the earliest studies on steviol glycosides were of limited value as the actual compositions of materials investigated and their questionable purities undermined drawing firm toxicological conclusions. These early studies reported a decrease in fertility with crude stevia preparations and increased mutagenic activity of the principle metabolite, steviol. Based on these and other 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, FDA was reluctant to authorize the use of stevia. These concerns included renal toxicity, effects on glucose metabolism, and inhibition of mitochondrial enzymes. Over the last decade and a half, the safety of steviol glycosides and rebaudioside A in particular have been extensively investigated employing comprehensive and modern toxicology protocols using scientifically accepted dosing regimens of purified and standardized test substances. The findings from these investigations are discussed below.<sup>12</sup>

JECFA encouraged the further elucidation of clinical effects on blood pressure and glucose metabolism on hypertensive and diabetic individuals, respectively, in parallel with normal human subjects. By 2006, sufficient data were generated for JECFA to satisfactorily establish a temporary ADI, which was finalized in 2008. Additional details on the JECFA reviews are discussed below.

#### 1. Summary of JECFA Reviews

Earlier at its 51<sup>st</sup> meeting, 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

<sup>12</sup> Recently, an additional subchronic study was published that investigated the effects of 97% pure stevioside in drinking water on body weight, organ relative weight, hematological and biochemical parameters, and enzyme activities in Sprague Dawley rats. This study is summarized in Appendix D and is discussed by the Expert Panel in Section VI.B.

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*.

Subsequently, additional data were generated on the metabolism of steviol glycosides and submitted to JECFA. This information suggested 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 new information also revealed that in *in vitro* studies steviol is mutagenic, while *in vivo* condition it is not mutagenic. 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. 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 63<sup>rd</sup> 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.

In 2007, at its 68<sup>th</sup> meeting, JECFA (WHO, 2007) concluded that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008. Subsequently, sufficient data had been received by JECFA 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.

In 2008, based on additional clinical studies, at its 69<sup>th</sup> meeting, JECFA finalized the evaluation of steviol glycosides (WHO, 2008) and raised the ADI to 0 – 4 mg/kg bw/day and removed the “temporary” designation. The summary of the Committee’s key conclusions in the final toxicology monograph addendum (WHO, 2009) 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**

In 2008, FSANZ completed a review of the safety of steviol glycosides for use as a sweetener in foods. 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. (2008), which was later published in 2008.

In their draft document, FSANZ 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).

### 3. Summary of EFSA Review of Steviol Glycosides

On March 10, 2010, EFSA adopted a scientific opinion on the safety of steviol glycosides (mixtures that comprise not less than 95% of stevioside and/or rebaudioside A) as a food additive. Earlier—in 1984, 1989 and 1999—the Scientific Committee for Food (SCF) evaluated stevioside as a sweetener. At the time, the SCF concluded that the use of stevioside was “toxicologically not acceptable” due to insufficient available data to assess its safety. However, in light of JECFA’s 2008 findings and in response to a June 2008 request by the European Commission, EFSA reevaluated the safety of steviol glycosides as a sweetener. As both rebaudioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for both glycosides, the EFSA Panel agreed that the results of toxicology studies on either stevioside or rebaudioside A are applicable for the safety assessment of steviol glycosides. Considering the available safety data (*in vitro* and *in vivo* animal studies and some human tolerance studies), the EFSA Panel concluded that steviol glycosides, complying with JECFA specifications, are not carcinogenic, genotoxic, or associated with any reproductive/developmental toxicity. The EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on the application of a 100-fold uncertainty factor to the NOAEL in the 2-year carcinogenicity study in the rat when administering 2.5% stevioside in the diet. This is equal to 967 mg stevioside/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day). Conservative estimates of steviol glycosides exposures both in adults and in children suggest that the ADI could possibly be exceeded by European consumers of certain ages and geographies at the maximum proposed use levels.

Recently, EFSA (2011) revised its exposure assessment of steviol glycosides from its uses as a food additive for children and adults and published the reduced usage levels in 16 foods by a factor of 1.5 to 3, with no changes for 12 food groups. Additionally, 15 other foods were removed, mainly within the category of desserts and other products, while 3 new food uses were added. The mean estimated exposure to steviol glycosides (equivalents) in European children (aged 1-14 years) ranged from 0.4 to 6.4 mg/kg bw/day and from 1.7 to 16.3 mg/kg bw/day at the 95<sup>th</sup> percentile. A correction was considered to be necessary for the consumption of non-alcoholic flavored drinks (soft drinks) by children, and the corrected exposure estimate at the 95<sup>th</sup> percentile for children ranged from 1.0 to 12.7 mg/kg bw/day. For adults, the mean and 97.5<sup>th</sup> percentile intakes were estimated to range from 1.9 to 2.3 and 5.6 to 6.8 mg/kg bw/day, respectively. Non-alcoholic flavored drinks (soft drinks) are the main contributors to the total

anticipated exposure to steviol glycosides for both consumer categories. For high consumers, EFSA noted that revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg/kg bw (steviol equivalent).

## **B. Safety Data on Rebaudioside A**

Since 2008, several well-designed toxicology studies that followed the current regulatory and other guidelines for such studies have been reported on purified rebaudioside A. These investigations included additional subchronic studies in rats and one in dogs, mutagenicity studies, reproduction and developmental studies in rats, and comparative pharmacokinetic studies with stevioside in rats and humans, as well as additional clinical studies.

### **1. Subchronic Studies**

Recently, Curry and Roberts (2008) reported the results of two repeat dose studies of rebaudioside A in Wistar rats. The results of these investigations suggest that administration of rebaudioside A to Han Wistar rats at dietary concentrations of up to 100,000 ppm (9938 and 11,728 mg/kg bw/day for males and females, respectively) for 4 weeks or 50,000 ppm (4161 and 4645 mg/kg bw/day for males and females, respectively) for 13 weeks did not present any evidence of systemic toxicity. In the 4-week study, rebaudioside A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000 and 100,000 ppm to male and female rats. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In the 13-week study, Wistar rats were fed diets containing rebaudioside A at dietary concentrations of 0, 12,500, 25,000 and 50,000 ppm. In high-dose male and females groups, reductions in body weight gain attributable to initial taste aversion and lower caloric density of the feed were observed. 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. No significant changes in other clinical pathology results, organ weights and functional observational battery test results were noted. Macroscopic and microscopic examinations of all organs 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 4161 and 4645 mg/kg bw/day in male and female rats, respectively (Curry and Roberts, 2008).

In another 90-day dietary admix toxicity study, effects of rebaudioside A (99.5% purity) at target exposure levels of 500, 1000 and 2000 mg/kg bw/day were tested in Cri:CD(SD) rats (Nikiforov and Eapen, 2008; Eapen, 2007). Each group consisted of 20/animals/sex. No treatment related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters were noted. There were no treatment related macroscopic, organ weight or microscopic findings. Significantly lower body weight gains were noted in the 2000 mg/kg bw/day group in males but not females. At the end of the dosing period, the body weight in males was 9.1% lower than the control group. Due to the small magnitude of difference from the control group value, the investigators did not consider this result to be adverse. The decrease was most likely due to the large proportion of the diet represented by the test material. The NOAEL was determined as ≥2000 mg/kg bw/day.

A 6-month dietary toxicity study in Beagle dogs (4/sex/group) was conducted to investigate the potential adverse effects of rebaudioside A (97.5% purity) at dosage levels of 0, 500, 1000 or 2000 mg/kg bw/day (Eapen, 2008). There were no unscheduled deaths during the course of the study. No treatment-related clinical observations were noted. Administration of rebaudioside A did not affect home cage, open field observations and functional observations and measurements. No differences in hematology findings, serum chemistry findings, or urinalysis findings between the groups were noted. Additionally, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. The investigators 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.

## 2. Mutagenicity Studies

In a set of *in vitro* and *in vivo* genotoxicity assays covering mutation, chromosome damage and DNA strand breakage, rebaudioside A consistently and uniformly revealed negative results (Pezzuto et al, 1985; Nakajima, 2000a; Nakajima, 2000b; Sekihashi et al., 2002. These studies are critically reviewed by Brusick (2008). JECFA also reviewed an unpublished chromosome aberration assay of rebaudioside A in cultured mammalian cells (Nakajima, 2000a) and did not find increases in chromosome aberrations.

Additionally, FDA also reviewed three unpublished studies on rebaudioside 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) submitted by Merisant as part of the GRAS Notification. All three studies demonstrated lack of mutagenic or genotoxic activity. Additionally, Williams and Burdock (2009) also reported lack of genotoxicity in another set of published studies that included *in vitro* mutagenicity assays with *Salmonella*, *E. coli*, and mouse lymphoma cells. These investigators also reported 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. The key mutagenicity testing results for rebaudioside A are summarized in Table 7.

## 3. Reproduction & Developmental Studies

In a two-generation reproductive toxicity study, rebaudioside A (97 % purity) at 0, 7,500, 12,500, and 25,000 ppm was administered in diet to male and female Han Wistar rats (Curry et al., 2008). Administration of rebaudioside A was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. Similarly, administration of rebaudioside A did not affect reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology in either the F<sub>0</sub> or F<sub>1</sub> generations. The survival and general condition of the F<sub>1</sub> and F<sub>2</sub> 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 highest dose tested).

The results from two unpublished studies with rebaudioside A (Sloter 2008a, b) further support the above described findings from published studies. In a two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were fed either basal diet or the diet containing rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter 2008a). For the F<sub>0</sub> and F<sub>1</sub> generations rebaudioside A doses were 0, 500, 1000 and 2000 mg/kg/day. At initiation of study, F<sub>0</sub> animals were approximately 7 weeks of age. The test diet was offered to the offspring selected to become the F<sub>1</sub> generation following weaning [beginning on postnatal day (PND) 21]. The F<sub>0</sub> and F<sub>1</sub> males continued to receive rebaudioside A throughout mating, continuing through the day of euthanasia. The F<sub>0</sub> and F<sub>1</sub> 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 as evaluated by estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints. Both for parental systemic and reproductive toxicity a dose level  $\geq 2000$  mg/kg bw/day (highest dose administered) was assigned to be the NOAEL.

In an embryo/fetal developmental toxicity study in rats (Sloter, 2008b), effects of rebaudioside A administered via gavage was tested. Rebaudioside A administration did not affect intrauterine growth and survival, 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  $\geq 2000$  mg/kg bw/day (highest dose administered) was considered to be the NOAEL for maternal and embryo/fetal developmental toxicity.

#### 4. Clinical Studies on Rebaudioside A

In a four week randomized, double-blind, placebo controlled trial, hemodynamic effects of rebaudioside A at a dose of 1000 mg/day rebaudioside A (97% purity) or placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) were investigated (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, administration of 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 investigators concluded that consumption of 1000 mg/day of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

In another trial, effects of 16 weeks of consumption of 1000 mg rebaudioside A (97% purity, n = 60) were compared to placebo (n = 62) in men and women (33-75 years of age) with type 2 diabetes mellitus (Maki, et al., 2008b). Changes in glycosylated hemoglobin levels did not differ significantly between the rebaudioside A ( $0.11 \pm 0.06\%$ , mean  $\pm$  standard error) and placebo ( $0.09 \pm 0.05\%$ ;  $p = 0.355$ ) groups. Similarly, no significant ( $p > 0.05$  for all) changes from baseline for rebaudioside A and placebo, respectively, in fasting glucose ( $7.5 \pm 3.7$  mg/dL and  $11.2 \pm 4.5$  mg/dL), insulin ( $1.0 \pm 0.64$   $\mu$ U/mL and  $3.3 \pm 1.5$   $\mu$ U/mL), and Cpeptide ( $0.13 \pm 0.09$  ng/mL and  $0.42 \pm 0.14$  ng/mL) were noted. No treatment related changes in blood pressure, body weight, and fasting lipids were noted. Rebaudioside A was well-tolerated, and records of

hypoglycemic episodes showed no excess versus placebo. Based on these results, the investigators suggested that chronic use of 1000 mg rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

**Table 7. 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 <i>E. coli</i> 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	Stevioside, 52%; Reb A, 22%	250 - 2000 mg/kg bw	Negative <sup>a</sup>	Sekishashi et al. (2002)
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Reb A	NS	1.2 - 55 mg/mL	Negative <sup>b</sup>	Nakajima (2000a)
Micronucleus formation	BDF1 mouse bone marrow	Reb A	NS	500-2000 mg/kg bw per day for 2 days	Negative <sup>c</sup>	Nakajima (2000b)
Forward mutation	<i>S. typhimurium</i> TM677	Reb A	NS	10 mg/plate	Negative <sup>b</sup>	Pezzuto et al. (1985)

NS = Not specified.

<sup>a</sup> Sacrificed at 3 hours and 24 hours.

<sup>b</sup> With or without metabolic activation (source not specified in original monograph).

<sup>c</sup> Sacrificed at 30 hours after 2nd administration.

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

In three recently completed studies, absorption and fate of rebaudioside A was systematically investigated in rats and humans.

For comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, rebaudioside A, toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol were examined in rats (Roberts and Renwick, 2008). Orally administered single doses of the radiolabelled compounds were extensively and rapidly absorbed with plasma concentration-time profiles following similar patterns for stevioside and rebaudioside A. Within 72 hours of administration, elimination of radioactivity from plasma was essentially complete. 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 rebaudioside A and stevioside are handled in an almost identical manner in the rat after oral dosing.

In a randomized, double blind, cross-over study in healthy male subjects, Wheeler et al. (2008) assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of rebaudioside A and stevioside. Following administration of rebaudioside A or stevioside, steviol glucuronide appeared in the plasma of all subjects, with median  $T_{max}$  values of 12.00 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  $C_{max}$  value (1472 ng/ml) than administration of stevioside (1886 ng/mL). The geometric mean  $AUC_{0-t}$  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 investigators 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 investigation 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). Rebaudioside A and total steviol were detected in peripheral blood of rats during daily administration of 2000 mg/kg bw/day of rebaudioside A at extremely low levels, with mean plasma concentrations of approximately 0.6 and 12 ug/mL, respectively. Estimates of absorbed dose for rebaudioside 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 rebaudioside A and measured hydrolysis products expressed as *Total Rebaudioside 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.”<sup>13</sup>

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.”<sup>14</sup>

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:<sup>15</sup>

- 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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<sup>13</sup> See 21 CFR 170.3(i).

<sup>14</sup> See 21 CFR 170.30(a).

<sup>15</sup> See 62 FR 18938 (17 April 1997) which is accessible at <http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm>.

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. Discussion of Expert Safety Reviews of Steviol Glycosides

Because of their sweetness characteristics, steviol glycosides are unique in that they have viable uses as a non-nutritive sweetener in foods.<sup>16</sup> Periodic reviews by JECFA over the years indicate the progress of knowledge on the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple investigations with *in vivo* and *in vitro* models which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably, the initially reported concerns related to the effects of stevia leaves or crude extracts on fertility were refuted by the well-designed reproductive studies with purified steviol glycosides. All other concerns failed to manifest themselves at the doses employed in the long-term rat studies.

As discussed in Section V, at its fifty-first meeting, JECFA determined that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), to establish a temporary ADI of 0 - 2 mg/kg bw/day with an adequate margin of safety. The committee also critically reviewed the lack of carcinogenic response in well-conducted studies. These studies justified the Committee 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 are safe to use as a sugar substitute. Subsequently, the additional clinical data reviewed by JECFA allowed the Committee to establish a permanent ADI of 0 - 4 mg/kg bw/day

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<sup>16</sup> 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. Chatsudhipong and Muanprasat (2009) 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 contemporary purity specifications established by JECFA for use as a sweetener. If oral doses of steviol glycosides impart pharmacological effects, such effects would undoubtedly occur due to actions of the principle metabolite, steviol, but the pharmacological effects of steviol have not been comprehensively investigated.

(based on steviol equivalents) or 0 - 12 mg/kg bw for rebaudioside A. The GRAS Expert Panel critically reviewed the JECFA assessment and agrees with this reasoning.

The Panel discussed findings from a recently published exploratory subchronic toxicity study in rats by Awney et al. (2010), where a number of toxicological effects of stevioside treatment were reported. Critical review of the publication revealed the poor study design that included insufficient numbers of animals, group-housing with the potential for stress-related changes, unreliable access to steviol *via* drinking water resulting in suspect dosing calculations in group-housed cages, no indication of fasting prior to blood collection which affects many chemistry and hematological values, no urine collection and no histopathological evaluations for confirmation of findings beyond the controls. Additionally, the report did not adequately report mean or individual organ weight data and lacked comparison of study findings against laboratory historical control data. In contrast to the data presented by Awney et al. (2010), several well-designed and well-conducted subchronic toxicity studies did not reveal any adverse effects from rebaudioside A consumption.

The Panel also noted from a recent study that DNA damage was seen in a variety of organs as assessed by comet assay in rats given drinking water containing 4 mg/mL steviol glycosides for up to 45 days (Nunes et al., 2007). The methodology used in this study was questioned by several experts in the field (Geuns, 2007; Williams, 2007; Brusick, 2008). The Panel has reviewed the cited publications and agrees and discounts the importance of the Nunes et al. (2007) study.

The Panel has reviewed the findings from human clinical studies. The Panel noted that as regards to the 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). The new data presented to JECFA and also published by Barriocanal et al. (2008) 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. It is possible that JECFA may also have reviewed the preliminary results associated with the recently published clinical studies on rebaudioside A (Maki et al., 2008a, b). The Panel concludes that there will be no effects on blood pressure and glucose metabolism in humans at the doses of rebaudioside A expected from its use in food as a non-nutritive sweetener.

JECFA's review also included anticipated dietary patterns and the use concentrations expected in various foods in order to calculate an estimated daily intake (EDI) (WHO, 2003, 2006). Based on the assumption of 100% substitution of steviol glycosides for sugar, an EDI of 5 mg/kg bw/day of steviol was calculated for US consumption. JECFA noted 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 5, the highest dietary estimate for use in foods for rebaudioside A is 4.7 mg/kg bw/day. The Panel agrees with the JECFA ADI of 4 mg/kg bw/day based on steviol equivalents which

corresponds to 12 mg/kg bw/day for rebaudioside A and notes that the estimates as contained in Table 5 of anticipated dietary intake are below the ADI.

### C. Discussion of Safety of Rebaudioside A<sup>17</sup>

Since July 2008, over ten papers describing the results of a comprehensive research program by different groups on rebaudioside A have been published. These and some other unpublished studies formed the basis of the two initial GRAS notifications to FDA each by Cargill (GRN 253) and Merisant (GRN 252). Prior to this, a limited number of toxicology studies specifically on rebaudioside A were conducted. Even before these new studies were completed and as noted in the previous section, JECFA concluded 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.

Since a majority of the previous pharmacokinetic research was conducted with steviol glycosides, the presumed strategy adopted for the more recent research on rebaudioside A was to conduct a limited number of well-designed and executed toxicology studies on rebaudioside A itself and to demonstrate in rats and in humans that it is handled pharmacokinetically similarly to stevioside. This approach appears to have been undertaken to justify the JECFA-generated ADI without having to conduct a chronic study in rats with rebaudioside A. Additionally, the Merisant group conducted three mutagenicity assays on rebaudioside A 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.

In a review article, Carakostas et al. (2008) summarized the most recent research on rebaudioside A. This review summarized the findings of the Cargill research program as follows:

- Steviol glycosides, rebaudioside A, and stevioside are not genotoxic *in vitro*.
- In well-conducted *in vivo* assays, steviol glycosides, rebaudioside A, and stevioside have not been found to be genotoxic.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes, et al., 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.

<sup>17</sup> Questions about the safety of rebaudioside A were previously raised by Huxtable (2002) and Kobylewski and Eckhert (2008). Their respective concerns, as well as opposing views supporting the safety of designated food uses of rebaudioside A expressed by Expert Panels have been outlined in other GRAS notifications that were submitted to FDA. A more detailed account can be found in GRAS notifications 278, 287, 303, and 304.

- 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 the consumption estimates described by both JECFA and Renwick (2008) very conservatively represent a potential high user of rebaudioside A if this non-nutritive sweetener becomes widely available in food. As part of the present GRAS evaluation, the Panel adopts the JECFA EDI for application to GLG's purified rebaudioside A (≥ 95%).

Regarding the available aggregate safety information, the Panel has concluded that JECFA has critically and extensively evaluated the use of steviol glycosides in foods and agrees that, at the present time, the ADI for steviol glycosides of adequate purity as defined by JECFA specifications has been properly determined to be 4 mg/kg bw/person as steviol equivalents, which corresponds to 12 mg/kg bw/day for rebaudioside A on a dry weight basis. The Panel agrees that unwanted pharmacological effects are not likely to occur at this level and that high consumers of rebaudioside 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).

The Panel recognizes that JECFA is composed of dozens of scientists that are internationally known 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.

The studies conducted by Cargill 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 agrees with the conclusion of JECFA and the Cargill and Merisant Expert Panels 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 rebaudioside A, when added to food at levels up to full replacement of sugar on a sweetness equivalency basis, meets FDA's definition of safe.

#### **D. 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 published 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 a series of studies published during 2008 and cited earlier. The newest clinical studies that address JECFA's concern on unwanted pharmacological effects with steviol glycosides (Barriocanal et al., 2008) and with rebaudioside A (Maki et al., 2008 a, b) are also published in the peer-reviewed scientific literature.

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 earlier 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; AFSSA, 2009). Furthermore, the favorable scientific opinion on the safety of steviol glycosides use as a sweetener in foods as issued by EFSA in 2010 reinforces the safety determinations of many other qualified organizations (EFSA, 2010). In addition, a number of individual 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 questions” 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<sup>18</sup>

**GLG's purified rebaudioside A (≥ 95%) as expressed on a dry weight basis is Generally Recognized As Safe when consumed as a general purpose non-nutritive sweetener in foods other than infant formulas and meat and poultry products 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  
Chair

(b) (6)

Robert S. McQuate, Ph.D.

(b) (6)

Madhusudan G. Soni, Ph.D., FACN

May 4, 2011

<sup>18</sup> 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](http://www.gras-associates.com). 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. Soni's curriculum vitae can be accessed at <http://www.soniassociates.net>. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety. All three individuals have previously served on multiple GRAS Expert Panels. Dr. Kraska served as Chair of the Panel.

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## **APPENDIX A**

### **JECFA Steviol Glycosides Specifications & Analytical Method**

#### **2010 – JECFA Specifications for Steviol Glycosides**

## STEVIOLE GLYCOSIDES

Prepared at the 73<sup>rd</sup> JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 69<sup>th</sup> JECFA (2008) and published in FAO JECFA Monographs 5 (2008). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69<sup>th</sup> 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 the 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 B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside which are 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-16-en-18-oic acid, β-D-glucopyranosyl ester

### C.A.S. number

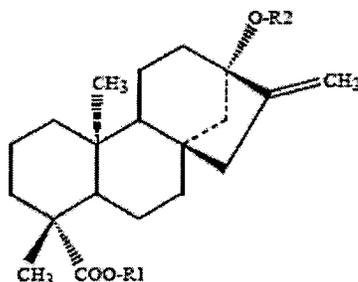
Stevioside: 57817-89-7  
Rebaudioside A: 58543-16-1

### Chemical formula

Stevioside: C<sub>38</sub>H<sub>60</sub>O<sub>18</sub>  
Rebaudioside A: C<sub>44</sub>H<sub>70</sub>O<sub>23</sub>

Structural Formula

The nine named steviol glycosides:



<u>Compound name</u>	<u>R1</u>	<u>R2</u>
<i>Stevioside</i>	$\beta$ -Glc	$\beta$ -Glc- $\beta$ -Glc(2→1)
<i>Rebaudioside A</i>	$\beta$ -Glc	$\beta$ -Glc- $\beta$ -Glc(2→1)   $\beta$ -Glc(3→1)
<i>Rebaudioside B</i>	H	$\beta$ -Glc- $\beta$ -Glc(2→1)   $\beta$ -Glc(3→1)
<i>Rebaudioside C</i>	$\beta$ -Glc	$\beta$ -Glc- $\alpha$ -Rha(2→1)   $\beta$ -Glc(3→1)
<i>Rebaudioside D</i>	$\beta$ -Glc- $\beta$ -Glc(2→1)	$\beta$ -Glc- $\beta$ -Glc(2→1)   $\beta$ -Glc(3→1)
<i>Rebaudioside F</i>	$\beta$ -Glc	$\beta$ -Glc- $\beta$ -Xyl(2→1)   $\beta$ -Glc(3→1)
<i>Dulcoside A</i>	$\beta$ -Glc	$\beta$ -Glc- $\alpha$ -Rha(2→1)
<i>Rubusoside</i>	$\beta$ -Glc	$\beta$ -Glc
<i>Steviolbioside</i>	H	$\beta$ -Glc- $\beta$ -Glc(2→1)

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

Formula weight

Stevioside: 804.88  
 Rebaudioside A: 967.03

Assay	Not less than 95% of the total of the nine named steviol glycosides on the dried basis.
<b>DESCRIPTION</b>	White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.
<b>FUNCTIONAL USES</b>	Sweetener
<b>CHARACTERISTICS</b>	
IDENTIFICATION	
<u>Solubility</u> (Vol. 4)	Freely soluble in water
<u>Stevioside and rebaudioside A</u>	The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.
<u>pH</u> (Vol. 4)	Between 4.5 and 7.0 (1 in 100 solution)
PURITY	
<u>Total ash</u> (Vol. 4)	Not more than 1%
<u>Loss on drying</u> (Vol. 4)	Not more than 6% (105°, 2h)
<u>Residual solvents</u> (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)
<u>Arsenic</u> (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
<u>Lead</u> (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").
<b>METHOD OF ASSAY</b>	Determine the percentages of the individual steviol glycosides by HPLC (Vol. 4) under the following conditions.  <u>Reagents</u> Acetonitrile: more than 95% transmittance at 210 nm.  <u>Standards</u> Stevioside: more than 99.0% purity on the dried basis. Rebaudioside A: more than 99.0% purity on the dried basis. Mixture of nine steviol glycosides standard solution: Containing stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and

steviolbioside. This solution is diluted with water-acetonitrile (7:3) accordingly and is used for the confirmation of retention times. Standards are available from Wako Pure Chemical Industries, Ltd. Japan and ChromaDex, USA.

Standard solution

Accurately weigh 50 mg of stevioside and rebaudioside A standard into each of two 50-ml volumetric flasks. Dissolve and make up to volume with water-acetonitrile (7:3).

Sample solution

Accurately weigh 50-100 mg of sample into a 50-ml volumetric flask. Dissolve and make up to volume with water-acetonitrile (7:3).

Procedure

Inject 5 µl of sample solution under the following conditions.  
Column: Capcell pak C<sub>18</sub> MG II (Shiseido Co.Ltd) or Luna 5µ C18(2) 100A (Phenomenex) or equivalent (length: 250 mm; inner diameter: 4.6 mm, particle size: 5µm)  
Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L sodium phosphate buffer (pH 2.6)  
Flow rate: 1.0 ml/min  
Detector: UV at 210 nm  
Column temperature: 40°  
Record the chromatogram for about 30 min.

Identification of the peaks and Calculation

Identify the peaks from the sample solution by comparing the retention time with the peaks from the mixture of nine steviol glycosides standard solution (see under figure). Measure the peak areas for the nine steviol glycosides from the sample solution. Measure the peak area for stevioside and rebaudioside A from their standard solutions. Calculate the percentage of each of the eight steviol glycosides except rebaudioside A in the sample from the formula:

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

Calculate the percentage of rebaudioside A in the sample from the formula:

$$\%Rebaudioside\ A = [W_R/W] \times [A_x/A_R] \times 100$$

where

- X is each steviol glycoside;
- W<sub>S</sub> is the amount (mg) calculated on the dried basis of stevioside in the standard solution;
- W<sub>R</sub> is the amount (mg) calculated on the dried basis of rebaudioside A in the standard solution;
- W is the amount (mg) calculated on the dried basis of sample in the sample solution;
- A<sub>S</sub> is the peak area for stevioside from the standard solution;
- A<sub>R</sub> is the peak area for rebaudioside from the standard solution;

$A_X$  is the peak area of X for the sample solution; and  $f_X$  is the ratio of the formula weight of X to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

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

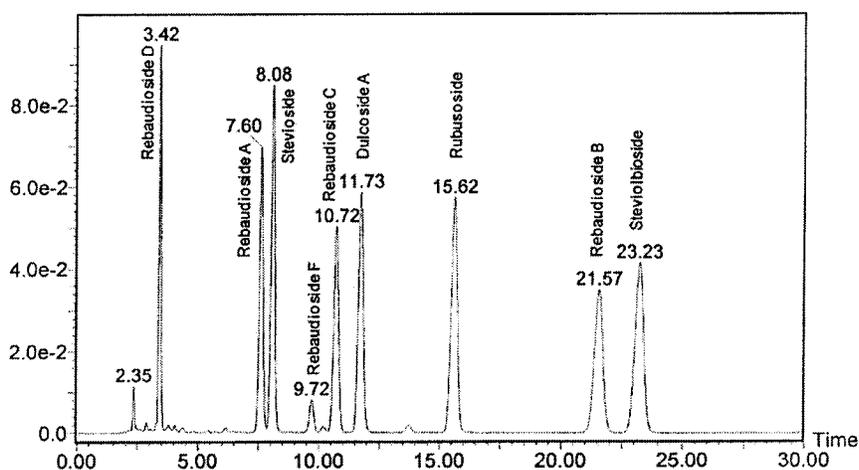


Figure. Chromatogram of mixture of nine steviol glycosides standard solution

Column: Capcell pak C<sub>18</sub> MG II

Concentration: 0.5 mg/ml each except rebaudioside F (about 0.1 mg/ml)

## **APPENDIX B**

### **Manufacturing Information for Production of High Purity Rebaudioside A ( $\geq 95\%$ )**

- B-1 Process Flow Diagram for Primary Stevia Extract (Rebaudioside A-Rich)**
- B-2 Process Flow Diagram for RA95**

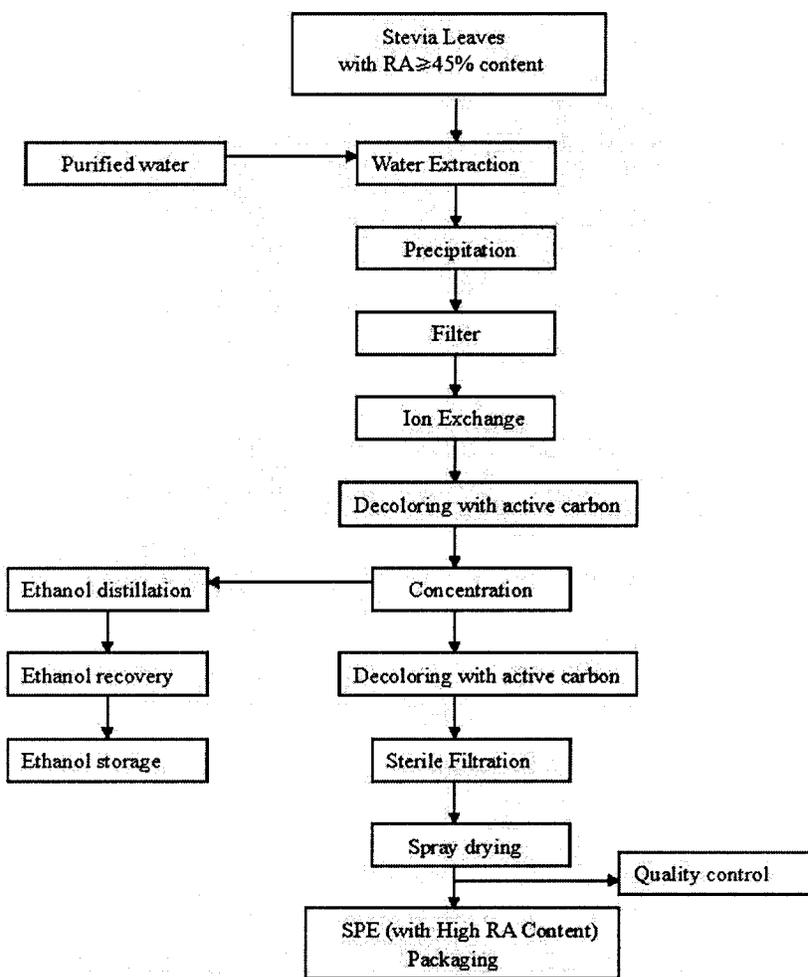
### Appendix B-1



File No. GLG-QA-STD-085  
Reviewed by: Zhang Lai - QC Manager  
Approved by: Kevin Li - Vice President

#### Stevia Primary Extract (with High RA Content)

#### Process Flow Chart



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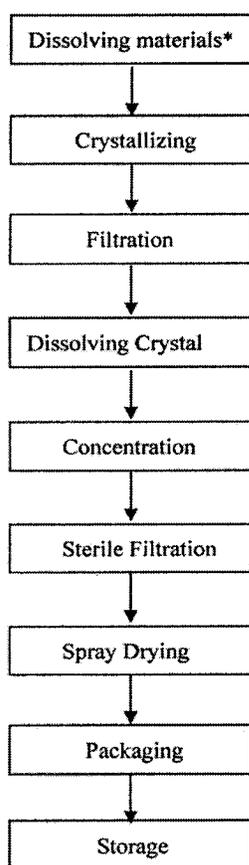
## Appendix B-2



# GLG Life Tech Corporation

File No. GLG-QA-STD-089  
Reviewed by: Zhang Lei - QC Manager  
Approved by: Kevin Li - Vice President

### Rebpure RA95 Process Flow Chart



\*Materials: Stevia Primary Extract with TSG  $\geq 87\%$  including RA  $\geq 45\%$   
Specialty Solvent to be used in above RA95 process is Ethanol only

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## **APPENDIX C**

- Appendix C-1            GLG RA95 Certificates of Analysis**
- Appendix C-2 (Part 1) Eurofins Analyses of GLG RA95:  
Methodology**
- Appendix C-2 (Part 2) Eurofins Analyses of GLG RA95:  
Data & Chromatograms**
- Appendix C-3            GLG RA95 Pesticide Residue Analyses**

Appendix C-1



Certificate of Analysis



Research and Development  
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 GLG-QA-COA-18

Product: Rebpure™ RAS5      Manufacturing Date: Aug 4th, 2010  
 Lot Number: (b) (6)      Country of Origin: China  
 Shelf Life: 2 years

Product Description: Rebpure™ RAS5 is a highly purified extract containing primarily rebaudioside A (MW: 967.03) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages

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 Canada      Web: www.glglifetech.com

Manufacturing By: Qingdao Runhao Rebiana High Tech Co., Ltd      Phone: +86.532.55553333  
 Qingdao Export Processing Zone, Chengyang District      Fax: +86.532.55566968  
 Qingdao, Shandong, China 266400

GLG Qingdao Runhao Rebiana High Tech Co., Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Date of Analysis: Aug 9th, 2010

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 95%	96.9%	JECFA HPLC
Total Steviol Glycosides	≥ 97%	99.64%	JECFA HPLC
Solubility	Freely soluble in water	Freely soluble in water	USP
pH	4.5 - 7.0	5.44	USP
Residue on Ignition	< 1.0%	0.07%	USP
Loss on Drying	≤ 4.0%	2.40%	USP
Specific Rotation [α] <sub>D</sub> <sup>20</sup>	-29° to -31°	-31°	USP
Lead (Pb)	< 1 ppm	0.1ppm	AFS
Arsenic (As)	< 1 ppm	0.03ppm	AFS
Residual Solvents – Ethanol	≤ 0.5%	0.15%	USP
– Methanol	≤ 0.02%	0.01%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10 cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10 cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: QUALIFIED

Note: This product should be stored sealed in a cool, dry place.

Analyzed by: (b) (6)      Date: 09/08/2010  
 Checked by: (b) (6)      Date: 09/08/2010  
 Approved by: (b) (6) (Quality Manager)      Date: 09/08/2010

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## Certificate of Analysis



Research and Development  
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 www.glglifetech.com  
 GLG-QA-COA-10

**Product:** Rebpure™ RA85  
**Lot Number:** (b) (6)  
**Manufacturing Date:** Aug. 4th, 2010  
**Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Rebpure™ RA85 is a highly purified extract containing primarily rebaudioside A (MW: 967.03) from Stevia rebaudiana Berton| leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages

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 Web: www.glglifetech.com

**Manufacturing By:** Qingdao Runhao Reblana High Tech Co., Ltd  
 Qingdao Export Processing Zone, Chengyang District,  
 Qingdao, Shandong, China 266400  
 Phone: +86.532.55553333  
 Fax: +86.532.55566968

GLG Qingdao Runhao Reblana High Tech Co., Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

**Date of Analysis:** Aug. 9th, 2010

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 95%	96.13%	JECFA HPLC
Total Steviol Glycosides	≥ 97%	98.7%	JECFA HPLC
Solubility	Freely soluble in water	Freely soluble in water	USP
pH	4.5 - 7.0	5.4	USP
Residue on Ignition	< 1.0%	0.06%	USP
Loss on Drying	≤ 4.0%	2.20%	USP
Specific Rotation (α) <sub>D</sub> <sup>20</sup>	-29° to -31°	-31°	USP
Lead (Pb)	< 1 ppm	0.1ppm	AFS
Arsenic (As)	< 1 ppm	0.02ppm	AFS
Residual Solvents – Ethanol	≤ 0.5%	0.11%	USP
– Methanol	≤ 0.02%	0.01%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10 cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10 cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

**Conclusion** QUALIFIED

Note: This product should be stored sealed in a cool, dry place.

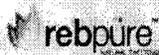
Analyzed by: (b) (6) Date: 09/08/2010

Checked by: (b) (6) Date: 09/08/2010

Approved by: (b) (6) (Quality Manager) Date: 09/08/2010

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### Certificate of Analysis



Research and Development  
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 www.glglifetech.com  
 GLG-QA-00A-10

**Product:** Rebpure™ RA95      **Manufacturing Date:** Aug 3rd, 2010  
**Lot Number:** (b) (6)      **Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Rebpure™ RA95 is a highly purified extract containing primarily rebaudioside A (MW: 967.03) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

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 Canada      Web: www.glglifetech.com

**Manufacturing By:** Qingdao Runhao Reblana High Tech Co., Ltd      Phone: +86.532.55553333  
 Qingdao Export Processing Zone, Chengyang District      Fax: +86.532.55566968  
 Qingdao, Shandong, China 266400

GLG Qingdao Runhao Reblana High Tech Co., Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

**Date of Analysis:** Aug 8th, 2010

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 95%	95.84%	JECFA HPLC
Total Steviol Glycosides	≥ 97%	98.48%	JECFA HPLC
Solubility	Freely soluble in water	Freely soluble in water	USP
pH	4.5 - 7.0	5.4	USP
Residue on Ignition	< 1.0%	0.06%	USP
Loss on Drying	≤ 4.0%	2.50%	USP
Specific Rotation [α] <sup>D</sup> / <sub>1%</sub>	-29° to -31°	-31°	USP
Lead (Pb)	< 1 ppm	0.1ppm	AFS
Arsenic (As)	< 1 ppm	0.04ppm	AFS
Residual Solvents - Ethanol	≤ 0.5%	0.12%	USP
- Methanol	≤ 0.02%	0.01%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10 cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10 cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

**Conclusion**      **QUALIFIED**

Note: This product should be stored sealed in a cool, dry place.

Analyzed by: \_\_\_\_\_ (b) (6)      Date: 08/08/2010  
 Checked by: \_\_\_\_\_ (b) (6)      Date: 08/08/2010  
 Approved by: \_\_\_\_\_ (b) (6) (Quality Manager)      Date: 08/08/2010

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### Certificate of Analysis



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 GLG-QA-COA-10

Product: Rebpure™ RA85      Manufacturing Date: Aug. 7th, 2010  
 Lot Number: (b) (6)      Country of Origin: China  
 Shelf Life: 2 years

**Product Description:** Rebpure™ RA85 is a highly purified extract containing primarily rebaudioside A (MW: 967.03) from Stevia rebaudiana (Bertoni) leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

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**Manufacturing By:** Qingdao Runhao Rebiana High Tech Co., Ltd      Phone: +86.532.55553333  
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 Qingdao, Shandong, China 266400

GLG Qingdao Runhao Rebiana High Tech Co., Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Date of Analysis: Aug. 12th, 2010

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 95%	96.2%	JECFA HPLC
Total Steviol Glycosides	≥ 97%	97.72%	JECFA HPLC
Solubility	Freely soluble in water	Freely soluble in water	USP
pH	4.5 - 7.0	4.76	USP
Residue on Ignition	< 1.0%	0.05%	USP
Loss on Drying	≤ 4.0%	2.40%	USP
Specific Rotation (α) <sub>D</sub> <sup>20</sup>	-29° to -31°	-31°	USP
Lead (Pb)	< 1 ppm	0.1ppm	AFS
Arsenic (As)	< 1 ppm	0.02ppm	AFS
Residual Solvents – Ethanol	≤ 0.5%	0.13%	USP
– Methanol	≤ 0.02%	0.01%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10 cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10 cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: **QUALIFIED**

Note: This product should be stored sealed in a cool, dry place.

Analyzed by: (b) (6)      Date: 12/08/2010  
 Checked by: (b) (6)      Date: 12/08/2010  
 Approved by: (Quality Manager)      Date: 12/08/2010

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### Certificate of Analysis



Research and Development  
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 GLG-QA-COA-10

**Product:** Rebpure™ RA95      **Manufacturing Date:** Aug. 6th, 2010  
**Lot Number:** (b) (6) 112      **Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Rebpure™ RA95 is a highly purified extract containing primarily rebaudioside A (MW: 967.03) from Stevia rebaudiana Barton leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

**Distributed By:** GLG Life Tech Corporation      Phone: 1.604.641.1368  
 999 Canada Place, Suite 519      Fax: 1.604.844.2830  
 Vancouver, B.C. V6C 3E1      Email: sales@glglifetech.com  
 Canada      Web: www.glglifetech.com

**Manufacturing By:** Qingdao Runhao Rebiana High Tech Co., Ltd      Phone: +86 532 55553333  
 Qingdao Export Processing Zone, Chengyang District      Fax: +86 532 55566968  
 Qingdao, Shandong, China 266400

GLG Qingdao Runhao Rebiana High Tech Co., Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

**Date of Analysis:** Aug. 11th, 2010

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 95%	96.3%	JECFA HPLC
Total Steviol Glycosides	≥ 97%	98.92%	JECFA HPLC
Solubility	Freely soluble in water	Freely soluble in water	USP
pH	4.5 - 7.0	5.41	USP
Residue on Ignition	< 1.0%	0.07%	USP
Loss on Drying	≤ 4.0%	2.30%	USP
Specific Rotation (α) <sup>D</sup> <sub>20</sub>	-29° to -31°	-31°	USP
Lead (Pb)	< 1 ppm	0.1ppm	AFS
Arsenic (As)	< 1 ppm	0.04ppm	AFS
Residual Solvents – Ethanol	≤ 0.5%	0.12%	USP
– Methanol	≤ 0.02%	0.01%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10 cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10 cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: **QUALIFIED**

Note: This product should be stored sealed in a cool, dry place.

Analyzed by: (b) (6)      Date: 11/08/2010  
 Checked by: (b) (6)      Date: 11/08/2010  
 Approved by: (b) (6) (Quality Manager)      Date: 11/08/2010

Disclaimers: This document contains confidential information that is intended only for the use of the party to whom it is addressed. Any disclosure, copying or distribution or use of the contents herein to a third party is prohibited.

999 Canada Place, 519 World Trade Centre \* Vancouver, B.C. \* Canada \* V6C 3E1  
 Phone: 1.604.641.1368 \* Fax: 1.604.844.2830 \* Email: sales@glglifetech.com \* Web: glglifetech.com

**Appendix C-2 (Part 1)**

(b) (6)



**Method Verification  
for the Determination of Rebaudioside A and Related  
Steviol Glycosides by High Performance Liquid  
Chromatography (HPLC) and Purity Analysis of Five  
Production Samples**

Prepared by: (b) (6)

Reviewed by:



Approved by: \_\_\_\_\_  
James Kempland  
GLG Life Tech Corporation

Date Issued: February, 2011

(b) (6)

Method Verification, Rebpure RA95, GLG  
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**I. Study Identification**

**1. Study Title:**

Method Verification for the Determination of Rebaudioside A and Related Steviol Glycosides by High Performance Liquid Chromatography (HPLC). *This report represents a replacement report for the initial report issued in October of 2010. It includes additional information and data regarding calibration, method validation, sample preparation and loss on drying. The attached data is also included to replace the data previously submitted in October of 2010.*

**2. Study Objective:**

The objective of this study is to verify the assay for rebaudioside A and related glycosides as listed in JEFCA (2008) in the GLG supplied Rebpure RA95 Stevia leaf extracts.

**3. Study Coordinator/Performing Laboratory:**

Jules Skamarack, (b) (6)

**4. Study Monitor(s):**

Marisel Esguerra, (b) (6)

**5. Test Materials:**

*Stevia rebaudiana* Leaf extracts

- (1) Rebpure RA95, Powder, Lot #GLG\_RA95-1008003, Serving = 100g, Eurofins sample number 740-2010-00006994
- (2) Rebpure RA95, Powder, Lot #GLG\_RA95-1008006, Serving = 100g, Eurofins sample number 740-2010-00006995
- (3) Rebpure RA95, Powder, Lot #GLG\_RA95-1008008, Serving = 100g, Eurofins sample number 740-2010-00006996
- (4) Rebpure RA95, Powder, Lot #GLG\_RA95-1008012, Serving = 100g, Eurofins sample number 740-2010-00006997
- (5) Rebpure RA95, Powder, Lot #GLG\_RA95-1008015, Serving = 100g, Eurofins sample number 740-2010-00006998

**6. Test Reagents:**

- (1) Acetonitrile, HPLC Grade  
Burdick & Jackson Lot# DC134 P/N AH015
- (2) Rebaudioside A, Lot. F01077 from USP C.A.S # 58543-16-1 calibration standard
- (3) Steviolbioside, Lot. 19349-2871-16 from ChromaDex C.A.S # 41093-60-1 retention time marker
- (4) Rebaudioside B, Lot. 18227-101 from ChromaDex C.A.S # 58543-17-2 retention time marker
- (5) Stevioside, Lot. 19351-0364 from ChromaDex C.A.S # 57817-89-7 retention time marker
- (6) Rebaudioside C, Lot. 00018228-3202 from ChromaDex C.A.S # 63550-99-2 retention time marker

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Method Verification, Rebpure RA95, GLG  
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- (7) Phosphoric Acid, Fischer Chemical Company P/N A260
- (8) Positive control sample identified as Eurofins control # LCKK149-1 monitored for rebaudioside A concentration.

Mobile Phase Preparation: (see attached method) 80% HPLC grade acetonitrile: 20% Milli-Q water (pH adjusted to 3.0 with phosphoric acid) filtered through 0.5 µm filter (V/V).

#### 7. Method References:

High Performance Liquid Chromatographic Determination of Individual Sweet Diterpenoid Glycosides of *Stevia rebaudiana*, W.A.Court, Agriculture & Food Canada Pest Management Research Centre, P.O. Box 186, Ontario, N4B 2W9

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

## II. Study Description

### 1. Scope:

This is applicable to the determination of rebaudioside A, and related Stevia glycosides in 5 raw material samples.

### 2. Test Materials:

- (1) Rebpure RA95, Powder, Lot #GLG\_RA95-1008003, Serving = 100g, Eurofins sample number 740-2010-00006994
- (2) Rebpure RA95, Powder, Lot #GLG\_RA95-1008006, Serving = 100g, Eurofins sample number 740-2010-00006995
- (3) Rebpure RA95, Powder, Lot #GLG\_RA95-1008008, Serving = 100g, Eurofins sample number 740-2010-00006996
- (4) Rebpure RA95, Powder, Lot #GLG\_RA95-1008012, Serving = 100g, Eurofins sample number 740-2010-00006997
- (5) Rebpure RA95, Powder, Lot #GLG\_RA95-1008015, Serving = 100g, Eurofins sample number 740-2010-00006998

#### A. Sample Preparation.

- 1. The RA 95 samples were dried at 105 degrees Centigrade for two hours as directed by JECFA.
- 2. On a microbalance, accurately weigh  $80.0 \pm 1$  mg of dried RA 95 samples; quantitatively transfer to a flask and quantitatively dilute (class A glassware) to a 40 mL volume with mobile phase and cap. Dissolve using sonication if necessary. Cool to room temperature, filter and place in auto sampler vials for analysis. Concentration is approximately 2 mg/mL rebaudioside A with related steviol glycosides where the actual sample concentration for each sample is listed on the sample chromatograms in the *ESTD Percent Report* area under *Sample Amount*.

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Method Verification, Rebpure RA95, GLG  
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**3. Reference Standard: Rebaudioside A**

**A. Stock standard.**

1. The standard for rebaudioside A was dried at 105 degrees Centigrade for two hours as directed by JECFA.
2. On a microbalance, accurately weigh  $10.0 \pm 1$  mg of rebaudioside A USP standard; quantitatively transfer to a 5-mL volumetric flask with mobile phase. Dissolve using heat if necessary. Cool to room temperature and dilute to volume with mobile phase. Concentration is approximately 2 mg/mL rebaudioside A.
3. Steviolbioside, rebaudioside B, stevioside and rebaudioside C reference materials were run as qualitative retention time markers. A sufficient amount of each material to create a detectable **peak** from the method was placed in an injection vial and solubilized with mobile phase.

**B. Reference standard preparation (USP rebaudioside A).** A single point calibration is used per JECFA for determination of high purity samples and individual glycosides. To accommodate this, the stock reference material is used as prepared. The concentration is adjusted for standard purity and is listed below for rebaudioside and stevioside. Individual glycosides are calculated from the USP rebaudioside A reference standard using the conversion factors listed in JECFA 2008 and the attached methodology.

**Reference Standard Concentration Rebaudioside A (mg/ml)**  
**2.10253628**

**4. Verification Study:**

**A. Primary method:**

The method was modified to use a single point calibration to provide greater accuracy in determining the purity of a near pure material. A single point calibration is recommended by JECFA (2008) A copy of the method "Determination of Rebaudioside A and Related Steviol Glycosides by HPLC – modified JECFA KK149" is attached. The method is validated and is included under our current ISO 17025 scope of accreditation (Certificate # 2942.01). A copy of the validation report is available upon request.

**B. Secondary method (Loss on Drying):**

Loss on drying was performed as per JECFA 2008 requirements. The method consists of drying the sample at 105 degrees Centigrade for 2 hours.

The listed specification is not more than 6%. For the purposes of this study approximately 1 g of sample material was dried at 105 degrees Centigrade for 2 hours. All samples meet or exceed the specification. The raw data is included in the appropriate addendum and is reported below:

Sample 6994	Result (%w/w)
Loss on Drying	1.7842
Sample 6995	Result (%w/w)
Loss on Drying	1.7865
Sample 6996	Result (%w/w)
Loss on Drying	1.1728

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Method Verification, Rebpure RA95, GLG  
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Sample 6997	Result (%w/w)
Loss on Drying	1.9050
Sample 6998	Result (%w/w)
Loss on Drying	1.4080

B. Single Point Calibration:

1. A single point calibration is used by injecting the reference stock standard a minimum of five times. This is a modification from the attached method (KK149) which is used to achieve greater accuracy on high purity analyses and is the published practice for JECFA 2008.

a. Calculate response factors. RSD between injections must be  $\leq 1.5\%$

2. Results, rebaudioside A;

a. Response factors RSD between levels (amount) was found to be 0.00866284 and passed the criteria. The calibration data is included under the appropriate attachment.

C. Selectivity: For purposes of this study, selectivity is specificity

1. Preparation solvent blank analysis:

a. Preparation solvent blanks are to be free of peaks.

1. Result: Blanks were shown to be free of peaks. (file # 2010-09-24\1FA-0101.D and -0102D.)

2. Demonstrate separation of the two major peaks, stevioside and rebaudioside A:

1. Separation was demonstrated during the study with actual retention times of 4.107 minutes (stevioside) and 5.303 minutes (rebaudioside A) (file # 2010-09-24\1DC-2401D included in attachments)

D. System Suitability:

1. The rebaudioside A standard solution is injected five times prior to sample analysis and after every five to six sample injections and at the end of the analysis sequence.

a. Acceptance criteria: The system is considered suitable if the retention times of the standard peaks do not deviate more than 0.5 minutes and the RSD of the peak areas for rebaudioside A are less than 2% for the five replicate injections. Raw data is included in the calibration table attachment

a. Results: Average retention time 5.315 minutes with a RSD of 0.057. The actual deviation between the longest and shortest retention time is 0.008 minutes. All criteria pass.

b. Results: The RSD of the five replicate injections of the rebaudioside A standard solution is 0.0129536.

2. USP tailing factor was determined for the rebaudioside B peak from the sample matrix. The tailing factor should be not more than (NMT) 2.0.

a. Results: The tailing factor passed the criteria (USP) at approximately 0.976.

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Method Verification, Rebpure RA95, GLG  
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3. Column Efficiency, Not less than (NLT) 5000 theoretical plate count, using the Rebaudioside B peak from the sample solution.
  - a. **Results: All theoretical plate count calculations were greater than 5000 with the halfwidth method calculating at 7500 or greater. All pass the criteria.**
4. USP tailing factor was determined for the rebaudioside A standard peak. The tailing factor should be not more than (NMT) 2.0.
  - a. **Results: The tailing factor passed the criteria (USP) at approximately 0.943.**
5. Column Efficiency, Not less than (NLT) 5000 theoretical plate count, using the Rebaudioside A standard peak..
  - a. **Results: All theoretical plate count calculations were greater than 5000 with the halfwidth method calculating at 7400 or greater. All pass the criteria.**

E. Precision:

1. For each sample, perform 3 replicate sample preparations. The acceptance criteria for the RSD for each set of 3 analyses must be ≤ 5% for the total steviol glycoside value.
  - a. **Results: RSDs for all samples pass the criteria.**

Sample 6994	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Stevibioside	0.041864	0.042068	0.046449	0.0434703	5.9402649
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.7854	1.7892	1.7934	1.7893333	0.2236401
Stevioside	0.71318	0.72292	0.71679	0.7176233	0.6874185
Rebaudioside C	0.18118	0.18587	0.18847	0.1851733	1.9952091
Unknown	0.10237	0.10494	0.10127	0.1319267	1.8310594
Rebaudioside A	98.819	98.849	98.922	98.863333	0.0535839
Total	101.64	101.69	101.77	101.7	0.0844783
Sample 6995	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Stevibioside	0.03337	0.030297	0.032426	0.032031	4.9143815
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.63399	1.6509	1.6365	1.6404633	0.5562633
Stevioside	0.82252	0.83430	0.83202	0.8296133	0.7531087
Rebaudioside C	0.40488	0.40709	0.41203	0.408	0.897263
Unknown	0.13079	0.15138	0.13291	0.13836	8.1854332
Rebaudioside A	98.590	98.772	98.237	98.533	0.2760664
Total	101.62	101.65	101.28	101.58333	0.2822939
Sample 6996	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Stevibioside	<0.01	<0.01	<0.01	n/a	n/a
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.5087	1.5839	1.6015	1.5980333	0.7983729
Stevioside	0.10982	0.10261	0.10697	0.1084667	3.4108993

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Method Verification, Rebpure RA95, GLG  
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Rebaudioside C	<0.01	<0.01	<0.01	n/a	n/a
Unknown	0.097512	0.099393	0.095445	0.09745	2.0264034
Rebaudioside A	98.137	98.154	98.059	98.116667	0.0518314
Total	99.953	99.939	99.864	99.918667	0.0478964
<b>Sample 6997</b>	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Stevioside	0.049992	0.044519	0.048481	0.0479973	6.2985606
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	0.99517	0.99589	0.99509	0.9953833	0.0442649
Stevioside	1.3802	1.37275	1.3517	1.36155	0.7777706
Rebaudioside C	0.36649	0.37927	0.35066	0.3654733	3.9215091
Unknown	0.16217	0.173933	0.15079	0.1622677	7.1301246
Rebaudioside A	98.908	98.487	98.668	98.687	0.2129411
Total	101.84	101.45	101.57	101.62	0.1965655
<b>Sample 6998</b>	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Stevioside	0.036319	0.038327	0.030492	0.035046	11.812363
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	2.4156	2.4142	2.4074	2.4124	0.1818248
Stevioside	0.63547	0.64686	0.63887	0.6404	0.9130403
Rebaudioside C	0.074426	0.078958	0.070418	0.073934	4.4602521
Unknown	0.093307	0.099687	0.093398	n/a	n/a
Rebaudioside A	98.626	98.410	98.043	98.359667	0.305876
Total	99.881	99.688	99.284	99.617	0.3055928

### 5. Conclusion:

Method verification was performed on the listed samples using the Eurofins method KK149, *Steviol Glycosides (HPLC) (JECFA 2008, modified)* (JECFA, Joint FAO/WHO Expert Committee on Food Additives). KK149 is a validated method and included under our ISO 17025 scope of accreditation as listed in the appropriate attachment. The purpose of this study is to verify the performance of method KK149 on 5 lots of test material (samples) submitted and to confirm the composition and identification of said submitted test materials.

To verify method performance on these samples the following parameters were determined: calibration, selectivity, system suitability, and precision. Criteria for each parameter was designed to meet or exceed industry standards (AOAC, USP, WHO).

Linearity measures the performance of the analytical instrumentation and methodology in regards to standardization against reference material with a known purity over a specified range of concentrations. The method was shown to be linear during the initial validation. A single point calibration was used to provide greater accuracy in determining the purity of a near pure material. A single point calibration is recommended by JECFA.

Selectivity for this study measured and confirmed that the analysis was free from interferences; as shown from the samples, and reagent blank test results. This study also confirmed that the method can determine the difference between closely related compounds, as shown in the separation of the two major peaks; stevioside and

(b) (6)

Method Verification, Rebpure RA95, GLG  
Page 8 of 8

rebaudioside A. Furthermore this study has proven that the method can properly identify compounds from the test material by retention time.

System suitability further confirms the performance of the method on test materials and standards and the equipments ability to respond consistently over time. Retention time indicates that the peak of interest is stable from sample to sample as the analysis progresses through time, where tailing factor and theoretical plate count describe the condition of the analytical column used for separation. When the criteria are met as it was in this study it indicates that the equipment is acceptable for quantitation and identification.

Precision is performed to confirm the test material can be prepared and analyzed repeatedly with no statistically significant difference between results. This is not only a measure of the ability of the method to perform appropriately on multiple analyses but also assesses the homogeneity of the test material. On this study each test material was prepared in triplicate. To evaluate repeatability on this data the relative standard deviation (RSD) was calculated. The RSD is calculated as the standard deviation divided by the average (of the measured values). All study RSDs passed the criteria. This result indicates the test material submitted (samples) were homogenous.

Attached sample chromatograms show final data results in percent by weight on the dry weight basis. After the samples are dried according to specification, the dried samples are then prepared for analysis as described above. Therefore, an additional correction to the result listed on the chromatogram is not necessary. The Agilent Chem Station software calculates the final result using internal algorithms based on the reference standard calibration level in mg/mL as stated in the *Calibration Report* and the sample preparation concentration (from the *Sample Amount* area of the sample chromatogram) also in mg/mL. Samples and standards are prepared as close in concentration to each other as possible to improve the accuracy of the analysis. Additional hand calculations are not necessary thereby reducing additional errors due to manual calculations

Results of this study further indicate that the KK149 is appropriate and verified for the submitted test material. The five test materials meet the listed specifications as provided on the Rebpure RA95 certificates of analyses (C of As) for identification and content. Identification was confirmed by showing that the peak retention time for rebaudioside A in the chromatogram of the *raw material sample preparation* corresponds to that in the chromatogram of the *standard preparation*, as obtained in the *Assay*. Standard and sample preparations are described above and in the attached method copy. Additionally the concentration of rebaudioside A in each of the samples meet or exceed the specification of  $\geq 95\%$  rebaudioside A. Copies of the submitted C of As are contained in the appropriate attachment.

**Appendix C-2 (Part 2)**

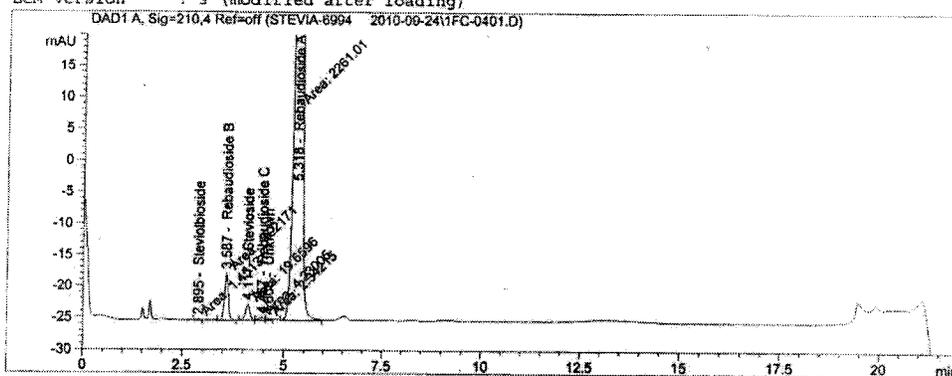
**SAMPLE CHROMATOGRAMS  
(PRECISION  
AND  
IDENTIFICATION)**

Sample Name: 10-6994 A

```

=====
Acq. Operator   : (b)                               Seq. Line :    4
Acq. Instrument : HPLC 10                           Location  : P1-F-03
Injection Date  : 9/24/2010 10:43:51 PM             Inj       :    1
                                                    Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-6994     2010-09-24\STEVIA.M
Last changed   : 9/24/2010 4:25:37 PM by (b)
Analysis Method: C:\CHEM32\10\DATA\STEVIA-6994     2010-09-24\STEVIA.M
Last changed   : 9/27/2010 11:02:00 AM by (b) (6)
              (modified after loading)
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : (b)
ECM Path       : ..\ma\LC\HPLC-10\Data\STEVIA-6994   2010-09-24.SC.SSizip
ECM Version    : 3 (modified after loading)
  
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ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 2.01500 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
2.895	MM	1.45131	5.81236e-4	4.18636e-2		Steviolbioside
3.431		-	-	-		Dulcoside A
3.587	MF	49.21711	7.30948e-4	1.785367		Rebaudioside B
4.117	MF	19.65959	7.30948e-4	0.713158		Stevioside
4.457	MF	4.23005	8.63047e-4	0.181178		Rebaudioside C
4.608	MF	2.34215	8.80670e-4	0.102365		Unknown
5.318	MM	2261.00879	8.80666e-4	98.818567		Rebaudioside A

HPLC 10 9/27/2010 11:02:51 AM (b) (6)

GRAS Assessment – GLG Life Tech Corporation  
Rebaudioside A (≥ 95%)  
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DATA FILE C:\GMS2\10\DATA\01EVIA-0294 2010-09-24\1FC-0401.D  
Sample Name: 10-6994 A

Totals : 101.642499

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

=====  
\*\*\* End of Report \*\*\*

HPLC 10 9/27/2010 11:02:51 AM (b) [REDACTED]

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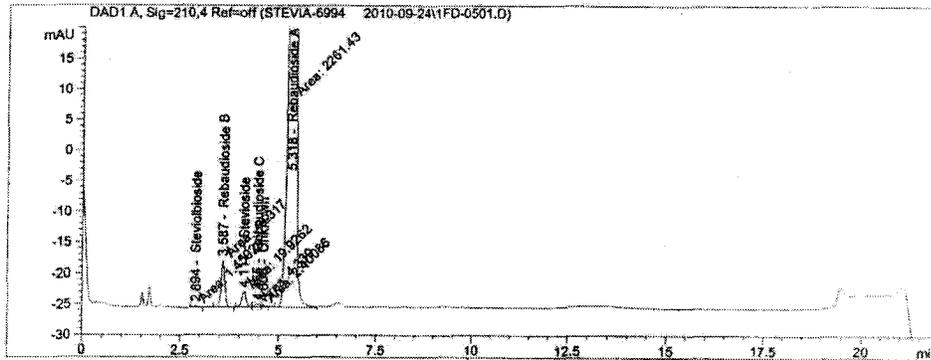
Sample Name: 10-6994 B

```

=====
Acq. Operator   : (b) (6) a                               Seq. Line :    5
Acq. Instrument : HPLC 10                               Location  : Pl-F-04
Injection Date  : 9/24/2010 11:23:34 PM                 Inj       :    1
                                                    Inj Volume: 5.0 µl

Acq. Method    : C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed   : 9/24/2010 4:25:37 PM by (b) (6) a
Analysis Method : C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed   : 9/27/2010 11:02:58 AM by (b) (6) a
Method Info    : Steviol Glycosides by HPLC (modified SECFA)

ECM Server     : http://us05gqlc/ecmwg
ECM Operator   : (b) (6) a
ECM Path       : Petaluma, CA \HPLC-10\Data\STEVIA-6994  2010-09-24.SC.SSIzip
ECM Version    : 4
  
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ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier    : 1.0000
Dilution      : 1.0000
Sample Amount  : 2.01475 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.894	MM	1.45925	5.81236e-4	4.20981e-2		Steviolbioside
3.431						Dulcoside A
3.587	MF	49.31699	7.30948e-4	1.789212		Rebaudioside B
4.117	MF	19.92619	7.30948e-4	0.722919		Stevioside
4.455	MF	4.33900	8.63047e-4	0.185867		Rebaudioside C
4.600	MF	2.40086	8.80670e-4	0.104944		Unknown
5.318	MM	2261.43311	8.80666e-4	98.849377		Rebaudioside A

Totals : 101.694417

HPLC 10 9/27/2010 11:04:59 AM (b) (6)

Page 1 of 2

GRAS Assessment – GLG Life Tech Corporation  
Rebaudioside A (≥ 95%)  
Page 75 of 112

Sample Name: 10-6994 B

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

\*\*\*\*\*  
\*\*\* End of Report \*\*\*

HPLC 10 9/27/2010 11:04:59 AM Ma (b) (6)

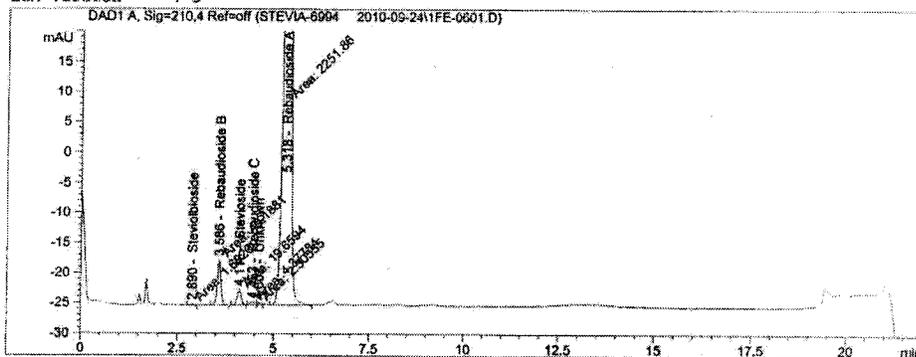
Page 2 of 2

Sample Name: 10-6994 C

```

=====
Acq. Operator   : (b) (6)                               Seq. Line :    6
Acq. Instrument : HPLC 10                               Location  : PI-F-05
Injection Date  : 9/25/2010 12:03:19 AM                 Inj       :    1
                                                    Inj Volume: 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed    : 9/24/2010 4:25:37 PM by (b) (6) a
Analysis Method : C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed    : 9/27/2010 11:02:58 AM by (b) (6) a
Method Info     : Steviol Glycosides by HPLC (modified GCFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : (b) (6) a
ECM Path       : C:\Data\LC-10\Data\STEVIA-6994         2010-09-24.SC.SSizip
ECM Version    : 5
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 2.00475 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.890	MM	1.60209	5.81236e-4	4.64493e-2		Steviolbioside
3.431		-	-	-		Dulcoside A
3.586	MF	49.18806	7.30948e-4	1.793436		Rebaudioside B
4.116	MF	19.65944	7.30948e-4	0.716799		Stevioside
4.453	MF	4.37784	8.63047e-4	0.188466		Rebaudioside C
4.602	MF	2.30535	8.80670e-4	0.101272		Unknown
5.318	MM	2251.85547	8.80666e-4	98.921717		Rebaudioside A

Totals : 101.768140

HPLC 10 9/27/2010 11:07:13 AM (b) (6)

GRAS Assessment – GLG Life Tech Corporation  
Rebaudioside A (≥ 95%)  
Page 77 of 112

Sample Name: 10-6994 C

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Warning : Calibrated compound(s) not found

\*\*\*\*\*  
\*\*\* End of Report \*\*\*

HPLC 10 9/27/2010 11:07:13 AM (b)

Page 2 of 2

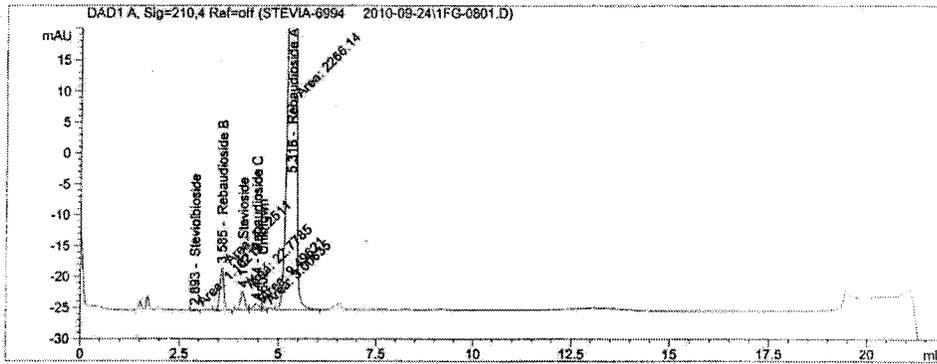
GRAS Assessment – GLG Life Tech Corporation  
 Rebaudioside A (≥ 95%)  
 Page 78 of 112

Sample Name: 10-6995 A

```

=====
Acq. Operator   : (b) (6)                               Seq. Line :    8
Acq. Instrument : HPLC 10                               Location  : PI-F-07
Injection Date  : 9/25/2010 1:22:47 AM                  Inj       :    1
                                                    Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed   : 9/24/2010 4:25:37 PM by (b) (6)
Analysis Method: C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed   : 9/27/2010 11:02:58 AM by (b) (6)
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05gglc/ecmwg
ECM Operator   : (b) (6)
ECM Path       : Petaluma\LC\HPLC-10\Data\STEVIA-6994   2010-09-24.SC.SSizip
ECM Version    : 6
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 2.02425 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.893	MM	1.16219	5.81236e-4	3.33707e-2		Steviolbioside
3.431		-	-	-		Dulcoside A
3.585	MF	45.25105	7.30948e-4	1.633996		Rebaudioside B
4.115	FM	22.77847	7.30946e-4	0.822521		Stevioside
4.454	MF	9.49621	8.63047e-4	0.404875		Rebaudioside C
4.602	MF	3.00635	8.80670e-4	0.130794		Unknown
5.316	MM	2266.13940	8.80666e-4	98.590218		Rebaudioside A

Totals : 101.615775

HPLC 10 9/27/2010 11:09:27 AM (b) (6)

Page 1 of 2

GRAS Assessment – GLG Life Tech Corporation  
Rebaudioside A (≥ 95%)  
Page 79 of 112

Sample Name: 10-6995 A

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

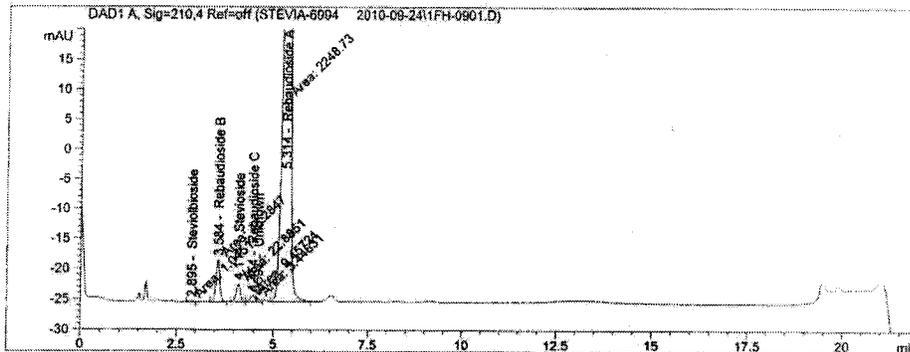
\*\*\*\*\*  
\*\*\* End of Report \*\*\*

Sample Name: 10-6995 B

```

=====
Acq. Operator   : (b) (6) a                      Seq. Line :    9
Acq. Instrument : HPLC 10                          Location  : F1-F-08
Injection Date  : 9/25/2010 2:02:29 AM             Inj       :    1
                                                    Inj Volume: 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-6994   2010-09-24\STEVIA.M
Last changed    : 9/24/2010 4:25:37 PM by (b) (6)
Analysis Method : C:\CHEM32\10\DATA\STEVIA-6994   2010-09-24\STEVIA.M
Last changed    : 9/27/2010 11:02:58 AM by (b) (6)
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : M (b) (6)
ECM Path       : Petaluma\LC\HPLC-10\Data\STEVIA-6994 2010-09-24.SC.SSIzip
ECM Version    : 7
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier    : 1.0000
Dilution      : 1.0000
Sample Amount  : 2.00500 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area %	Amount	Grp	Name
2.895	MM	1.04511	5.81236e-4	3.02970e-2		Steviolbioside
3.431						Dulcoside A
3.584	MF	45.28473	7.30948e-4	1.650912		Rebaudioside B
4.114	MF	22.88506	7.30948e-4	0.834304		Stevioside
4.454	MF	9.45724	8.63047e-4	0.407085		Rebaudioside C
4.597	FM	3.44631	8.80670e-4	0.151375		Unknown
5.314	MM	2248.72876	8.80666e-4	98.772046		Rebaudioside A

Totals : 101.846018

HPLC 10 9/27/2010 11:11:01 AM (b) (6)

GRAS Assessment – GLG Life Tech Corporation  
Rebaudioside A ( $\geq 95\%$ )  
Page 81 of 112

Sample Name: 10-6995 B

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Warning : Calibrated compound(s) not found

\*\*\*\*\*  
\*\*\* End of Report \*\*\*

HPLC 10 9/27/2010 11:11:01 AM (b) (6)

Page 2 of 2

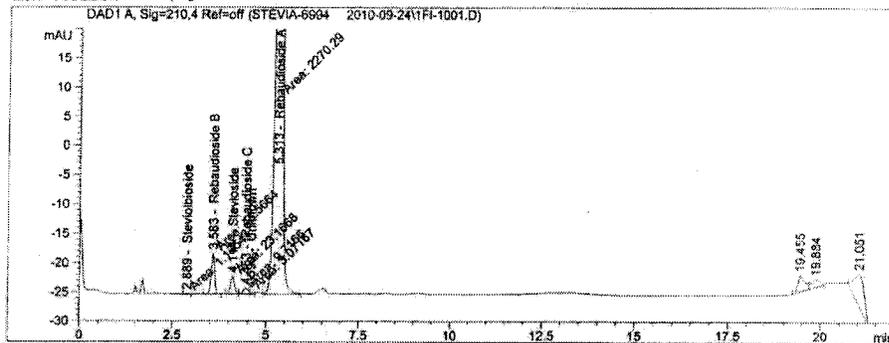
GRAS Assessment – GLG Life Tech Corporation  
 Rebaudioside A (≥ 95%)  
 Page 82 of 112

Sample Name: 10-6995 C

```

=====
Acq. Operator   : (b) (6)                               Seq. Line : 10
Acq. Instrument : HPLC 10                               Location  : P1-F-09
Injection Date  : 9/25/2010 2:42:12 AM                 Inj       : 1
                                                    Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed   : 9/24/2010 4:25:37 PM by (b) (6)
Analysis Method: C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed   : 9/27/2010 11:02:58 AM by (b) (6)
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05gslc/ecmwg
ECM Operator   : (b) (6)
ECM Path       : Pecaluma\LC\HPLC-10\Data\STEVIA-6994   2010-09-24.SC.SSizip
ECM Version    : 8
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 2.03525 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Ant/Area	Amount %	Grp	Name
2.889	MM	1.13541	5.81236e-4	3.24255e-2		Steviolbioside
3.431		-	-	-		Dulcoside A
3.583	MF	45.56639	7.30948e-4	1.636490		Rebaudioside B
4.114	MF	23.16676	7.30948e-4	0.832021		Stevioside
4.453	MF	9.71660	8.63047e-4	0.412032		Rebaudioside C
4.590	MF	3.07167	8.80670e-4	0.132914		Unknown
5.313	MM	2270.28931	8.80666e-4	98.236933		Rebaudioside A

Totals : 101.282814

HPLC 10 9/27/2010 11:11:56 AM (b) (6)

Page 1 of 2

Sample Name: 10-6995 C

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

\*\*\*\*\*  
\*\*\* End of Report \*\*\*

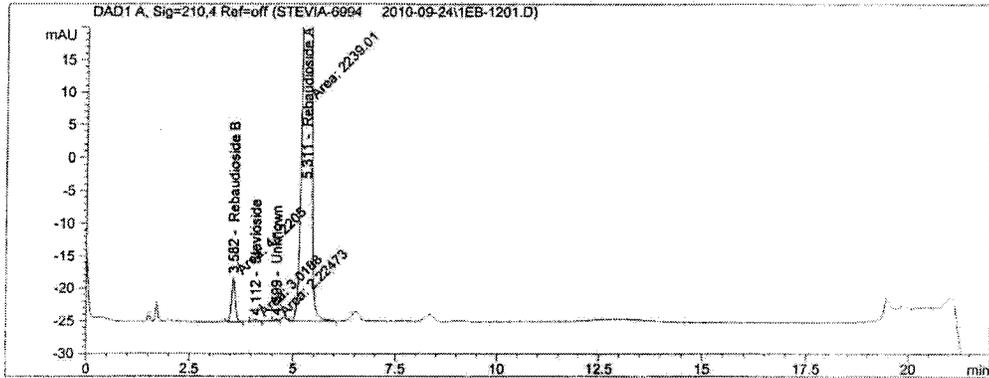
GRAS Assessment – GLG Life Tech Corporation  
 Rebaudioside A (≥ 95%)  
 Page 84 of 112

Sample Name: 10-6996 A

```

=====
Acq. Operator   : (b) (6)                               Seq. Line : 12
Acq. Instrument : HPLC 10                               Location  : P1-B-02
Injection Date  : 9/25/2010 4:01:38 AM                 Inj       : 1
                                                    Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-6994        2010-09-24\STEVIA.M
Last changed   : 9/24/2010 4:25:37 PM by (b) (6)
Analysis Method : C:\CHEM32\10\DATA\STEVIA-6994        2010-09-24\STEVIA.M
Last changed   : 9/27/2010 11:02:58 AM by (b) (6)
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : (b) (6)
ECM Path       : Petaluma\LC\HPLC-10\Data\STEVIA-6994  2010-09-24.SC.SSizip
ECM Version    : 9
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 2.00925 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.889	-	-	-	-	-	Steviolbioside
3.431	-	-	-	-	-	Dulcoside A
3.582	MM	44.22051	7.30948e-4	1.608704	-	Rebaudioside B
4.112	MM	3.01880	7.30948e-4	0.109821	-	Stevioside
4.449	-	-	-	-	-	Rebaudioside C
4.599	MF	2.22473	8.80670e-4	9.75117e-2	-	Unknown
5.311	MM	2239.00708	8.80666e-4	98.137015	-	Rebaudioside A

Totals : 99.953052

HPLC 10 9/27/2010 11:16:12 AM (b) (6) E

Page 1 of 2

GRAS Assessment – GLG Life Tech Corporation  
Rebaudioside A (≥ 95%)  
Page 85 of 112

DATA FILE C:\CHEM32\10\DATA\STEVIA-6996 2010-09-29\185-1201.D  
Sample Name: 10-6996 A

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

\*\*\*\*\*  
\*\*\* End of Report \*\*\*

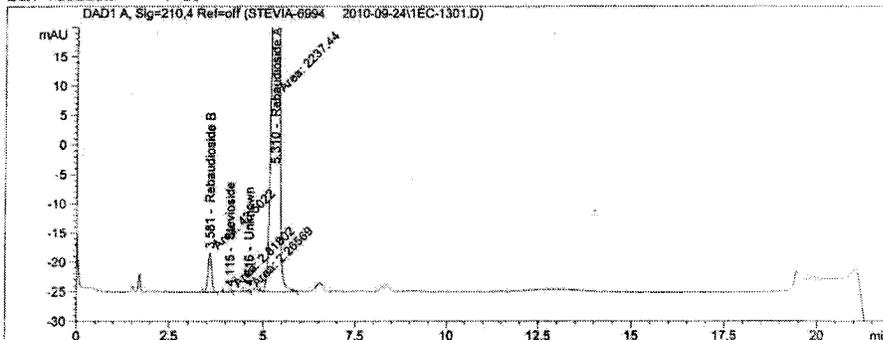
GRAS Assessment – GLG Life Tech Corporation  
 Rebaudioside A (≥ 95%)  
 Page 86 of 112

Data File C:\CHEM32\10\DATA\STEVIA-6994 2010-09-24\IEC-1301.D  
 Sample Name: 10-6996 B

```

=====
Acq. Operator   : (b) (6)                               Seq. Line : 13
Acq. Instrument : HPK 110                               Location  : PI-E-03
Injection Date  : 9/25/2010 4:41:21 AM                 Inj       : 1
                                                    Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-6994 2010-09-24\STEVIA.M
Last changed   : 9/24/2010 4:25:37 PM by (b) (6) ra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-6994 2010-09-24\STEVIA.M
Last changed   : 9/27/2010 11:02:58 AM by (b) (6) a
Method Info    : Steviol Glycosides by HPLC (MODIFIED) (UCFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : (b) (6) a
ECM Path       : C:\Program Files\Agilent\LC-10\Data\STEVIA-6994 2010-09-24.SC.SSizip
ECM Version    : 10
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 2.00750 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount	Grp	Name
2.889		-	-	-		Steviolbioside
3.431		-	-	-		Dulcoside A
3.581	MM	43.50223	7.30948e-4	1.583954		Rebaudioside B
4.115	MM	2.81802	7.30948e-4	0.102606		Stevioside
4.449		-	-	-		Rebaudioside C
4.616	MM	2.26569	8.80670e-4	9.93933e-2		Unknown
5.310	MM	2237.44360	8.80666e-4	98.153976		Rebaudioside A

Totals : 99.939930

HPLC 10 9/27/2010 11:17:45 AM (b) (6)

Page 1 of 2

DATA FILE C:\CHEM32\10\DATA\1018718-0994 2010-09-24 11:00-1301.D  
Sample Name: 10-6996 B

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

\*\*\*\*\*  
\*\*\* End of Report \*\*\*

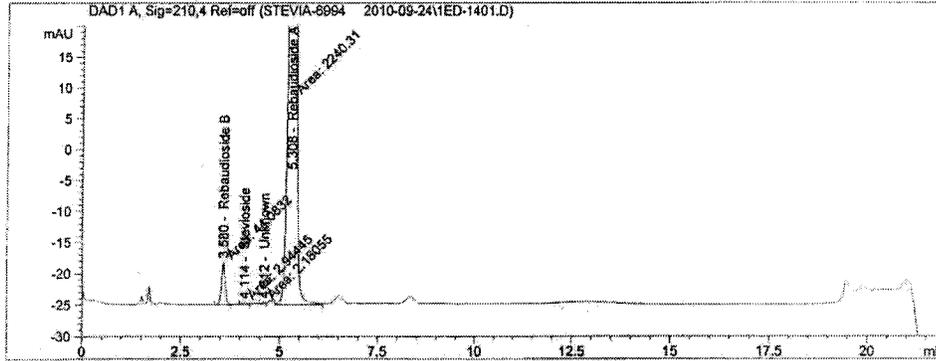
GRAS Assessment – GLG Life Tech Corporation  
 Rebaudioside A (≥ 95%)  
 Page 88 of 112

Data File C:\CHEM32\10\DATA\STEVIA-6994 2010-09-24\IED-1401.D  
 Sample Name: 10-6996 C

```

=====
Acq. Operator   : (b) (6)                               Seq. Line : 14
Acq. Instrument : HPLC 10                               Location  : P1-E-04
Injection Date  : 9/25/2010 5:21:05 AM                 Inj       : 1
                                                    Inj Volume: 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-6994       2010-09-24\STEVIA.M
Last changed    : 9/24/2010 4:25:37 PM b(6) a
Analysis Method : C:\CHEM32\10\DATA\STEVIA-6994       2010-09-24\STEVIA.M
Last changed    : 9/27/2010 11:02:58 AM b(6) a
Method Info     : Steviol Glycosides by HPLC (Modified JSCFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : M: (b) (6) a
ECM Path        : P:\Data\LC-10\Data\STEVIA-6994       2010-09-24.SC.SSI.zip
ECM Version     : 11
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:     : 1.0000
Sample Amount: : 2.01200 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.889		-	-	-		Steviolbioside
3.431		-	-	-		Dulcoside A
3.580	MM	44.08324	7.30948e-4	1.601519		Rebaudioside B
4.114	MM	2.94445	7.30948e-4	0.106970		Stevioside
4.449		-	-	-		Rebaudioside C
4.612	MM	2.18055	8.80670e-4	9.54447e-2		Unknown
5.308	MM	2240.30933	8.80666e-4	98.059882		Rebaudioside A

Totals : 99.863815

HPLC 10 9/27/2010 11:19:41 AM (b) (6)

Page 1 of 2

FILE: \\10-6996\DATA\01072010\0924 2010-09-24\180-1401.D  
Sample Name: 10-6996 C

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

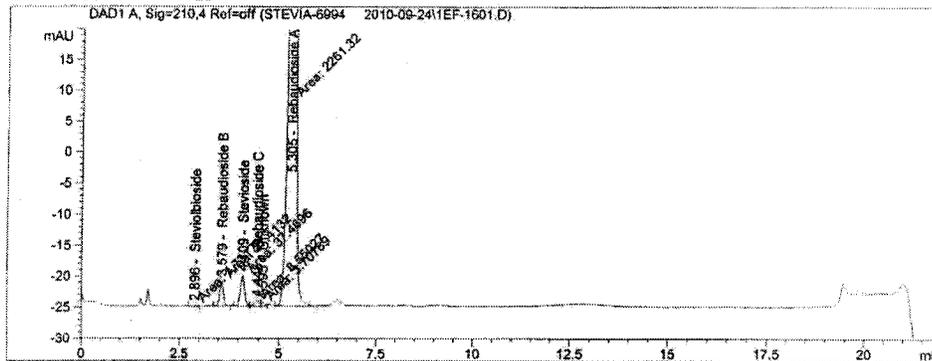
\*\*\*\*\*  
\*\*\* End of Report \*\*\*

Sample Name: 10-6997 A

```

=====
Acq. Operator   : (b) (6) a                      Seq. Line : 16
Acq. Instrument : HPLC 10                          Location  : P1-E-06
Injection Date  : 9/25/2010 6:40:32 AM             Inj       : 1
                                                    Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-6994    2010-09-24\STEVIA.M
Last changed   : 9/24/2010 4:25:37 PM by (b) (6) a
Analysis Method : C:\CHEM32\10\DATA\STEVIA-6994    2010-09-24\STEVIA.M
Last changed   : 9/27/2010 11:02:58 AM by (b) (6) a
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : (b) (6) a
ECM Path      : Petaluma\LC-10\Data\STEVIA-6994    2010-09-24.SC.SSIzip
ECM Version    : 12
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:     : 1.0000
Sample Amount: : 2.01350 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.896	MM	1.73179	5.81236e-4	4.99916e-2		Steviolbioside
3.431		-	-	-		Dulcoside A
3.579	MF	27.41322	7.30948e-4	0.995165		Rebaudioside B
4.109	MF	37.46959	7.30948e-4	1.360235		Stevioside
4.446	MF	8.55027	8.63047e-4	0.366490		Rebaudioside C
4.595	MF	3.70789	8.80670e-4	0.162177		Unknown
5.305	MM	2261.32397	8.80666e-4	98.905970		Rebaudioside A

Totals : 101.840038

HPLC 10 9/27/2010 11:21:06 AM (b) (6)

Data File C:\CHEM32\10\DATA\01EVLK-0994 2010-09-24\1EF-1601.D  
Sample Name: 10-6997 A

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

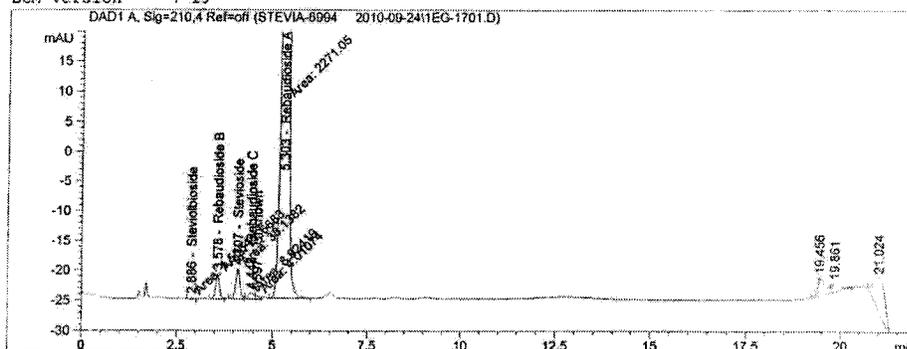
=====  
\*\*\* End of Report \*\*\*

Sample Name: 10-6997 B

```

=====
Acq. Operator   : (b) (6)                               Seq. Line : 17
Acq. Instrument : HPLC 10                               Location  : F1-E-07
Injection Date  : 9/25/2010 7:20:17 AM                 Inj       : 1
                                                    Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed   : 9/24/2010 4:25:37 PM by (b) (6)
Analysis Method: C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed   : 9/27/2010 11:02:58 AM by (b) (6)
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05hgic/ecmwg
ECM Operator   : (b) (6)
ECM Path      : Petaluma\LC\HPLC-10\Data\STEVIA-6994   2010-09-24.SC.SSizip
ECM Version    : 13
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 2.03075 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

Signal 1: DADI A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.886	MM	1.55541	5.81236e-4	4.45185e-2		Steviolbioside
3.431						Dulcoside A
3.578	MM	27.66830	7.30948e-4	0.995893		Rebaudioside B
4.107	MF	38.13818	7.30948e-4	1.372745		Stevioside
4.448	MF	8.92419	8.63047e-4	0.379269		Rebaudioside C
4.597	FM	4.01074	8.80670e-4	0.173933		Unknown
5.303	MM	2271.04614	8.80666e-4	98.487440		Rebaudioside A

Totals : 101.453798

HPLC 10 9/27/2010 11:22:40 AM (b) (6)

GRAS Assessment – GLG Life Tech Corporation  
Rebaudioside A (≥ 95%)  
Page 93 of 112

DATA FILE C:\CHEM32\10\DATA\STEVIA-6994 2010-09-24\IRG-1701.D  
Sample Name: 10-6997 B

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

\*\*\*\*\*  
\*\*\* End of Report \*\*\*

HPLC 10 9/27/2010 11:22:40 AM (b) (6)

Page 2 of 2

GRAS Assessment – GLG Life Tech Corporation  
 Rebaudioside A (≥ 95%)  
 Page 94 of 112

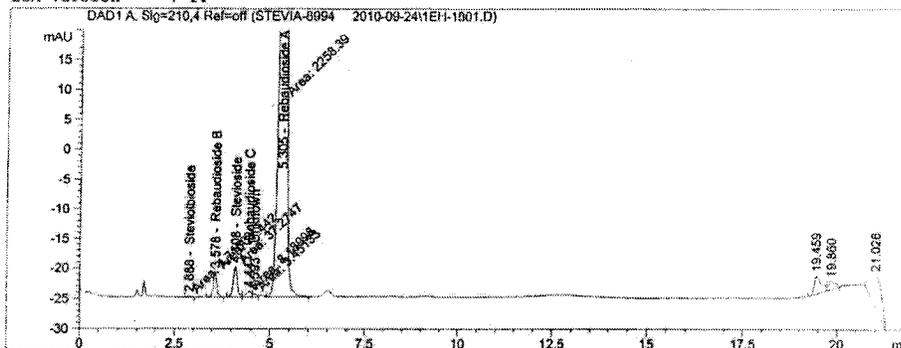
Sample Name: 10-6997 C

=====

Acq. Operator : (b) (6) Seq. Line : 18  
 Acq. Instrument : HPLC 10 Location : P1-E-08  
 Injection Date : 9/25/2010 8:00:01 AM Inj : 1  
 Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\10\DATA\STEVIA-6994 2010-09-24\STEVIA.M  
 Last changed : 9/24/2010 4:25:37 PM by (b) (6)  
 Analysis Method : C:\CHEM32\10\DATA\STEVIA-6994 2010-09-24\STEVIA.M  
 Last changed : 9/27/2010 11:02:58 AM by (b) (6)  
 Method Info : Steviol Glycosides by HPLC (modified JBCFA)

ECM Server : http://us05sqli.ecmwg  
 ECM Operator : (b) (6)  
 ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-6994 2010-09-24.SC.SSI2ip  
 ECM Version : 14



ESTD Percent Report

Sorted By : Signal  
 Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Sample Amount : 2.01575 [mg/mL]  
 Use Multiplier & Dilution Factor with ISTDs

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp Name
2.888	FM	1.71601	5.81236e-4	4.94807e-2	Steviolbioside
3.431	-	-	-	-	Dulcoside A
3.578	MF	27.44200	7.30948e-4	0.995097	Rebaudioside B
4.108	MF	37.27473	7.30948e-4	1.351650	Stevioside
4.447	MF	8.18998	8.63047e-4	0.350656	Rebaudioside C
4.593	MF	3.45133	8.80670e-4	0.150787	Unknown
5.305	MM	2258.39282	8.80666e-4	98.667510	Rebaudioside A

Totals : 101.565181

HPLC 10 9/27/2010 11:23:35 AM (b) (6)

Page 1 of 2

Data File C:\chem52\10\DATA\STEVIA-6994 2010-09-24\IEH-1801.D  
Sample Name: 10-6997 C

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

\*\*\*\*\*  
\*\*\* End of Report \*\*\*

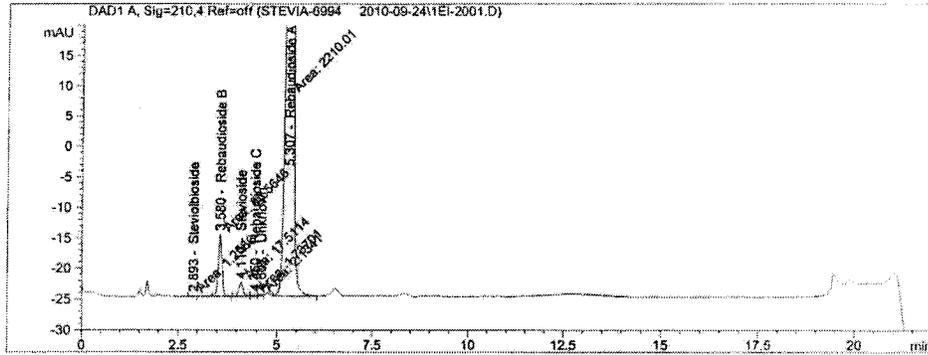
GRAS Assessment – GLG Life Tech Corporation  
 Rebaudioside A (≥ 95%)  
 Page 96 of 112

Sample Name: 10-6998 A

```

=====
Acq. Operator   : (b) (6) a                               Seq. Line : 20
Acq. Instrument :                                           Location  : P1-E-09
Injection Date  : 9/25/2010 9:19:28 AM                      Inj       : 1
                                                    Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-6994             2010-09-24\STEVIA.M
Last changed   : 9/24/2010 4:25:37 PM by (b) (6) a
Analysis Method: C:\CHEM32\10\DATA\STEVIA-6994             2010-09-24\STEVIA.M
Last changed   : 9/27/2010 11:02:58 AM by (b) (6) a
Method Info    : Steviol Glycosides by HPLC (modified SACFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : (b) (6) a
ECM Path       : Petaluma\LC\In-10\Data\STEVIA-6994         2010-09-24.SC.SSIzlp
ECM Version    : 15
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 2.01425 (mg/mL)
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime (min)	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.893	MM	1.25860	5.81236e-4	3.63185e-2		Steviolbioside
3.431						Dulcoside A
3.580	MF	66.56462	7.30948e-4	2.415553		Rebaudioside B
4.110	MF	17.51143	7.30948e-4	0.635469		Stevioside
4.450	MF	1.73701	8.63047e-4	7.44257e-2		Rebaudioside C
4.607	FM	2.13410	8.80670e-4	9.33070e-2		Unknown
5.307	MM	2210.01294	8.80666e-4	96.625732		Rebaudioside A

Totals : 99.880806

HPLC 10 9/27/2010 11:25:24 AM (b) (6)

Page 1 of 2

-----  
Sample Name: 10-6998 A

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

=====  
\*\*\* End of Report \*\*\*

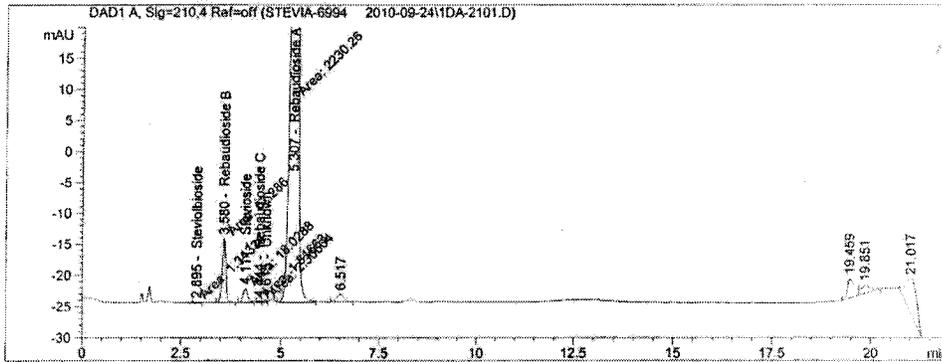
GRAS Assessment – GLG Life Tech Corporation  
 Rebaudioside A (≥ 95%)  
 Page 98 of 112

DATA FILE C:\CHEM32\10\DATA\STEVIA-6994 2010-09-24\IDA-2101.D  
 Sample Name: 10-6998 B

```

=====
Acq. Operator   : (b) (6) [redacted] Seq. Line : 21
Acq. Instrument : HPLC 10 Location : P1-D-01
Injection Date  : 9/25/2010 9:59:12 AM Inj : 1
                                           Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-6994 2010-09-24\STEVIA.M
Last changed    : 9/24/2010 4:25:37 PM by (b) (6) [redacted]
Analysis Method : C:\CHEM32\10\DATA\STEVIA-6994 2010-09-24\STEVIA.M
Last changed    : 9/27/2010 11:02:58 AM by (b) (6) [redacted]
Method Info     : Steviol Glycosides by HPLC (modified USCPA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : (b) (6) [redacted]
ECM Path        : F:\CALIBRA\LC\HPLC-10\Data\STEVIA-6994 2010-09-24.SC.SSizip
ECM Version     : 16
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 2.03725 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.895	MM	1.34335	5.81236e-4	3.83265e-2		Steviolbioside
3.431		-	-	-		Dulcoside A
3.580	MM	67.28596	7.30948e-4	2.414163		Rebaudioside B
4.111	MF	18.02884	7.30948e-4	0.646859		Stevioside
4.444	MF	1.81663	8.63047e-4	7.69583e-2		Rebaudioside C
4.615	MF	2.30604	8.80670e-4	9.96865e-2		Unknown
5.307	MM	2230.25806	8.80666e-4	96.410014		Rebaudioside A

Totals : 99.686008

HPLC 10 9/27/2010 11:28:27 AM (b) (6) [redacted]

Sample Name: 10-6998 B

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

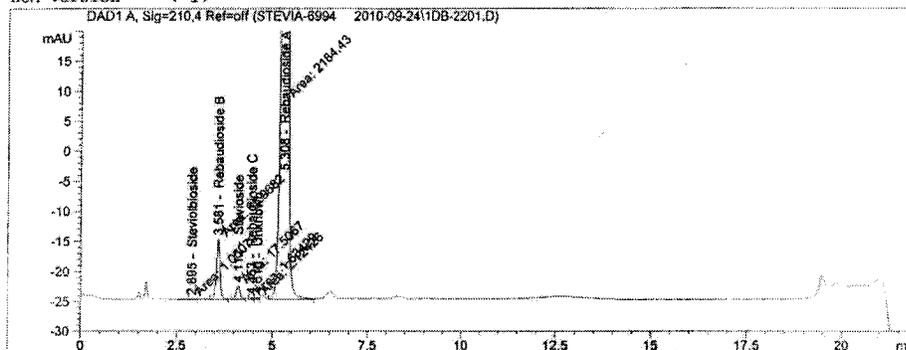
\*\*\*\*\*  
\*\*\* End of Report \*\*\*

Sample Name: 10-6998 C

```

=====
Acq. Operator   : (b) (6)                               Seq. Line : 22
Acq. Instrument : HPLC 10                               Location  : P1-D-02
Injection Date  : 9/25/2010 10:38:55 AM                 Inj       : 1
                                                    Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed   : 9/24/2010 4:25:37 PM by (b) (6)
Analysis Method: C:\CHEM32\10\DATA\STEVIA-6994         2010-09-24\STEVIA.M
Last changed   : 9/27/2010 11:02:58 AM by (b) (6)
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : (b) (6)
ECM Path       : Petaluma\LC\HPLC-10\Data\STEVIA-6994   2010-09-24.SC.SSIZip
ECM Version    : 17
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : Monday, September 27, 2010 10:57:03 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 2.00300 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

Signal 1: DAD1 A, Sig=210.4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.895	MM	1.05079	5.81236e-4	3.04922e-2		Steviolbioside
3.431		-	-	-		Dulcoside A
3.581	MF	65.96823	7.30948e-4	2.407356		Rebaudioside B
4.112	MF	17.50669	7.30948e-4	0.638866		Stevioside
4.453	FM	1.63429	8.63047e-4	7.04180e-2		Rebaudioside C
4.610	MM	2.12426	8.80670e-4	9.33983e-2		Unknown
5.308	MM	2184.42529	8.80666e-4	96.043417		Rebaudioside A

Totals : 99.283947

HPLC 10 9/27/2010 11:31:54 AM (b) (6)

Sample Name: 10-6998 C

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)  
Warning : Calibrated compound(s) not found

=====  
\*\*\* End of Report \*\*\*

### Appendix C-3

Test Report                      No.: QDFDO101207589FD                      Date: Jan 05, 2011

Client name: Qingdao Runhao Rebiana High Tech Co., Ltd  
 Client address: North Chenggang Road, Qingdao Export processing zone, Hetao, Chengyang District, Qingdao City

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	QDFDO101207589FD	Stevia extracts	(b) (6)

Manufacture: Qingdao Runhao Rebiana High Tech Co., Ltd  
 SGS reference No.: SHAFD1018844801  
 Date of receipt: Dec 28, 2010,  
 Testing period: Dec 28, 2010 ~ Jan 05, 2011

**TEST(S) REQUESTED:**

Selected test(s) as requested by applicant:  
 -- To determine the Organochlorine, Pyrethroid, Organophosphorous Pesticide Residues Quantification Content of the submitted samples.

**TEST RESULT(S):**

Code	Test Items		Test method	Unit	MDL	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	Bendiocarb	悉虫威	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
5	Benfluralin	乙丁氟灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
6	Benfuresate	呋草磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
7	BHC-a	α-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
8	BHC-β	β-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
9	BHC-r	林丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
10	BHC-δ	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. ND=Not detected, NR=Not Recoveried
3. The test was carried out by a SGS laboratory.

Signed for and on behalf of SGS

(b) (6)

Authorized Signature

Test Report No.: QDFDO101207589FD Date: Jan 05, 2011

Code	Test Items		Test method	Unit	MDL	Test Result
11	Bifenthrin	联苯菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
12	Bromophos ethyl	乙基溴硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
13	Bromopropylate	溴螨酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
14	Butachlor	丁草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
15	Carbophenothion	三硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
16	Chlorbenside	氯杀螨	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
17	Chlordane-cis	顺式-氯丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
18	oxy-Chlordane	氧氯丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
19	trans-Chlordane	反式氯丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
20	Chlorfenapyr	虫螨腈	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
21	Chlorfenson	杀螨酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
22	Chlorfenvinphos	毒虫畏	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
23	Chlorobenzilate	乙酯杀螨醇	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
24	Chlorpyrifos	毒死蜱	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
25	Chlorpyrifos-methyl	甲基毒死蜱	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
26	Chlozolinate	克氯得	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
27	Cyfluthrin	氟氯氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
28	λ-Cyhalothrin	λ-氟氯氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
29	Cypermethrin	氰氯菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
30	o,p'-DDD	o,p'-滴滴滴	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
31	p,p'-DDD	p,p'-滴滴滴	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
32	o,p'-DDE	o,p'-滴滴伊	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND

Test Report

No.: QDFDO101207589FD

Date: Jan 05, 2011

Code	Test Items		Test method	Unit	MDL	Test Result
33	p,p'-DDE	p,p'-滴滴伊	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
34	o,p'-DDT	o,p'-滴滴涕	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
35	p,p'-DDT	p,p'-滴滴涕	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
36	Deltamethrin	溴氰菊酯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
38	Diazinon	二嗪磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
38	Dichlofenthion	除线磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
39	Dicofol	三氯杀螨醇	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
40	Dieldrin	狄氏剂	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
41	Dimethenamid	二甲吩草胺	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
42	Dimethylvinphos	甲基毒虫畏	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
43	Diphenamid	双苯酰草胺	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
44	Disulfoton	乙拌磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
45	a-Endosulfan	a-硫丹	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
46	β-Endosulfan	β-硫丹	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
47	Endosulfan sulfate	硫丹硫酸酯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
48	EPN	苯硫磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
49	Ethalfuralin	乙丁烯氟灵	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
50	Ethion	乙硫磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
51	Ethofumesate	乙氧呋草黄	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
52	Ethoprophos	灭线磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
53	Fenchlorphos	皮蝇磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
54	Fenitrothion	杀螟硫磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND

Page 3 of 9

Test Report

No.: QDFDO101207589FD

Date: Jan 05, 2011

Code	Test Items		Test method	Unit	MDL	Test Result
55	Fenpropathrin	甲氧菊酯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
56	Fensulfothion	丰索磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
57	Fenthion	倍硫磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
58	Fenvalerate	氟戊菊酯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
59	Flusilazole	氟硅唑	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
60	tau-Fluvalinate	氟胺氟菊酯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
61	Fonofos	地虫硫磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
62	Furalaxyl	呋霜灵	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
63	Heptachlor	七氯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
64	Heptenophos	庚烯磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
65	Hexachlorobenzene	六氯苯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
66	Isofenphos	异柳磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
67	Metalaxyl	甲霜灵	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
68	Methyl-pentachlorophenyl sulfide	甲基五氯苯硫醚	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
69	Napropamide	敌草胺	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
70	Nitrothal-isopropyl	敌菌酯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
71	Paclobutrazol	多效唑	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
72	Penconazole	戊菌唑	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
73	Pendimethalin	二甲戊乐灵	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
74	Pentachloroaniline	五氯苯胺	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
75	Pentachloroanisole	五氯茴香醚	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
76	Permethrin	氯菊酯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND

Page 4 of 9

Test Report

No.: QDFDO101207589FD

Date: Jan 05, 2011

Code	Test Items		Test method	Unit	MDL	Test Result
77	Phenthoate	稻丰散	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
78	Phorate	甲拌磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
79	Pirimiphos-ethyl	乙基嘧啶磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
80	Pirimiphos-methyl	甲基嘧啶磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
81	Procymidone	腐霉利	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
82	Profenophos	丙溴磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
83	Prometryn	扑草净	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
84	Propargite	炔螨特	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
85	Propiconazole	丙环唑	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
86	Propyzamide	炔苯酰草胺	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
87	Quinalphos	啶硫磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
88	Quintozene	五氯硝基苯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
89	Safrotin	巴胺磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
90	Salithion	蔬果磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
91	Sulfotep	治螟磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
92	Tebuconazole	戊唑醇	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
93	Tecnazene	四氯硝基苯	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
94	Terbufos	特丁硫磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
95	Tetrachlorvinphos	杀虫畏	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
96	Tetradifon	四氯杀螨酮	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND

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Code	Test Items		Test method	Unit	MDL	Test Result
97	Tolclofos-methyl	甲基立枯磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
98	Triazophos	三唑磷	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND
99	Trifluralin	氟乐灵	With reference to US FDA PAM GB/T 19648-2006	mg/kg	0.01	ND

Code	Test Items		Test method	Unit	MDL	Test Result
1	Acetamiprid	啮虫腈	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
2	Acetochlor	乙草胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
3	Aldoxycarb	虱灭威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
4	Aramite	杀螨特	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
5	Anilofos	莎稗磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
6	Azinophos methyl	保棉磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
7	Azoxystrobin/Pyroxyrobin	肟啉菌酯/啉菌酯	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
8	Bensulfuron-methyl	笨嘧磺隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
9	Bitertanol	联苯三唑醇	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
10	Buprofezin	噁嗪酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
11	Carbaryl	甲基威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
12	Carbendazim	多菌灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
13	Carbofuran	虫螨威/克百威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
14	Carbofuran-3-hydroxy	3-羟基虫螨威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
15	Chlorbufam	氟草灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
16	Chloroxuron	枯草隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
17	Clethodim	烯草酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND

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Code	Test Items		Test method	Unit	MDL	Test Result
18	Cyanazine	草净津	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
19	Cyprodinil	啉菌环胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
20	Dichlorvos	敌敌畏	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
21	Diethofencarb	乙霉威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
22	Difenoconazole	苯醚甲环唑	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
23	Dimethoate	乐果	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
24	Dimethomorph	烯酰吗啉	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
25	Edifenphos	克瘟散	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
26	Esprocarb	戊草丹	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
27	Ethiofencarb	乙硫甲威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
28	Fenoxycarb	苯醚威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
29	Fenpropimorph	丁苯吗啉	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
30	Fentrazamide	四唑喹啉	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
31	Haloxyp-ethoxyethyl	氟吡乙禾灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
32	Haloxyp-methyl	氟吡甲禾灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
33	Hexythiazox	噻嗪酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
34	Imazalil	抑霉唑/烯菌灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
35	Iprovalicarb	丙森锌	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
36	Isoprocarb	异丙威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
37	Isoprothiolane	稻瘟灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
38	Isoproturon	异丙隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
39	Lenacil	环草啶	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND

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Code	Test Items		Test method	Unit	MDL	Test Result
40	Linuron	利谷隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
41	Malathion	马拉硫磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
42	Mefenacet	苯噻酰草胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
43	Melthamidophos	甲胺磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
44	Methidathion	杀扑磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
45	Methiocarb	灭虫威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
46	Metolachlor	异丙甲草胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
47	Mevinphos	速灭磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
48	Monocrotophos	久效磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
49	Nicosulfuron	烟嘧磺隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
50	Omethoate	氧化乐果	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
51	Oxadixyl	恶霜灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
52	Oxadizon	恶草酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
53	Oxamyl	杀线威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
54	Oxydemeton-methyl	砒吸磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
55	Parathion	对硫磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
56	Phosalone	伏杀硫磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
57	Phosmet	亚胺硫磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
58	Pirimicarb	抗蚜威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
59	Prochloraz	咪鲜胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
60	Promecarb	猛杀威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
61	Propamocarb	霜霉威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	NR
62	Propoxur	残杀威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
63	Pymetrozin	拒嗒酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND

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Date: Jan 05, 2011

Code	Test Items		Test method	Unit	MDL	Test Result
64	Pyrazophos	吡菌磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
65	Pyridaphenthion	吡啶磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
66	Pyrimethanil	嘧霉胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
67	Quizalofop-ethyl	禾草克	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
68	Rimsulfuron	嘧啶磺隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
69	Spinosad	艾克敌	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
70	Spiroxamine	(谷物和葡萄杀菌剂)	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
71	Tebufenozide	虫酰肼	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
72	Tebufenpyrad	吡蚜胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
73	Thiabendazole	噻菌灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
74	Thiacloprid	噻虫啉	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
75	Thifensulfuron-methyl	阔叶散	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
76	Thiodicarb	硫双威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
77	Thiofanox-sulfone	久效威酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
78	Thiofanox-sulfoxid	久效威亚酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
79	Tolfenpyrad	唑虫酰胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
80	Triasulfuron	醚苯磺隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
81	Triflumizole	氟菌唑	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
82	Triflusulfuron-methyl	氟胺磺隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
83	Vamidothion	完灭硫磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND

Sample description: sample in the bag.

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 \*\*\*End of report\*\*\*

## **APPENDIX D**

### **Summary Evaluation of Stevioside Subchronic Study**

## Appendix D

### Summary of Study on Stevioside by Awney et al., 2010<sup>19</sup>

In a recently published exploratory subchronic toxicity study, Awney et al. (2010) investigated the effects of 97% pure stevioside on bodyweight, organ relative weight, hematological and biochemical parameters and enzyme activities in Sprague Dawley. In this 12-week toxicity study, groups of male rats (8/group) were given drinking water containing stevioside. The groups were assigned to drink distilled water (control), low-dose stevioside solution (15 mg/kg/day), high-dose stevioside solution (1500 mg/kg/day) or low-dose stevioside (15 mg/kg/day) plus inulin solution for 12 weeks as the sole source of liquid. Fluid intake was recorded daily and levels of test articles were adjusted weekly to receive the appropriate target concentration. Low dose stevioside (15 mg/kg bw/day) administration without or with inulin for 12 weeks did not reveal any adverse effects on body weight, organs relative weight, hematological and biochemical parameters or enzymes activities. However, treatment with high dose stevioside caused significant changes in several investigated toxicological parameters. Among the hematological parameters, significant changes were noted in all except WBCs, RBCs, and PCV% and in all clinical chemistry parameters except proteins, total lipids, serum alanine aminotransferase (ATL) and aspartate aminotransferase (AST). These data suggest the NOEL of 15mg/kg/day. However, critical review of the publication reveals that the study was poorly designed and implemented. Design deficiencies include: insufficient numbers of animals, group-housing with the potential for stress-related changes, unreliable access to steviol via drinking water resulting in suspect dosing calculations in group-housed cages, no indication of fasting prior to blood collection which affects many chemistry and hematological values, no urine collection and no histopathological evaluations for confirmation of findings beyond the controls. In addition to these study design deficiencies, the report fails to adequately present mean or individual organ weight data and, in general, there appears to be inadequate comparison of study findings against laboratory historical control data. Any one of these oversights could have adversely affected the results and/or interpretation of the hematological and chemistry data.

In addition to the above described parameters, tartrate-resistant alkaline phosphatase (TRAP) levels were measured and found to be significantly decreased (Awney et al., 2010). TRAP is an enzyme that is expressed by bone-resorbing osteoclasts, inflammatory macrophages and dendritic cells. This enzyme was not measured in any previous steviol glycoside studies nor has it been adequately vetted for application in toxicological studies. These investigators did not identify the specific TRAP isomer measured, the methodology employed, the handling of the samples, or any historical data on TRAP levels. The significance and relevance of this poorly documented toxicological endpoint which lacks histopathological confirmation does not appear to have a distinct role in determining the toxicological profile of a material in a test animal. The data presented by Awney et al (2010) are probably not representative of changes due to the subchronic dietary administration of steviol glycoside because of overall poor study design and reliance on the findings of the untested enzyme TRAP. The preponderance of the data from several well designed studies on steviol glycoside suggests that differences noted in hematological and chemistry data are probably random, nonspecific and not toxicologically significant.

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<sup>19</sup> Awney, H.A., Massoud, M.I. El-Maghrabi, S., 2010. Long-term feeding effect of stevioside sweetener on some toxicological parameters of growing male rats. *Journal of Applied Toxicology*, Online Publication: 19 NOV 2010; DOI: 10.1002/jat. 1604.

SUBMISSION END

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