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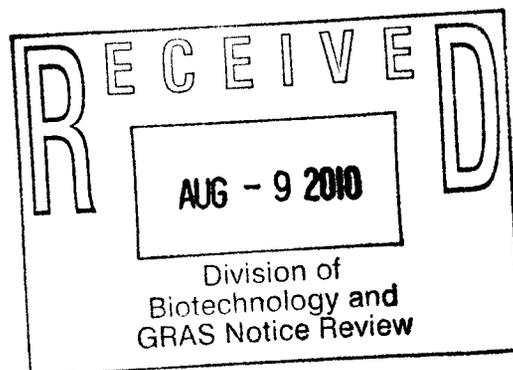
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August 6, 2010

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



Attention: Dr. Robert L. Martin

Re: GRAS Notification – High Purity Steviol Glycosides ($\geq 97\%$)

Dear Dr. Martin:

On behalf of GLG Life Tech Ltd. of Vancouver, British Columbia, Canada, we are submitting for FDA review a GRAS notification for High Purity Steviol Glycosides ($\geq 97\%$). The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

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

We look forward to your feedback.

Sincerely,

(b) (6)

Robert S. McQuate, Ph.D.
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Enclosure: GRAS Notification – High Purity Steviol Glycosides ($\geq 97\%$) (in triplicate)

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GRAS ASSESSMENT

OF

HIGH PURITY STEVIOL GLYCOSIDES ($\geq 97\%$)

Food Usage Conditions for General Recognition of Safety

For

GLG Life Tech, Ltd.
Vancouver, British Columbia
CANADA

Evaluation by GRAS Expert Panel

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

August 6, 2010



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I. GRAS EXEMPTION CLAIM

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

GLG Life Tech, Ltd's high purity steviol glycosides products with rebaudioside A and stevioside as the principal components, which are sold under the name of BlendSure™ and which meet the specifications for high purity steviol glycosides (≥ 97%) as described below, has been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made by an appropriately convened panel of experts who are qualified by scientific training and experience; the GRAS evaluation is based on scientific procedures as described in the following sections; and the evaluation accurately reflects the conditions of the stevia-derived sweetener's intended use in foods.

Signed:

(b) (6)



August 6, 2010

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

Date

B. Name & Address of Notifier

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

As the notifier, GLG Life Tech, Ltd. ("GLG") accepts responsibility for the GRAS determination that has been made for the high purity steviol glycosides as described in the subject notification; consequently, the high purity steviol glycosides preparations meeting the conditions described herein are exempt from premarket approval requirements for food ingredients.

¹ See 62 FR 18938 (17 April 1997) which is accessible at <http://www.gpo.gov/fdsys/pkg/FR-1997-04-17/html/97-9706.htm>.

C. Common Name & Identity of the Notified Substance

High purity steviol glycosides with rebaudioside A and stevioside as the principal components; also see Section III.A.

D. Conditions of Intended Use in Food

The high purity steviol glycosides 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 infant formulas and meat and poultry products, at per serving levels that reflect good manufacturing practices principles in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, the high purity steviol glycosides ($\geq 97\%$) with rebaudioside A and stevioside as the principal components have been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

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

I. INTRODUCTION

A. Objective

At the request of GLG, GRAS Associates, LLC (“GA”) has undertaken an independent safety evaluation of GLG’s BlendSure™ preparations. The preparations are composed of highly purified steviol glycosides with rebaudioside A and stevioside as the principal components. The purpose of the evaluation is to ascertain whether the intended food uses of high purity steviol glycosides (≥ 97%) as a general purpose non-nutritive sweetener are generally recognized as safe, i.e., GRAS, under the intended conditions of use as described in Section IV.A.

B. Foreword

GLG provided GA with substantial background information needed to enable the GRAS assessment to be undertaken. In particular, the information provided addressed the safety/toxicity of steviol glycosides; the history of use of stevia in food; and compositional details, specifications, and method of preparation of GLG’s BlendSure™. 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 so-called “doses” or use levels, is critical in extrapolating to safe exposures for highly purified steviol glycosides 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.

GLG supplied the requested safety/toxicity studies and consumption/exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through August 3, 2010. A GRAS assessment based primarily on the composite safety information, that is, based on scientific procedures, was undertaken. Those references that were deemed pertinent to the objective at hand are listed in Section VIII.

C. Summary of Regulatory History of Stevia & Stevia-Derived Sweeteners

In South America and in several countries in Asia, including China, Japan, and Korea, stevia derived-sweeteners are permitted as a food additive. In recent years, the subject sweeteners have received food usage approvals in Mexico, Australia, New Zealand, and Switzerland. 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 subsequently these petitions were withdrawn. It appears that the previously available safety data—including purity considerations—for stevia, stevioside, or steviol glycosides were inadequate. Since 2008, the US FDA has issued “no questions” letters in response to the multiple GRAS notifications filed on Reb A and steviol glycosides.

Based on information from FDA's GRAS Notice Inventory² website as of August 3, 2010, the agency has received 12 notices on rebaudioside A or steviol glycosides. Ten of these notices have received “no questions” letters from the FDA, while two 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 questions” 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, and PureCircle USA, Inc. Each of these firms received a “no questions” letter from FDA.³ Additionally, two notifications submitted to FDA by GLG Life Tech Corp. and NOW Foods 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 51st, 63rd, 68th and 73rd 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 63rd 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 69th meeting, the temporary status of the ADI was removed, and the ADI was raised to 0-4 mg/kg bw/day (on a steviol basis) as a result of the JECFA review of recently completed clinical studies with steviol glycosides (WHO, 2008). 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

² Accessible at: <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true>.

³ 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, and GRAS notification 323 was submitted by PureCircle USA; 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 questions” letters.

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 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 (FAO/WHO, 2009), and JECFA recently took final action in approving the modified steviol glycosides specifications to include Rebaudioside D and Rebaudioside F (FAO, 2010).

In 2008, Switzerland's Federal Office for Public Health (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).

Stevia-derived sweeteners are not permitted as an ingredient in conventional food in Hong Kong. This appears to be related to a lack of review of new data on the sweeteners rather than a safety concern. Although the government website cites permission to use stevia (Hong Kong Government, 2002), Hong Kong maintains that stevia is not permitted as a sweetener. The Hong Kong Government was reported to be waiting for the JECFA determination on the safety of steviol glycosides. However, no further official actions have been noted since JECFA's final resolution was reported in June 2008 or following subsequent JECFA actions in 2009 or 2010.

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).

Earlier, 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."⁴ 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 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.

⁴ See <http://www.maff.gov.uk/food/novel/980924.html>.

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). As per FDA food regulation, 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 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.⁵

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

⁵ See 21 CFR 170.3(i)(3).

⁶ See 62 FR 18938 (17 April 1997) which is accessible at <http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm>.

III. CHEMISTRY & MANUFACTURE OF HIGH PURITY STEVIOL GLYCOSIDES & RELATED GLG PRODUCT

A. Common or Usual Name

High purity steviol glycosides is the common or usual name of the non-nutritive sweetener derived from *Stevia rebaudiana* Bertoni that is the subject of the GRAS evaluation, and the specific materials that are the subject of this safety evaluation are identified as BlendSure™ as produced and sold by GLG. BlendSure™ products are considered to be part of the GLG Sweet Success™ line, and they consist of distinct blends of highly purified Reb A and stevioside. Rebaudioside A and stevioside are manufactured separately and are mixed as per the desired ratio to obtain the blend. The compositional features of the subject high purity steviol glycosides (≥ 97%) are described in more detail in this Section. JECFA adopted the term, steviol glycosides, for the family of chemical substances with sweetness properties that are derived from stevia. The term, stevia, is used more broadly to describe the plant or crude extracts of the plant.

B. Chemistry of Steviol Glycosides

The following chemistry related description of steviol glycoside is taken from the original JECFA monograph (WHO, 2000).

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

The predominant sweetener components of stevia extracts have been identified as stevioside and Reb A. The chemical identities and key chemical identifiers for the two major components are presented in Table 1.

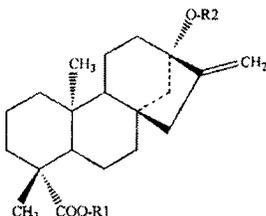
In the most recent Chemical and Technical Assessment (FAO, 2007b), JECFA identified the sweetener components. They updated the list of common glycosides and their chemical structures, which are slightly different from compounds depicted in older publications (Nanayakkara et al., 1987; Suttajit et al.,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

Table 1. Chemical Identity of Stevioside & Rebaudioside A

| STEVIOSIDE | |
|-------------------------|--|
| Common name | Stevioside |
| Chemical name | 13-[2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester |
| Chemical formula | C ₃₈ H ₆₀ O ₁₈ |
| Formula weight | 804.88 |
| CAS Number | 57817-89-7 |
| REBAUDIOSIDE A | |
| Common Name | Rebaudioside A |
| Chemical name | 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D- glucopyranosyl) oxy] kaur-16-en-18-oic acid, β-D- glucopyranosyl ester |
| Chemical formula | C ₄₄ H ₇₀ O ₂₃ |
| Formula weight | 967.03 |
| CAS Number | 58543-16-1 |

In a number of reviews by different authors (Kingham, 2002, Kennelly, 2002, Geuns, 2003), the structures of the components of steviol glycosides have been described. Through a series of chemical reactions and analyses the structures, stereochemistry, and absolute configurations of steviol and isosteviol were established over a 20-year period after the seminal work of Bridel and Lavieille (1931) in France. The work by Ogawa et al. (1980; cited in Brandle et al., 1998) on synthetic transformation of steviol into stevioside supported the proposed structures. Two other sweet glycosides, Reb A and B, were obtained from methanol extracts of stevia leaves, along with the major sweet principle constituent, stevioside, and a minor constituent steviolbioside that which was first prepared from stevioside by alkaline hydrolysis by Wood et al. (1955; cited in Brandle et al., 1998). Subsequently, it was suggested that Reb B was an artifact formed from Reb A during isolation (Brandle et al., 1998; Kennelly, 2002). Furthermore, stevioside can be converted both chemically and enzymatically to Reb A. Further fractionation led to the isolation and identification of three other sweet glycosides respectively named Reb C, D, and E. It was reported that Reb A and D could be converted to Reb B by alkaline hydrolysis showing that only the ester functionality differed (Brandle et al., 1998). Dulcosides A and B were also described by Kobayashi et al. (1977). Subsequently, dulcoside B and Reb were shown to be structurally identical.

Figure 1. Chemical Structures of Various Steviol Glycosides^{a, b}



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

^a From FAO, 2007b.

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

C. Accepted Identity Specifications for Food Grade Steviol Glycosides

In addition to the manufacturing process, the composition of *Stevia rebaudiana Bertoni* extract depends upon the composition of the harvested leaves, which, in turn, is influenced by soil, climate, etc. (FAO, 2007b). JECFA recommended that food grade specifications for steviol glycosides consist of a minimum of 95% on a dried weight basis of seven specific steviol glycosides (FAO, 2007a, see Appendix A-1), and this has more recently been expanded to include the original seven specific steviol glycosides plus Reb D and Reb F (FAO, 2010). The component glycosides of particular interest for their sweetening property are stevioside and Reb A. In addition to the newly added Reb D and Reb F, the other five glycosides that are found at substantially lower levels in the preparations of steviol glycosides and recognized by JECFA consist of Reb C, dulcoside A, rubusoside, steviolbioside, and Reb B. JECFA updated the

specifications for steviol glycosides in 2008 (FAO, 2008, see Appendix A-2) and then again in 2010 as noted in Section II.C; see Appendix A-3.

D. Manufacturing Processes

In the published scientific and patent literature, manufacturing processes for stevia-derived sweeteners have been described. GLG's high purity steviol glycosides ($\geq 97\%$) manufacturing process is also discussed in the following sections.

1. Scientific & Patent Literature

Steviol glycosides are typically obtained by hot water or alcohols (ethanol or methanol) extraction of *Stevia rebaudiana* Bertoni leaves. This extract is a dark particulate solution containing all the active principles plus leaf pigments, soluble polysaccharides, and other impurities. In some processes, the "grease" from the leaves is removed before the extraction by employing solvents such as chloroform or hexane (Kingham, 2002). JECFA also cited that the typical manufacture starts with extracting leaves with hot water, and the aqueous extract is then 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. There are several extraction patents for the isolation of steviol glycosides. Kinghorn (2002) has 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 such as ultrafiltration, metallic ions, supercritical fluid extraction with CO₂ and extract clarification with zeolite have been employed.

2. Processing to Provide GLG's High Purity Steviol Glycosides ($\geq 97\%$)

The manufacturing process employed by GLG is fairly typical and similar to that yielding other related stevia-derived sweetener products on the market. High purity rebaudioside A and stevioside are prepared separately and mixed as per the desired ratio to obtain the blend. The ethanol and methanol used in the purification process comply with FCC 5th Edition specifications for these solvents. The ion exchange resins used in the manufacturing comply with 21 CFR 173.65. The GLG steviol glycosides are 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.

The source of GLG's steviol glycosides preparations is the leaves of the *Stevia rebaudiana* (Bertoni) plant. 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 recently submitted GRAS notification (GRN 329). The process is summarized by flow diagrams in Appendix B-1 and B-2. In brief, steviol glycosides are obtained by the extraction of

stevia leaves with water. Leaves from different varieties of stevia plants are used for stevioside and 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 desorbed 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 stevioside or rebaudioside A. These extracts thus obtained are further processed to obtain the high purity stevioside and rebaudioside A. These processes are summarized by the flow diagrams in Appendix B-3 and B-4. 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 and/or methanol to obtain high purity rebaudioside A or stevioside. The highly purified rebaudioside A and stevioside thus obtained are blended at a desired ratio as depicted in the flow chart in Appendix B-5. The content of rebaudioside A or stevioside in the final BlendSure™ products is $\geq 95\%$, while the total steviol glycosides content is $\geq 97\%$.

E. Product Specifications & Supporting Methods

1. JECFA Specifications

At its 68th meeting, JECFA finalized food grade specifications for steviol glycosides. These specifications were published in FAO JECFA Monograph 4 (FAO, 2007a). JECFA specifications for steviol glycosides---subsequently revised (FAO, 2008) to reflect the composition and recommended analytical methods---are presented in Appendix A-2. Even more recently, we see that JECFA expanded the specifications to include Reb D and Reb F as found in Appendix A-3.

2. Specifications for GLG BlendSure™ Products

GLG has adopted product specifications for BlendSure™ products to meet or exceed current JECFA recommendations. A comparison of the specifications provided by GLG for the final dried product and those from JECFA are presented in Table 2. Also included in Table 2 are data for different blends of GLG's BlendSure™. GLG intends to use three blends that contain Reb A and stevioside ratios of 57:38, 71:24, and 76:19, respectively. The total steviol glycosides content of each of the product blends is $\geq 97\%$. These blends will be marketed under the trade names BlendSure™ 6.0; BlendSure™ 7.5; and BlendSure™ 8.0, respectively. Specification sheets for these products along with label claims are presented in Appendix C-1. Analyses demonstrating that 5 production batches of BlendSure™ 6.0 and BlendSure™ 7.5 meet the required specifications are provided in Appendix C-2 and Appendix C-3. Regarding the batch data for BlendSure™ 8.0---which is similar to the two other blended products---this blend is also prepared by mixing rebaudioside A and stevioside, and it is unlikely that the batch data will differ from the standard specifications. Secondly, the specifications and identity for rebaudioside A and stevioside that are used to prepare the blend, have been well established. Hence, in our opinion, there is no need to have batch data for BlendSure™ 8.0. An analytical report related to the identity of the ingredients from multiple batches of BlendSure™ 6.0, along with details of the

methodology, is included as Appendix C-4. This report demonstrates that the products meet the purity criteria. Finally, Appendix C-5 presents data on pesticide residues.

F. Stability Data for Stevioside & Rebaudioside A

Steviol glycosides have been reported to be stable over the pH range 3-9 and can be heated at 100°C for 1 hour. However, at pH levels greater than 9 under these conditions it rapidly decomposes (Kinghorn, 2002). The pH 10 steviobioside would be the major decomposition product produced from stevioside by alkaline hydrolysis. 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.

Extensive stability data of rebaudioside A have been reported in the GRAS notices submitted by Cargill (2008) and Merisant (2008). Merisant (2008) conducted experiments with rebaudioside A as a powder, as a pure sweetener in solution, and as an ingredient in 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).

In the GRAS notification by Cargill (2008), extensive stability testing on rebaudioside A as a powder under various storage conditions and under a range of pH and temperatures was reported. Additionally, in this notification rebaudioside A stability in several representative food matrices at room temperature and elevated temperatures was also reported. 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).

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Table 2. Specifications for GLG High Purity Steviol Glycosides BlendSure™ Products

| PARAMETER | JECFA ^a SPECIFICATIONS | SPECIFICATIONS BLENDSURE™ 6.0 | SPECIFICATIONS BLENDSURE™ 7.5 | SPECIFICATIONS BLENDSURE™ 8.0 | METHOD |
|------------------------------|--|-------------------------------------|-------------------------------------|-------------------------------------|-----------------------|
| PHYSICAL | | | | | |
| Appearance | White to light yellow powder | White, Off-white hygroscopic powder | White, Off-white hygroscopic powder | White, Off-white hygroscopic powder | Organoleptic |
| Flavor | Sweet | Sweet | Sweet | Sweet | Organoleptic |
| Aroma | Odorless or slight characteristic odor | Sweet | Sweet | Sweet | Organoleptic |
| Particle size (mesh) | NS | 80-100 mesh | 80-100 mesh | 80-100 mesh | Ro Tap 25 g for 5 min |
| CHEMICAL | | | | | |
| Rebaudioside A (%) | NS | ≥ 57 | ≥ 71 | ≥ 76 | JECFA HPLC |
| Stevioside (STV) (%) | NS | ≥ 38 | ≥ 24 | ≥ 19 | JECFA HPLC |
| Total Steviol glycosides (%) | ≥ 95 | ≥ 97 | ≥ 97 | ≥ 97 | JECFA HPLC |
| Total metals (ppm) | NS | ≤ 10 | NS | ≤ 10 | AFS |
| Arsenic (ppm) | ≤ 1 | ≤ 1 | ≤ 1 | ≤ 1 | AFS |
| Lead (ppm) | ≤ 1 | ≤ 1 | ≤ 1 | ≤ 1 | AFS |
| Ethanol (ppm) | ≤ 5000 | ≤ 5000 | ≤ 5000 | ≤ 5000 | GC |
| Methanol (ppm) | ≤ 200 | ≤ 200 | ≤ 200 | ≤ 200 | GC |
| Loss on drying | ≤ 6 | ≤ 4.0 | ≤ 4.0 | ≤ 4.0 | 105°C, 2 hrs |
| pH | | 4.5-7.0 | 4.5-7.0 | 4.5-7.0 | 1 in 100 solution |
| Residue on ignition (%) | ≤ 1.0 | ≤ 1.0 | ≤ 1.0 | ≤ 1.0 | FCC 6 |
| MICROBIOLOGICAL | | | | | |
| Total Plate Count, CFU/g | NA | < 1000 | < 1000 | < 1000 | FDA BAM |
| Yeast and Mold, CFU/g | NA | < 100 | < 100 | < 100 | FDA BAM |
| <i>E. Coli</i> | NA | Negative | Negative | Negative | FDA BAM |
| <i>Staphylococcus aureus</i> | NA | Negative | Negative | Negative | FDA BAM |
| <i>Salmonella sp.</i> | NA | Negative | Negative | Negative | FDA BAM |
| Pesticides | None detected | None detected | | | |

^aPrepared at 69th JECFA (2008); NS = not specified; NA = not applicable

Cargill (2008; published as Clos et al., 2008) also conducted photostability studies on the dry powder and mock beverages to ascertain Reb A behavior under defined conditions of fluorescent and near UV light exposure. Reb A was determined to be photostable under the defined conditions of analysis. The authors stated that the observation of better stability than in the work by Chang and Cook (1983) is due to differences in analytical methods. From the stability testing

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

In addition to the above described stability reports for purified rebaudioside A, in a GRAS notification by Sunwin (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 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 support the position that high purity steviol glycosides are well-suited for the intended food uses of steviol glycosides as reported by GLG.

IV. INTENDED DIETARY USES

A. Intended Uses

The subject GLG high purity steviol glycosides preparations with Reb A and stevioside as the principal components are intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener that is added into foods in general. GLG does not intend to incorporate its BlendSure™ products into infant formulas or meat and poultry products. The high purity steviol glycosides with Reb A and stevioside as the principal sweetening components will function as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19). The use levels will vary by food category, but the levels are self-limiting due to organoleptic factors and consumer taste considerations. The amounts of high purity steviol glycosides 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.⁷

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

As part of their safety deliberations, both JECFA and FSANZ reviewed estimates of possible daily consumption of mixed steviol glycosides. In addition, Merisant and Cargill estimated the consumption of Reb A in their submissions to FDA. Estimated maximum use levels in various foods as evaluated by JECFA are summarized in Table 3.

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

| FOOD USES | MAXIMUM USE LEVEL REPORTED^a (mg STEVIOL GLYCOSIDES / kg OF FOOD) | MAXIMUM USE LEVEL CALCULATED^b (mg STEVIOL EQUIVALENTS / kg OF FOOD) |
|--------------------|--|---|
| Desserts | 500 | 200 |
| Cold confectionery | 500 | 200 |
| Pickles | 1000 | 400 |
| Sweetcorn | 200 | 80 |
| Biscuits | 300 | 120 |
| Beverages | 500 | 200 |
| Yogurt | 500 | 200 |
| Sauces | 1000 | 400 |
| Delicacies | 1000 | 400 |
| Bread | 160 | 64 |

^a From WHO, 2006. ^b Calculated by Expert Panel by multiplying by ratio of molecular weight of steviol to molecular weight of stevioside.

⁷ See 21 CFR 182.1(b)(1).

In the GRAS Notification by Merisant, expected levels of use for Reb A for various food applications were listed. 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 90th percentile daily consumption of rebaudioside A was estimated as 2.0 and 4.7 mg/kg bw/day, respectively. Specific food uses and use levels are given in Table 4. 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.

Table 4. Proposed Uses & Levels of Rebaudioside A by Merisant^a

| FOOD USES | REB A (PPM) |
|---|---------------------|
| Tabletop sweeteners | 30,000 ^b |
| Sweetened ready-to-drink teas | 90-450 |
| Fruit juice drinks | 150-500 |
| Diet soft drinks | 150-500 |
| Energy drinks | 150 |
| Flavored water | 150 |
| Cereals (oatmeal, cold cereal, cereal bars) | 150 |

^a Merisant, 2008. ^b Reb A content of sachet prior to dilution and not representative of "as consumed."

C. Estimated Daily Intake

GLG intends to use its BlendSure™ products in foods other than infant formulas and meat and poultry products as a general-purpose sweetener and as a table top sweetener. The very conservative consumer intake estimates provided by JECFA (as shown above in Table 3) were utilized to gauge the potential human exposures of the subject steviol glycosides in foods as reported in the US and in other countries. 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 was prepared for the European Commission by the Scientific Committee on Food. JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars, at the lowest reported relative sweetness ratio for

steviol glycosides and sucrose, which is 200:1. The intakes ranged from 1.3 mg/kg bw/day with the African diet to 3.5 mg/kg bw/day with the European diet.

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

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

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

^a WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme.

^b These estimates were prepared in parallel to those for the international estimates; it was assumed that all dietary sugars in diets in Japan and the US would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200:1.

JECFA concluded that the replacement estimates were highly conservative---that is, the calculated dietary exposure overestimates likely consumption---and that true dietary intakes of steviol glycosides (as steviol) would probably be 20 – 30% of these values or 1.0 - 1.5 mg/kg bw/day on a steviol basis. Similarly, FSANZ (2008) 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 for the mean and 90th percentile consumer, respectively. FSANZ examined consumption in other age groups and concluded there was no safety concern in children of any age. Merisant also calculated a dietary estimate for Reb A of 2.0 mg/kg bw/day for the average consumer and 4.7 mg/kg bw/day for a 90th percentile consumer. On a steviol equivalent basis, the Merisant estimates would be 0.7 and 1.6 mg/kg bw/day, respectively. 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).

In concert with the JECFA intake estimates, the anticipated human exposures as projected independently and with different approaches by both Merisant and Cargill in compiling their GRAS dossiers for Reb A (Merisant, 2008 and Cargill, 2008) tended to converge to yield estimated daily intakes (EDIs) in the range 0.4 - 1.6 mg/kg bw/day on a steviol basis or 1.3 - 4.7 mg/kg bw/day on a Reb A basis. The actual daily intakes of stevioside and Reb A from products such as BlendSure™ offerings and from Merisant, Cargill, McNeil Nutritional, Blue California, Sweet Green Fields, Wisdom Natural Brands, Sunwin and WILD Flavors, Pyure Brands, and other manufacturers' and suppliers' products cannot yet be determined with accuracy.

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 is 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 above discussion, the intake estimates presented here 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.B.1.

D. Other Information on Human Exposure to Stevia

For about 20 years, consumers in Japan and Brazil, where stevia had been approved as a food additive, have been using stevia extracts as non-caloric sweeteners.⁸ It is 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). Stevia usage as a dietary supplement is presently permitted in the US, Canada, Australia and New Zealand. It has been widely used in China and Japan in food and in dietary supplements. In the US, stevia is available in packets containing 60 - 90 mg steviol glycoside for home supplement uses, such as in beverages or other foods. It is estimated that sales of stevia in the US reached \$45 million in 2005 (The Food Institute Report, 2006). No estimates are available on the daily consumption levels of steviol glycosides consumed in the US *via* dietary supplements in the form of capsules, softgels, tablets, etc. In South America, stevia is commonly used as a treatment for type 2 diabetes (Hawke, 2003). However, elevated doses in the range of 1 gram per person per day or more were reported to be necessary to achieve this therapeutic effect (Gregersen et al., 2004).

⁸ See Raintree Nutrition Tropical Plant Database (www.rain-tree.com/stevia.htm).

V. SAFETY DATA FOR STEVIOL GLYCOSIDES

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

As GLG's high purity steviol glycosides ($\geq 97\%$) blends primarily contain Reb A and stevioside, the scientific data on each component are relevant to the present safety assessment. A number of reviewers have assessed the biological, toxicological and clinical data on stevia and steviol glycosides (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) and continues to do so (FAO/WHO, 2009). Recently FSANZ (2008) also evaluated steviol glycosides for use in food. The majority of these reviews primarily focused on mixtures of steviol glycosides. The early reviews tracked the development of better toxicology studies on purer samples of steviol glycosides. These reviews followed with keen interest whether effects of concern seen in various toxicology studies, such as the decrease in fertility with crude stevia preparations and the mutagenic activity of the principle metabolite steviol, would be manifest in comprehensive studies using modern test protocols with pure test materials. Additionally, JECFA encouraged the further elucidation of clinical effects on blood pressure and glucose metabolism in hypertensive and diabetic individuals, respectively, in comparison to normal human subjects. By 2006, sufficient favorable data were generated for JECFA to yield a temporary ADI, which was finalized at an elevated level in 2008. More details on the JECFA reviews are discussed in Section V.A.1. The key toxicology and clinical data on steviol glycosides (primarily stevioside), more recently developed data on Reb A, and data on the principle metabolite, steviol, as evaluated by JECFA and other reviewers are summarized in Sections V.B, V.C and V.D, respectively.

1. Summary of Interim & Final JECFA Reviews

Earlier at its 51st 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 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 were 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 63rd meeting (WHO, 2006):

The Committee noted that most of the data requested at its fifty-first meeting, e.g., data on the metabolism of stevioside in humans, and on the activity of steviol in suitable studies of genotoxicity *in vivo*, had been made available. The Committee concluded that stevioside and rebaudioside A are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*.

The NOEL for stevioside was 970 mg/kg bw/day in a long-term study (Toyoda et al., 1997) evaluated by the Committee at its fifty-first meeting. The Committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to about 12.5–25 mg/kg bw/day (equivalent to 5–10 mg/kg bw/day expressed as steviol). The evidence available at present was inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g., those with hypotension or diabetes).

The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg/kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg/kg bw/day (or 383 mg/kg bw/day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. The Committee noted that this temporary ADI only applies to products complying with the specifications.

The Committee required additional information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics.

In 2007, at its 68th 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 69th 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., 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.

B. Safety Data on Stevioside & Stevia Extracts that are Predominantly Stevioside

This Section summarizes studies on stevioside or stevia extracts that were identified compositionally as predominantly stevioside. Related studies on Reb A are discussed in Section V.C.

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

Several studies in rats (Wingard et al., 1980; Nakayama et al., 1986; Koyama et al., 2003a) and other animal models, including chickens (Geuns et al., 2003a), hamsters (Hutapea et al., 1999), and pigs (Geuns et al., 2003b) indicate that stevioside is not readily absorbed from the GI tract. Available evidence from *in vitro* metabolism studies suggests that bacteria in the colon of rats and humans can transform various stevia glycosides into steviol (Gardana et al., 2003). Steviol was shown to be more readily transported with *in vitro* intestinal preparations than various steviosides (Geuns, 2003, Koyama et al., 2003b). Slow absorption of steviol was indicated by detection in the plasma of rats given oral stevioside (Wang et al., 2004). However, Sung (2002) did not detect plasma steviol following oral administration of steviosides to rats. In studies with human and rat liver extracts, Koyama et al. (2003b) demonstrated that steviol can be converted to various glucuronides. Excretion of metabolites of stevioside after oral doses has been shown in urine and feces in rats (Sung, 2002) and hamsters (Hutapea et al., 1999). Oral doses in pigs led to the detection of metabolites in feces but not in urine (Geuns et al., 2003b).

In a human study with 10 healthy subjects, Geuns et al. (2006) measured blood, urine and fecal metabolites in subjects that received 3 doses of 250 mg of purified stevioside (>97%) 3 times a day for 3 days. Urine was collected for 24 hours on day 3 and blood and fecal samples were also taken on day 3. Free steviol was detected in feces but not in blood or urine. Steviol glucuronide

was detected in blood, urine and feces. Approximately 76% of the total steviol equivalents dosed were recovered in urine and feces. Based on these measurements, the authors concluded that there was complete conversion of stevioside in the colon to steviol, which was absorbed and rapidly converted to the glucuronide.

In a recent publication, Renwick and Tarka (2008) reviewed studies on microbial hydrolysis of steviol glycoside. The reviewers concluded that stevioside and Reb A are not absorbed directly and both are converted to steviol by gut microbiota in rats and in humans. This hydrolysis occurs more slowly for Reb A than for stevioside. Studies have shown that steviol-16, 17 epoxide is not a microbial metabolite. The authors concluded that there is a single hydrolysis product and that toxicological studies on stevioside are relevant to the safety assessment for Reb A.

2. Acute Toxicity Studies

The LD₅₀ studies of stevioside (purity, 96%) following administration of a single oral dose to rodents are summarized in Table 6. No lethality was seen within 14 days after administration, and no clinical signs of toxicity or morphological or histopathological changes were found, indicating that stevioside is relatively harmless.

Table 6. Acute Toxicity of Stevioside (Purity 96%) Given Orally to Rodents

| SPECIES | SEX | LD ₅₀ (g/kg bw) | REFERENCE |
|---------|-----------------|----------------------------|-------------------------|
| Mouse | Male and Female | >15 | Toskulkao et al. (1997) |
| Mouse | Male | > 2 | Medon et al. (1982) |
| Rat | Male and Female | >15 | Toskulkao et al. (1997) |
| Hamster | Male and Female | >15 | Toskulkao et al. (1997) |

3. Subchronic Toxicity Studies

In three published studies, subchronic toxicity of stevioside was investigated in rats following oral administration. In addition, a reproduction study in hamsters included subchronic phases on the F₀, F₁ and F₂ generations. These studies are summarized in Table 7. One of these studies was particularly important because it served as a range-finding study for two subsequent chronic studies. In this 13-week toxicity study, Fischer 344 rats (10/sex/group) were given doses of 0, 0.31, 0.62, 1.25, 2.5, or 5% in the diet (equivalent to 160, 310, 630, 1300, and 2500 mg/kg bw/day) to determine the appropriate doses for a two-year carcinogenicity study. None of the animals died during the administration period, and there was no difference in body-weight gain between the control and treated groups during administration or in food consumption in the latter part of the study. The activity of lactic dehydrogenase and the incidence of single-cell necrosis in the liver were increased in all groups of treated males. The authors considered these effects to be nonspecific, because of the lack of a clear dose-response relationship, the relatively low severity, and their limitation to males. Other statistically significant differences in hematological and biochemical parameters were also considered to be of minor toxicological significance. The

authors concluded that a concentration of 5% in the diet was a suitable maximum tolerable dose of stevioside for a two-year study in rats (Aze et al., 1991).

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

Table 7. Summary of Subchronic Studies on Stevioside

| STUDY | ANIMAL MODEL/ GROUP SIZE | TEST MATERIAL/ SAMPLE PURITY | DOSES / DURATION | AUTHOR ASSIGNED NOEL (mg/kg bw/day) | RESULTS AND REMARKS |
|--|---|------------------------------|--|-------------------------------------|---|
| Aze et al., 1991 ^a | F344 rat/ 10 females and 10 males in each of 6 groups | Stevioside/ Not reported | 0, 0.31, 0.62, 1.25, 2.5, 5% in diet/13 weeks | Not reported | No effects observed on mortality, body weight or food consumption. Clinical chemistry investigation revealed increased LDH levels and histopathological investigation indicated increased incidence of single-cell liver necrosis in all male treated groups, but not in a clear dose-response relationship. Investigators did not consider these changes to be treatment related due to the small magnitude and low severity of changes, the lack of a clear dose relationship and the limitation to males only. Organ weights, urine chemistry and gross necropsy not discussed. Authors concluded that 5% stevioside in diet is a tolerable dose for a 2 year study. |
| Yodyingyuad and Bunyawong, 1991 ^a | Hamster/ four groups of 20 (10 male, 10 female) | Stevioside/ 90% | 0, 0.5, 1.0, 2.5 g/kg bw/day/ duration unclear/ 3 months | 2500 | F ₀ , F ₁ and F ₂ generations in reproductive study were dosed for 90 days. Histological examination showed no effect at any dose. Weights of organs, blood analysis, urine chemistry and gross necropsy not discussed. The F ₁ and F ₂ hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents). |
| Mitsuhashi, 1976 ^b | Rat (strain not reported) | Stevioside/ Not reported | Dietary concentrations up to 7%/ 3 months | Not reported | No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy and histopathology not discussed. |
| Akashi and Yokoyama, 1975 ^b | Rat (strain not reported) | Stevioside/ Not reported | Oral doses up to 2500 mg/kg bw/3 months | 2500 | No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy and histopathology not discussed. |

^a Abstract only. ^b As reported by Geuns, 2003.

4. Chronic Toxicity Studies

In three separate studies summarized in Table 8, chronic effects of stevioside have been studied. No treatment-related increase in tumor incidence was seen in any of these studies. In the most recent and well-documented study (additional study details were presented to JECFA in 2006), the apparent no observed adverse effect level (NOAEL) in F344 rats was the dietary level of 2.5% (test sample purity 96%, Toyoda et al., 1997). At 5% of the diet, statistically significant decreases in body weight, percent survival and kidney weight were noted. The author attributed these effects to various factors. The decrease in body weight was attributed to an inhibition of glucose utilization. The decrease in survival seemed to have been caused by an unusual late onset of large granular lymphocyte leukemia in high dose males. The authors reported that this tumor is rather common in F344 rats and that the overall incidence in male rats was actually within the historical control range experienced in the laboratory where studies were conducted. The authors attributed the decrease in kidney weight as probably due to a decrease in chronic inflammation found in the histopathological examination relative to control animals.

5. Reproductive & Developmental Toxicity Studies

The use of *S. rebaudiana* as an oral contraceptive has been reported by Indians in Paraguay (Planas and Kuc, 1968; Schwartzman et al., 1977). In experimental studies in rats, crude stevia leaf extract has been shown to inhibit fertility (Planas and Kuc, 1968). Reproductive toxicity studies have been conducted with orally administered purified stevioside as tabulated in Table 8. No effect on fertility or reproductive parameters was seen in a three-generation study in hamsters at doses up to 2500 mg/kg/day (Yodyingyud and Bunyawong, 1991). There was an absence of statistically significant effects at doses up to 3% (equivalent to 3000 mg/kg bw/day; sample purity 96%; Mori et al., 1981). Similar results were observed in an additional rat study that was reviewed by Geuns (2003) where limited information is available in English (Usami et al., 1995). In a recent study, no effect on pregnancy or developmental parameters were observed in Swiss albino mice with stevioside or aqueous stevia extract at doses up to 800 mg/kg bw/day in female mice (Kumar and Oommen, 2008). Further details on these studies to the extent available are presented in Table 9.

6. Mutagenicity & Genotoxicity Studies

In a series of studies mutagenic and genotoxic effects of stevia and stevioside were investigated. These studies are summarized in Table 10. All studies were negative with the exception of a comet assay done in rats (Nunes et al., 2007a). The methodology used in this study and the resulting conclusions have been questioned (Geuns, 2007; Nunes et al., 2007b and 2007c; Williams, 2007; and Brusick, 2008).

Table 8. Summary of Chronic Toxicity Studies on Stevioside

| STUDY | ANIMAL MODEL/ GROUP SIZE | TEST MATERIAL/ SAMPLE PURITY | DOSES/ DURATION | AUTHOR ASSIGNED NOAEL (mg/kg bw/day) | RESULTS AND REMARKS |
|--------------------------------|--|--|---|--|---|
| Toyoda et al., 1997 | F344 rat/ 50 per sex per group | 95.6% Stevioside | <i>Ad libitum</i> 0,2.5, 5% of diet/~24 months (104 weeks) | Author did not assign a NOAEL. (Mid-dose calculates to 970 in males; JECFA, 2006) | A significant decrease in survival rates in males receiving 5%. General condition, body weight, food intake, mortality, hematological, histopathological and organ weights were observed. Body weight gains dose-dependently decreased in both sexes. Kidney weights were significantly lower in 5% males and ovary, kidney and brain weights were significantly increased in 5% females. Tumors and non-neoplastic lesions found in all groups, and were not correlated to treatment. Conclusion was that stevioside is not carcinogenic under these experimental conditions. |
| Xili et al., 1992 ^a | Wistar rat/ 45 per sex per group | 85% Stevioside | 0, 0.2, 0.6, 1.2 % of diet/24 months | 794 (high dose) | After 6, 12 and 24 months five rats from each group were sacrificed for analysis. No effects observed on growth, food utilization, general appearance, mortality or lifespan. No changes in hematological, urinary or clinical biochemical values. Histopathological analysis showed that the neoplastic and non-neoplastic lesions were unrelated to the level of stevioside in the diet. |
| Yamada et al., 1985 | F344 rat/ 70 per sex per group, 30 per sex per group in low-dose | 95.2% Steviol glycosides (75% stevioside; 16% Reb A) | 0.1, 0.3, 1% of diet/22 months for males, 24 months for females | 550 (high dose) | At 6 and 12 months, 10 males and 10 females were sacrificed for analysis. General behavior, growth and mortality were same among groups throughout the experiment. At 6 months, protein urea was significantly increased in females, and blood glucose was increased in both sexes, although urinary glucose not detected. Weights of liver, kidney, heart, prostate and testes were increased in males at 6 months, and weight of ovaries was decreased in females in dose-dependent manner. Histopathological examination showed differences in various organs at 6 months that were unrelated to stevioside dose. These differences were not found at 12 months. Authors concluded that there were no significant changes after 2 years. |

^a Only abstract available.

7. Clinical Studies & Other Reports in Humans

In several studies, pharmacological and biochemical effects of crude extracts of stevia leaves and purified steviol glycosides have been investigated. The effects noted included glucose uptake, insulin secretion, and blood pressure (Geuns, 2003a). In South America, stevioside is used as a treatment for Type II diabetes. These effects were key concerns for JECFA. In 2006, JECFA summarized the available clinical studies of stevioside and further studies were recommended (WHO, 2006). Subsequently, several studies were conducted, and in 2009, JECFA reviewed these new studies (WHO, 2009). JECFA's summaries of the key studies are included below.

a. Studies Summarized in 2006

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

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

In a follow-up multi-center randomized, double-blind, placebo-controlled trial was conducted in hypertensive Chinese men and women (aged 20–75 years), 85 patients were given capsules containing 500 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 1500 mg of stevioside per day (equivalent to 21 mg/kg bw/day, as calculated by FSANZ, 2008). Eighty-nine patients were given a placebo. During the course of study, three patients in each group withdrew. There were no significant changes in body mass index or blood biochemistry parameters throughout the study. In the group receiving stevioside, mean systolic and diastolic blood pressures were significantly decreased compared with the baseline, commencing from about 1 week after the start of treatment. After 2 years, 6 out of 52 patients (11.5%) in the group receiving stevioside had left ventricular hypertrophy compared with 17 of 50 patients (34%) in the group receiving the placebo ($p < 0.001$). Eight patients in each group reported minor side effects (nausea, dizziness and asthenia), which led two patients in each group to withdraw from the study. Four patients in the group receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment. These effects subsequently resolved and the patients remained in the study (Hsieh et al., 2003).

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

Table 9. Summary of Reproductive Toxicity Studies on Steviol Glycosides

| STUDY | ANIMAL MODEL/ GROUP SIZE | TEST SAMPLE PURITY STEVIOSIDE (UNLESS OTHERWISE NOTED) | DOSES / DURATION | AUTHOR ASSIGNED NOAEL (mg/kg bw/day) | RESULTS AND REMARKS |
|--|--|--|---|--------------------------------------|--|
| Kumar and Commen, 2008 | Swiss albino mice/ 4 groups of 5 females | Not reported | 500 and 800 mg/kg bw/15 days | 800 | Stevioside and stevia extract (purity and composition not reported) did not have any effect on reproductive parameters in mice when administered to female mice before or during pregnancy. No changes seen in number of implantations or uterine resorptions. No gross anatomical or histopathologic effects seen in 16-day embryos. |
| Usami et al., 1995 ^a | Wistar Rat/4 groups of 25 or 26 pregnant rats | 95.6% ^b | 0, 250, 500, 1000 mg/kg bw/10 days | 1000 | Pregnant rats given doses of stevioside by gavage once a day on days 6-15 of gestation and were sacrificed on day 20 of gestation. Fetuses were examined for malformations in addition to maternal and fetal body weight, number of live fetuses, sex distribution, and numbers of resorptions or dead fetuses. No treatment-related effects observed. Authors concluded that orally administered stevioside is not teratogenic in rats. |
| Yodyingyuad and Bunyawong, 1991 | Hamster/ 10 male, 10 female per group (40 total) | 90% | 0, 500, 1000, 2500 mg/kg bw/day/ duration unclear/ 3 months | 2500 | Males from each group were mated to females from respective dose group. Each female was allowed to bear 3 litters during the course of experiment. Stevioside had no effect on pregnancies of females at any dose. The F ₁ and F ₂ hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents); showed normal growth and fertility. Histological examination showed no effect on reproductive organs at any dose. |
| Oliveira-Filho et al., 1989 ^a | Rat/number not reported | Not reported (Dried Stevia Leaves) | 0 or 0.67 g dried leaves /ml, 2 ml twice per day/ 60 days | Not reported | Prepubertal rats (25-30 days old) tested for glycemia; serum concentrations of thyroxine; tri-iodothyroxine; available binding sites in thyroid hormone-binding proteins; binding of ³ H-methyltrienolone (a specific ligand of androgen receptors) to prostate cytosol; zinc content of prostate, testis, submandibular salivary gland, and pancreas; water content of testes and prostate; body-weight gain; and final weights of testes, prostate, seminal vesicle, submandibular salivary gland, and adrenal. Only difference due to treatment was seminal vesicle weight, which fell to 60% compared to control. |
| Mori et al., 1981 | Rat/11 male, 11 female per group (44 total) | 96% | 0, 0.15, 0.75 or 3 % of feed/60 days | 2000 | Males given stevioside dose in diet for 60 days before and during mating with females who received same diet (as mated male) 14 days before mating and 7 days during gestation. No effect due to treatment on fertility or mating performance, and no effect of fetal development. Rats of each sex had slightly decreased body weight gain at highest dose with non-significant increase in number of dead and resorbed fetuses at highest dose. |
| Planas and Kuc, 1968 ^c | Rat/14 per group (28 total) | Not reported (Crude stevia extract) | 0 or 5% Crude stevia extract /18 days | Not reported | Extract given orally to adult female rats for 12 days, who were mated with untreated males during the last 6 days. Fertility reduced to 21% of fertility in control rats and remained reduced in a 50-60 day recovery. Histological examination, weights of organs, blood analysis, urine chemistry and gross necropsy not discussed. |

^a Only abstract available. ^b As reported by European Commission, 1999b.

Table 10. Mutagenicity & Genotoxicity Studies on Stevia Extracts & Stevioside

| END-POINT | TEST SYSTEM | MATERIAL | PURITY (%) | CONCENTRATION / DOSE | RESULT | REFERENCE |
|--------------------------|---|----------------|---------------------------|--|---|-------------------------|
| Reverse mutation | <i>S. typhimurium</i> TA97, TA98, TA100, TA102, TA104, TA1535, TA1537 | Stevioside | 83 | 5 mg/plate ^a 1 mg/plate ^b | Negative | Matsui et al. (1996) |
| Reverse mutation | <i>S. typhimurium</i> TA98, TA100 | Stevioside | 99 | 50 mg/plate | Negative ^c | Suttajit et al. (1993) |
| Forward mutation | <i>S. typhimurium</i> TM677 | Stevioside | 83 | 10 mg/plate | Negative ^c | Matsui et al. (1996) |
| Forward mutation | <i>S. typhimurium</i> TM677 | Stevioside | NS | 10 mg/plate | Negative ^c | Pezzuto et al. (1985) |
| Forward mutation | <i>S. typhimurium</i> TM677 | Stevioside | NS | Not specified | Negative ^c | Medon et al. (1982) |
| Gene mutation | Mouse lymphoma L5178Y cells, TK-locus | Stevioside | NS | 5 mg/mL | Negative ^{c,d} | Oh et al. (1999) |
| Gene mutation (umu) | <i>S. typhimurium</i> TA1535/pSK1002 | Stevioside | 83 | 5 mg/plate | Negative ^c | Matsui et al. (1996) |
| Gene mutation | <i>B. subtilis</i> H17 rec+, M45 rec- | Stevioside | 83 | 10 mg/disk | Negative ^c | Matsui et al. (1996) |
| Chromosomal aberration | Chinese hamster lung fibroblasts | Stevioside | 83 | 8 mg/mL 12 mg/mL | Negative | Matsui et al. (1996) |
| Chromosomal aberration | Human lymphocytes | Stevioside | NS | 10 mg/mL | Negative | Suttajit et al. (1993) |
| Chromosomal aberration | Chinese hamster lung fibroblasts | Stevioside | 85 | 12 mg/mL | Negative ^a | Ishidate et al. (1984) |
| DNA damage (comet assay) | Wistar rats; liver, brain and spleen | Stevioside | 88.62 | 4 mg/L (estimated to be 80 - 500 mg/kg bw/day) in drinking water for 45 days | Positive in all tissues examined, most notably in liver | Nunes et al. (2007a) |
| DNA damage (comet assay) | Male BDF1 mouse stomach, colon, liver | Stevia extract | Stevioside, 52; Reb A, 22 | 250 - 2000 mg/kg bw | Negative ^e | Sekihashi et al. (2002) |
| DNA damage (comet assay) | Male ddY mouse stomach, colon, liver, kidney, bladder, lung, brain, bone marrow | Stevia | NS | 2000 mg/kg bw | Negative ^e | Sasaki et al. (2002) |
| Micronucleus formation | ddY mouse bone marrow and regenerating liver | Stevioside | NS | 62.5 - 250 mg/kg bw | Negative | Oh et al. (1999) |
| Mutation | <i>D. melanogaster</i> Muller 5 strain | Stevioside | NS | 2% in feed | Negative | Kerr et al. (1983) |

NS = Not specified. ^a Without metabolic activation. ^b As calculated by Williams, 2007. ^c With and without metabolic activation (source not specified in original monograph). ^d Inadequate detail available. ^e Sacrificed at 3 hours and 24 hours.

In a randomized, double-blind trial designed, 48 hyperlipidemic volunteers were recruited to investigate the hypolipidemic and hepatotoxic potential of steviol glycoside extract. The extract used in this study was a product containing stevioside (73 ± 2%), rebaudioside A (24 ± 2%) and other plant polysaccharides (3%). The subjects were given two capsules, each containing 50 mg of steviol glycoside extract or placebo, twice daily (i.e., 200 mg/day, equivalent to 3.3 mg/kg bw/day assuming an average body weight of 60 kg), for 3 months. One subject from placebo group and three from treatment group failed to complete the study for personal reasons, not related to adverse reactions. At the end of the study, both groups showed decreased serum

concentrations of total cholesterol and of low-density lipoproteins. Analyses of serum concentrations of triglycerides, liver-derived enzymes and glucose indicated no adverse effects. The authors questioned the subjects' compliance with the dosing regime, in view of the similarity of effect between treatment and placebo (Anonymous, 2004a). In a follow-up study, 12 patients were given steviol glycosides extract in incremental doses of 3.25, 7.5 and 15 mg/kg bw/day for 30 days per dose. Preliminary results indicated no adverse responses in blood and urine biochemical parameters (Anonymous, 2004b).

b. Studies Summarized in 2009

In a short term study of stevioside in healthy subjects, 4 male and 5 female healthy volunteers (aged 21–29 years) were provided with capsules containing 250 mg stevioside (97% purity) to be consumed 3 times per day for 3 days (Temme et al., 2004). Doses, expressed as steviol, were 288 mg/day or 4.4 mg/kg bw/day for females and 3.9 mg/kg bw/day for males. Twenty-four hour urine samples were taken before dosing on day 1 and after dosing on day 3. Fasting blood samples were taken before dosing on day 1, and six samples were taken at different time points on day 3 after dosing. Fasting blood pressure measurements were taken before the first capsule and at six different time intervals after the first dose. Urine was analyzed for creatinine, sodium, potassium, calcium, and urea. Blood was analyzed for plasma glucose, plasma insulin, alkaline phosphatase, ALT, GPT, creatine kinase, and lactate dehydrogenase. The clinical analyses of blood, blood pressure, and urine showed no differences between samples taken before or after dosing.

In an unpublished double-blind, placebo-controlled trial study reviewed at the sixty-eighth JECFA meeting, 250 mg of a product containing 91.7% total steviol glycosides, including 64.5% stevioside and 18.9% rebaudioside A, was administered to groups of type 1 ($n = 8$) and type 2 diabetics ($n = 15$) and non-diabetics ($n = 15$) 3 times daily for 3 months. Control groups with the same number of subjects received a placebo. After 3 months, there were no significant changes in systolic or diastolic blood pressure, glycated haemoglobin (HbA1c), blood lipids, or renal or hepatic function. No adverse effects were reported. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Barriocanal et al., 2006, 2008). The Committee previously noted that this product did not meet the proposed specification of “not less than 95% steviol glycosides” and that the study was conducted in a small number of subjects.

A study of antihypertensive effects was conducted in previously untreated mild hypertensive patients with crude stevioside obtained from the leaves of *S. rebaudiana*. Patients with essential hypertension were subjected to a placebo phase for 4 weeks and then received either capsules containing placebo for 24 weeks or crude stevioside at consecutive doses of 3.75 mg/kg bw/day (7 weeks), 7.5 mg/kg bw/day (11 weeks) and 15 mg/kg bw/day (6 weeks). Comparison of patients receiving stevioside with those on placebo showed neither antihypertensive nor adverse effects of stevioside. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Ferri et al., 2006). The product in this study also did not meet the proposed specification.

In a long-term, randomized, double blinded, placebo-controlled study, Jeppesen et al. (2006) investigated the efficacy and tolerability of oral stevioside in patients with type 2 diabetes. In this study, 55 subjects received 500 mg stevioside (purity unspecified) or placebo (maize starch) 3 times daily for 3 months. Compared with the placebo, stevioside did not reduce the incremental area under the glucose response curve and maintained the insulin response and HbA1c and fasting blood glucose levels. HbA1c is an indicator of mean glucose levels and is used in identifying effects on the control of diabetes. No differences in lipids or blood pressure were observed. It is not clear whether this study was approved by the local ethics committee or met the requirements of the Declaration of Helsinki (Jeppesen et al., 2006).

A placebo-controlled double-blind trial was carried out in 49 hyperlipidemic patients (aged 20–70 years, number of males and females not supplied) not undergoing treatment. The study was approved by the local ethics committee and complied with the principles of the Declaration of Helsinki. Individuals were divided into two groups, with 24 subjects receiving placebo capsules and 25 receiving capsules containing a dose of 50 mg steviol glycosides (70% stevioside, 20% Rebaudioside A), equivalent to 1.04 mg steviol/kg bw/day, using the mean body weight of the treatment group, 72.7 kg. Two capsules were taken before lunch and two before dinner each day for 90 days. Six subjects withdrew from the study, four in the placebo group and two in the test group. Self-reported adverse reactions were recorded, and fasting blood samples were taken at the end of the study and analyzed for ALT, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), and triglycerides. No effects of treatment on ALT, AST, or GGT were found. Decreases in the total cholesterol and LDL were observed in both the stevioside group and the placebo group, which were not treatment related. No adverse effects were observed (Cavalcante da Silva et al., 2006). The Committee noted at its sixty-eighth meeting that the product used in this study did not meet the proposed specification.

C. Safety Data on Rebaudioside A⁹

Only limited studies were available on Reb A during the JECFA reviews. 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, although it is uncertain whether or not these studies were considered by JECFA during its 2008 deliberations. 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. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

Three recently completed studies have shed light on the absorption and fate of Reb A in rats and humans. For comparative purposes to determine whether toxicological studies conducted

⁹ Questions about the safety of rebaudioside A were previously raised by Huxtable (2002) and Kobylewski and Eckhart (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.

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 (Wheeler et al., 2008).

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 \approx 84%.

2. Subchronic Toxicity 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 Crl: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 \geq 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.

3. Reproductive & Developmental Toxicity 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 F₀ or F₁ generations. The survival and general condition of the F₁ and F₂ 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 F₀ and F₁ generations rebaudioside A doses were 0, 500, 1000 and 2000 mg/kg/day. At initiation of study, F₀ animals were approximately 7 weeks of age. The test diet was offered to the offspring selected to become the F₁ generation following weaning [beginning on postnatal day (PND) 21]. The F₀ and F₁ males continued to receive rebaudioside A throughout mating, continuing through the day of euthanasia. The F₀ and F₁ 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 ≥ 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 ≥ 2000 mg/kg bw/day (highest dose administered) was considered to be the NOAEL for maternal and embryo/fetal developmental toxicity.

4. Mutagenicity & Genotoxicity Studies

The mutagenicity and genotoxicity data on Reb A have greatly increased recently. 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 11.

5. Clinical Studies

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 ± 3.7 mg/dL and 11.2 ± 4.5 mg/dL), insulin (1.0 ± 0.64 μ U/mL and 3.3 ± 1.5 μ U/mL), and Cpeptide (0.13 ± 0.09 ng/mL and 0.42 ± 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

Table 11. Mutagenicity & Genotoxicity 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 | 5 Salmonella strains and 1 E coli strain with and without exogenous metabolic activation system | Reb A | | 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 | | Up to 5000 µg/mL | No mutagenic or clastogenic response | Williams and Burdock (2009) |
| Chromosome Aberration | Chinese Hamster V79 cells | reb A | | Up to 5000 µg/mL | | Williams and Burdock (2009) |
| Mouse Micronucleus | Micronucleus study consisted of 7 groups, each containing 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 | | Reb A | | Up to 750 mg/kg bw | No increase in micronuclei formation | Williams and Burdock (2009) |
| Unscheduled DNA Synthesis | <i>In vivo</i> rat | Reb A | | Up to 2000 mg/kg bw | No increase in unscheduled DNA synthesis | Williams and Burdock (2009) |
| DNA damage (comet assay) | <i>Male BDF1 mouse stomach, colon, liver</i> | Stevia extract | Stevioside, 52%; Reb A, 22% | 250 - 2000 mg/kg bw | Negative ^a | Sekihashi et al. (2002) |
| Chromosomal aberration | <i>CHL/IU Chinese hamster lung fibroblasts</i> | Reb A | NS | 1.2 - 55 mg/mL | Negative ^b | Nakajima (2000a) |
| Micronucleus formation | <i>BDF1 mouse bone marrow</i> | Reb A | NS | 500-2000 mg/kg bw per day for 2 days | Negative ^c | Nakajima (2000b) |
| Forward mutation | <i>S. typhimurium</i> TM677 | Reb A | NS | 10 mg/plate | Negative ^b | Pezzuto et al. (1985) |

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

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.

D. Studies on the Principal Metabolite: Steviol

In a number of studies, steviol, the principal mammalian metabolite of stevioside, has been investigated for its safety. The results of these studies are summarized in the following sections.

1. Acute Toxicity Studies

The oral LD₅₀ of steviol (purity, 90%) in male and female mice and rats was reported to be > 15 g/kg bw. In this study, only one of 15 animals died within 14 days of administration. The LD₅₀ values in hamsters given steviol orally were 5.2 g/kg bw in males and 6.1 g/kg bw in females. Histopathological examination of the kidneys revealed severe degeneration of the proximal tubular cells, and these structural alterations were correlated with increased serum blood urea nitrogen and creatinine. The authors concluded that the cause of death was acute renal failure (Toskulkao et al., 1997).

2. Developmental Toxicity Studies

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1000 mg/kg bw/day (only 12 animals at the highest dose) by gavage in corn oil on days 6 - 10 of gestation. A significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12) were observed at the three highest doses, and the number of live fetuses per litter and mean fetal weight decreased in parallel. Histopathological examination of the maternal kidneys showed a dose-dependent increase in the severity of effects on the convoluted tubules (dilatation, hyaline droplets). However, no dose-dependent teratogenic effects were seen. The NOEL was 250 mg/kg bw/day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

3. Mutagenicity & Genotoxicity Studies

In a number of studies mutagenicity and genotoxicity of steviol has been investigated. These studies reviewed by JECFA are summarized in Table 12.

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Table 12. Mutagenicity & Genotoxicity Studies on Steviol

| STUDY | IN VIVO/IN VITRO | SYSTEM | TEST SAMPLE PURITY | AUTHOR CONCLUSION | RESULTS AND REMARKS |
|--|------------------|---|--------------------|-------------------|---|
| Sekihashi et al., 2002 ^a | In Vivo/In Vitro | Comet Assay | Not reported | Negative | In <i>in vitro</i> study, steviol at 62.5, 125, 250 and 500 µg/ml did not damage DNA of TK6 and WTK1 cells in presence or absence of S9 mix. In <i>in vivo</i> study, mice sacrificed 3 or 24 hours after one-time oral administration of 250, 500, 1000 or 2000 mg/kg of steviol. Stomach, colon, kidneys, testis and liver DNA not damaged. An identical <i>in vivo</i> experiment with stevia extract performed, which also gave negative results. |
| Oh et al., 1999 ^b | In Vivo? | Cell Mutation and DNA damage | Not reported | Negative | Steviol gave negative results for cell mutation and DNA damage in cultured cells. |
| Matsui et al., 1996 ^c | In Vivo? | Mutagenicity and Chromosome aberration (Chinese hamster lung fibroblasts) | Not reported | Positive | Gene mutation and chromosomal aberration found in Chinese hamster lung fibroblasts after metabolic activation of steviol. In hamsters, several metabolites of stevioside found that have not been found in rats or humans. Therefore, experimental relevance should be questioned when hamsters are used. |
| Terai et al., 2002 ^a | In Vitro | Bacterial Mutagenicity | Not Reported | Positive | Steviol found to be mutagenic in Aroclor induced rat liver S9 fraction. 15-oxo-steviol found to be mutagenic at 10% level of steviol. Specific mutagenicity of lactone derivative in presence of S9 mixture 10x lower than that of derivative without S9 mixture. |
| Temcharoen et al., 1998 ^c | In Vitro | Bacterial Mutagenicity | Not Reported | Positive | Mutagenic effects of steviol and/or metabolites found in <i>S.typhimurium</i> TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene. Magnitude of increase of these mutations over the control not reported. |
| Klongpanichpak et al., 1997 ^c | In Vitro | Bacterial Mutagenicity | Not Reported | Negative | Steviol and stevioside inactive in TA strains of <i>S. typhimurium</i> , <i>e. coli</i> WP2, <i>uvrA/PKM101</i> and rec assay using <i>B. subtilis</i> even when microsomal activated fraction present. Magnitude of increase of these mutations over the control not reported. |
| Matsui et al., 1996 ^a | In Vitro | Bacterial Mutagenicity | Not Reported | Negative | Testing of Southern Blot technique with probe for gpt gene DNA of <i>E. coli</i> . The chromosomal DNA of TM677 and steviol-induced TM677 mutants digested by restriction enzymes and probed. No significant differences found in fragment length between wild-type and mutant DNA. |
| Matsui et al., 1996 ^a | In Vitro | Bacterial Mutagenicity | Not Reported | Both | Steviol weakly positive in umu test, either with or without metabolic activation. Steviol negative in reverse mutation and other bacterial assays even in presence of S9 activation. |
| Procinska et al., 1991 ^c | In Vitro | Bacterial Mutagenicity | Not Reported | Negative | The direct mutagenic activity of 15-oxo-steviol was refuted. |

| STUDY | IN VIVO/IN VITRO | SYSTEM | TEST SAMPLE PURITY | AUTHOR CONCLUSION | RESULTS AND REMARKS |
|---------------------------------------|------------------|-----------------------------------|--------------------|-------------------|---|
| Compadre et al., 1988 ^a | <i>In Vitro</i> | Bacterial Mutagenicity, Mass Spec | Not Reported | Positive | Mass spectral analysis of steviol and analogues under conditions known to produce a mutagenic response. 15-oxo-steviol, a product of the metabolite, 15-alpha-hydroxysteviol was found to be direct-acting mutagen. Magnitude of increase over control in assay not discussed. |
| Pezzuto et al., 1985 ^d | <i>In Vitro</i> | Bacterial Mutagenicity | Not Reported | Positive | Using <i>S. typhimurium</i> TM677 strain, steviol found to be highly mutagenic in presence of 9000 x g supernatant from livers of Aroclor 1254-pretreated rats. This mutagenicity dependent on pretreatment of rats with Aroclor and NADPH addition, as unmetabolized steviol was inactive. None of other metabolites tested was mutagenic. Authors concluded that structural features of requisite importance for the expression of mutagenic activity may include a hydroxy group at position 13 and an unsaturated bond joining the carbon atoms at positions 16 and 17. |
| Temacharoen et al., 2000 ^c | <i>In Vivo</i> | Micronucleus (rat) | 90% | Negative | Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals. |
| Temacharoen et al., 2000 ^c | <i>In Vivo</i> | Micronucleus (mouse) | 90% | Negative | Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals. |
| Matsui et al., 1996 ^a | <i>In Vivo</i> | Micronucleus (mouse) | Not Reported | Negative | Steviol did not increase number of micronuclei observed in this study. |
| Temacharoen et al., 2000 ^c | <i>In Vivo</i> | Micronucleus (hamster) | 90% | Negative | Very high doses (4 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals. |

^a Abstract only. ^b As reported in JECFA, 2006. ^c As reviewed by Geuns, 2003. ^d Full article.

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VI. DISCUSSION OF GRAS CRITERIA & PANEL SAFETY FINDINGS

A. GRAS Criteria

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

“...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance.”¹⁰

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

Furthermore, in discussing GRAS criteria, FDA notes that:

“...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.”

“General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information.”¹¹

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called “common knowledge element,” in terms of the two following component elements:¹²

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

¹⁰ See 21 CFR 170.3(i).

¹¹ See 21 CFR 170.30(a).

¹² See Footnote 1.

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

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

B. Panel Findings on Safety Studies of Steviol Glycosides

GLG’s BlendSure™ products with a minimum steviol glycosides content of 97% as identified in the subject notification meet the JECFA specifications in that the glycosides constitute 95% or more of the dry weight. Clearly, the products consist primarily of stevioside and Reb A. Both of these steviol glycosides have been extensively studied in clinical, pharmacokinetic and toxicological studies. A majority of these studies have been published in peer-reviewed journals. The Panel has reviewed the data on both glycosides as well as the data on steviol, the principal metabolite.

1. Safety Data on Stevioside & Stevia Extracts that are Predominantly Stevioside

Because of their sweetness characters, steviol glycosides are unique as they have viable uses as a non-nutritive sweetener in foods.¹³ Periodic reviews by JECFA over the years document the progression in acquiring knowledge of 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.

¹³ It has also been reported that steviol glycosides may have pharmacological properties, which can be used to treat certain disease conditions such as hypertension and Type 2 diabetes. Chatsudthipong and Muanprasat (2009) 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.

As discussed in Section V.A.1, at its fifty-first meeting, JECFA reasoned that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), on which to base a temporary ADI with an adequate margin of safety. The Committee was satisfied that the lack of carcinogenic response in these well-conducted studies justified their conclusion that the *in vitro* mutagenic activity of steviol, buttressed by the evidence of rapid biotransformation and elimination of absorbed steviol, did not present a risk of carcinogenic effects *in vivo*. In addition, they concluded that all common steviol glycosides share the same basic metabolic and excretory pathways. Therefore, JECFA has concluded that high purity preparations of various steviol glycosides are safe to use as a non-nutritive sweetener. The additional clinical data subsequently presented allowed JECFA to establish a permanent ADI of 0 - 4 mg/kg bw/day (based on steviol equivalents), which translates to 0 -10 mg/kg bw/day for stevioside (as determined from the ratio of molecular weights of steviol and stevioside). The estimated consumption levels for stevioside containing sweeteners summarized in Section IV are comfortably within the JECFA ADI.

The Panel also noted that in a recent study, 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., 2007a). 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 with the challenges made by these scientists, thereby discounting the conclusions from the Nunes et al. (2007a) study.

The Panel has reviewed the findings from human clinical studies related to pharmacological effects. 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 10 mg/kg bw/day of stevioside, and 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.

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2. Safety Data on Rebaudioside A

In addition to the information on steviol glycosides discussed in the previous section, there are recent additional data specific to Reb A. Since July 2008, over ten papers describing the results of a comprehensive research program by different groups on rebaudioside A have been published. These studies formed the basis of the Cargill GRAS notification (GRN 253, 2008). Several other studies were sponsored by Merisant, and these were also then submitted with their GRAS notification (GRN 252). Previously, only a limited number of toxicology studies and clinical studies on Reb A were conducted and reported. As in the previous section on steviol glycosides, JECFA had concluded, even before these new studies were completed, that seven common steviol glycosides are safe for use as sweetener preparations when present in any combination at a combined purity of 95% or more.

As a majority of the previous pharmacokinetic research was conducted with steviol glycosides, the presumed strategy adopted for the more recent research on Reb A was to conduct a limited number of well-designed and executed toxicology studies on Reb A itself and to demonstrate in rats and in humans that it is handled pharmacokinetically similarly to stevioside. These studies were also done to help justify using the JECFA-generated ADI (for steviol glycosides, expressed as steviol) without having to conduct a new chronic study in rats on Reb A. In addition, Merisant (see Table 11) as well as another group (Williams and Burdock, 2009) upgraded the mutagenicity and genotoxicity data available on Reb A with three assays that FDA reportedly believes are most predictive for carcinogenicity. The Cargill group also conducted two clinical studies to assure that Reb A does not have potentially adverse pharmacological effects on blood glucose and blood pressure as was previously demonstrated in some stevioside studies.

In two separate reviews by Carakostas et al. (2008) and Brusick (2008), the recent research on Reb A was summarized and combined with the body of knowledge on stevioside. These reviews summarized the findings of the Cargill research program as follows:

- Steviol glycosides, Reb A, and stevioside are not genotoxic *in vitro*.
- Steviol glycosides, Reb A, and stevioside have not been shown to be genotoxic *in vivo* in well-conducted assays.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes et al., 2007a) and was improperly interpreted as a positive response.
- Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.
- The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- The pharmacokinetic similarity between Reb 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 Reb A.

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- The dietary levels expected from consumption of Reb A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.

Regarding possible pharmacological effects of Reb A in decreasing blood pressure and blood glucose, there is a recently published clinical data study on Reb A (Maki et al., 2008a, b). The Panel has reviewed these clinical studies and concludes that there should be no effects on blood pressure and glucose metabolism in humans at the doses of Reb A expected from use in food as a non-nutritive sweetener.

C. Panel's Overall Conclusions

The Panel has critically reviewed the anticipated dietary patterns and the use concentrations expected for steviol glycosides in various foods considered by JECFA in order to calculate the EDI, and the Panel agrees with these assessments. For US consumption, based on the very conservative assumption of 100% substitution of steviol glycosides for all sugars, an EDI of 5 mg/kg bw/day steviol was calculated by JECFA. However, JECFA concluded that the replacement estimates were highly conservative and that this calculated intake of steviol glycosides (as steviol) would more likely be 20 - 30% of these values. Additionally, Renwick (2008) also concluded that if only Reb A were used as a total sugar replacement, the levels would be below the JECFA ADI. The Panel concurs that an EDI of 5 mg/kg bw/day steviol very conservatively represents a potential high user of steviol glycosides, if this non-nutritive sweetener becomes widely available in food. As part of this GRAS evaluation, the Panel agrees with the JECFA EDI for application to the GLG's high purity steviol glycosides products.

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 past 40 years. In addition to JECFA's safety assessment of steviol glycosides, both Merisant and Cargill took rather rigorous scientific approaches to demonstrate the safety of Reb 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 and, in turn, the safety of steviol glycosides. A series of pharmacokinetic studies with steviol glycosides and more recent data on Reb A demonstrate that Reb A is handled pharmacokinetically similarly to stevioside. Hence, the safety studies of Reb A are also applicable to the safety assessment of stevioside.

In consideration of the aggregate safety information available, the Panel concludes that JECFA has conducted an expert safety evaluation and agrees with JECFA's conclusion. The per person ADI for steviol glycosides of adequate purity as defined by JECFA specifications has been properly determined to be 4 mg/kg bw/day (as steviol equivalents). The Panel calculates that this is equivalent to 10 mg/kg bw/day for stevioside and 12 mg/kg bw/day for Reb A on a weight basis. The Panel agrees that adverse pharmacological effects are not likely to occur at this level and that even high consumers of steviol glycosides are not likely to exceed this level. Therefore, the Panel agrees with the JECFA-derived ADI as a safe intake level of steviol glycosides and that food uses meeting the JECFA specifications, within the intake limits determined by JECFA, can

be considered to be generally recognized as safe (GRAS) within the meaning of the Food, Drug, and Cosmetic Act.

The Panel considers the available qualitative and quantitative scientific evidence, including human and animal data, to be sufficient to establish safety-in-use with the designated ADI for high purity steviol glycosides. On the basis of scientific procedures,¹⁴ the Panel concludes that the intended use of high purity steviol glycosides ($\geq 97\%$) with Reb A and stevioside as the principal components, 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 for a GRAS Determination

The first common knowledge element for a GRAS determination requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The majority of the studies reviewed in 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 reviewed only a limited number of studies conducted specifically on Reb A. The collection of supporting data on Reb A has recently been enhanced by the publication of the 2008 studies. The newest clinical studies that address JECFA's concern with unwanted pharmacological effects due to steviol glycosides (Barriocanal et al., 2008) and with Reb A (Maki et al., 2008 a, b) have been published in the scientific literature.

To be sure, the Panel recognizes that the safety of steviol glycosides in human foods has been the subject of interest for many years. In addition to the reported substantial history of consumption of stevia, especially in South America and Asia, many scientific studies have been conducted and published. Some of the studies have raised safety concerns, and the Panel has given careful attention to such concerns. The overriding evidence, particularly with high purity steviol glycosides, has certainly diminished the Panel's concerns based on better study designs, better study execution, and new investigations that better reflect state-of-the-art toxicological and clinical 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 2008 JECFA final 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

¹⁴ 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

because of their specific expertise on various classes of food ingredients. In addition, FDA participates in JECFA deliberations.

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

The common knowledge element has been embellished by the many well-respected scientists that participated in the Cargill-sponsored research conducted on Reb A, most notably David Brusick, Nigel Brown, and Andrew Renwick. An assertion of “general recognition of safety” was also made by Carakostas et al. (2008). We also note that the favorable safety conclusions on McNeil Nutritionals GRAS notification on steviol glycosides with Reb A as a principal component were reported by the McNeil Expert Panel, along with FDA’s concurrence with Blue California’s GRAS designation for its high purity Reb A. Similar safety conclusions were noted with FDA “no questions” letters as issued to Sweet Green Fields, Wisdom Natural Brands, Sunwin and WILD Flavors, Pyure Brands, and PureCircle USA. In summary, many diverse groups of scientists from all corners of the globe 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 Reb A as submitted by Merisant and Cargill and the more recent comparable findings by FDA with the additional GRAS notifications cited above.

While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Panel believes that a wide and expanding consensus does exist in the scientific community to support a GRAS conclusion as outlined in this evaluation. The scientific community will undoubtedly conclude that concerns expressed by others over the years (Huxtable, 2002) are likely to be satisfied by newer data on more purified materials and the rigid specifications for purity published by JECFA for steviol glycosides, including Reb A. Most notably the concerns on effects of fertility with crude extract have been addressed with a number of reproductive effects in rats and hamsters with purer materials (Usami et al., 1995a; Yodyingyuad and Bunyawong, 1991; Mori et al., 1981). Several chronic rat studies with sufficiently high no effect levels---most notably the study by Toyoda et al. (1997)---are available to set an acceptable ADI based on FDA tested review methodology. The recent clinical studies put to rest the concern that effects on blood pressure and blood glucose will be seen at the dietary levels expected (Barriocanal et al., 2008). There is also a wide consensus that the body of newer research on Reb A is sufficient to establish safety, 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. CONCLUSIONS¹⁵

High purity steviol glycosides ($\geq 97\%$), with the designated compositions as established for GLG's BlendSure™ products, which are produced in accordance with FDA Good Manufacturing Practices requirements and which meet at a minimum the JECFA purity specifications for steviol glycosides, are Generally Recognized As Safe when consumed as a general purpose non-nutritive sweetener as defined in the subject notification and within the JECFA ADI of 4 mg/kg bw/day on a steviol equivalent 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 should not exceed the amount reasonably required to accomplish its intended technical effect.

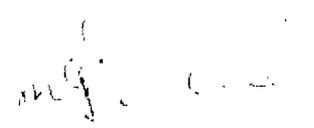
This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

(b) (6)

Richard C. Kraska, Ph.D., DABT
Chair

(b) (6)

Robert S. McQuate, Ph.D.


Madhusudan G. Soni, Ph.D., FACN

DATE: August 6, 2010

¹⁵ The detailed educational and professional credentials for two of the individuals serving on the Expert Panel can be found on the GRAS Associates website at 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/Soni%20CV.pdf>. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety. Each individual has 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

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

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

A-3 Updated JECFA Specifications for Steviol Glycosides -- 2010

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APPENDIX A-1

STEVIOLE GLYCOSIDES

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

SYNONYMS

INS no. 960

DEFINITION

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

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

Chemical name

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

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

C.A.S. number

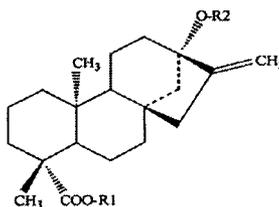
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: $C_{28}H_{60}O_{18}$
Rebaudioside A: $C_{44}H_{70}O_{23}$

Structural formula

The seven named steviol glycosides:



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

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

Formula weight Stevioside: 804.88
 Rebaudioside A: 967.03

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

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

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

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

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

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

PURITY

Total ash (Vol. 4) Not more than 1%

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

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

| | |
|-------------------------|--|
| <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). |

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

Standards

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

Mobile phase

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

Standard solutions

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

(b) Repeat with previously dried rebaudioside A standard.

Sample solution

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

Chromatography Conditions

Column: Supelcosil LC-NH2 or equivalent (length: 15-30 cm; inner diameter: 3.9-4.6 mm)

Mobile phase: A 80:20 mixture of acetonitrile and water (see above)

Flow rate: Adjust so that the retention time of rebaudioside A is about 21 min.

Injection volume: 5-10 µl

Detector: UV at 210 nm

Column temperature: 40°

Procedure

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

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

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

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

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

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

where

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

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

A_s is the peak area for stevioside from the standard solution

A_x is the peak area of X for the sample solution

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

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

APPENDIX A-2

STEVIOLE GLYCOSIDES

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

SYNONYMS

INS no. 960

DEFINITION

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

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

Chemical name

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

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

C.A.S. number

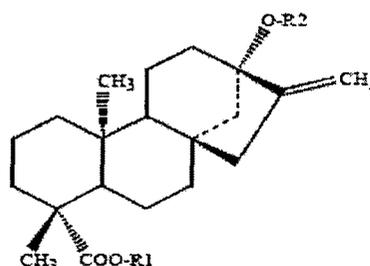
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: C₃₈H₆₀O₁₈
Rebaudioside A: C₄₄H₇₀O₂₃

Structural Formula

The seven named steviol glycosides:



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

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

Formula weight

Stevioside: 804.88
 Rebaudioside A: 967.03

Assay

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

DESCRIPTION

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

FUNCTIONAL USES

Sweetener

CHARACTERISTICS

IDENTIFICATION

| | |
|--------------------------------------|---|
| <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 Volume 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 Volume 4 (under "General Methods, Metallic Impurities"). |

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

Standards

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

Mobile phase

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

Standard solutions

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

Sample solution

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

the mobile phase.

Chromatography Conditions

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

Procedure

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

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

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

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

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

$$\%X = [Ws/W] \times [fxAx/As] \times 100$$

where

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

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

Appendix A-3

STEVIOI GLYCOSIDES

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 69th 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 69th JECFA (2008).

SYNONYMS

INS no. 960

DEFINITION

The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and 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

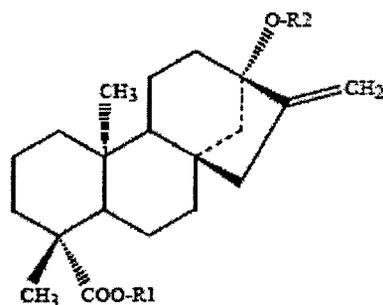
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: C₃₈H₆₀O₁₈
Rebaudioside A: C₄₄H₇₀O₂₃

Structural Formula

The nine named steviol glycosides:



| <u>Compound name</u> | <u>R1</u> | <u>R2</u> |
|-----------------------|---------------------------------|--|
| <i>Stevioside</i> | β -Glc | β -Glc- β -Glc(2→1) |
| <i>Rebaudioside A</i> | β -Glc | β -Glc- β -Glc(2→1) β -Glc(3→1) |
| <i>Rebaudioside B</i> | H | β -Glc- β -Glc(2→1) β -Glc(3→1) |
| <i>Rebaudioside C</i> | β -Glc | β -Glc- α -Rha(2→1) β -Glc(3→1) |
| <i>Rebaudioside D</i> | β -Glc- β -Glc(2→1) | β -Glc- β -Glc(2→1) β -Glc(3→1) |
| <i>Rebaudioside F</i> | β -Glc | β -Glc- β -Xyl(2→1) β -Glc(3→1) |
| <i>Dulcoside A</i> | β -Glc | β -Glc- α -Rha(2→1) |
| <i>Rubusoside</i> | β -Glc | β -Glc |
| <i>Steviolbioside</i> | H | β -Glc- β -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. |
| DESCRIPTION | White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose. |
| FUNCTIONAL USES | Sweetener |
| CHARACTERISTICS | |
| IDENTIFICATION | |
| <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"). |
| METHOD OF ASSAY | 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 C₁₈ 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_S is the amount (mg) calculated on the dried basis of stevioside in the standard solution;
- W_R 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_S is the peak area for stevioside from the standard solution;
- A_R 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).

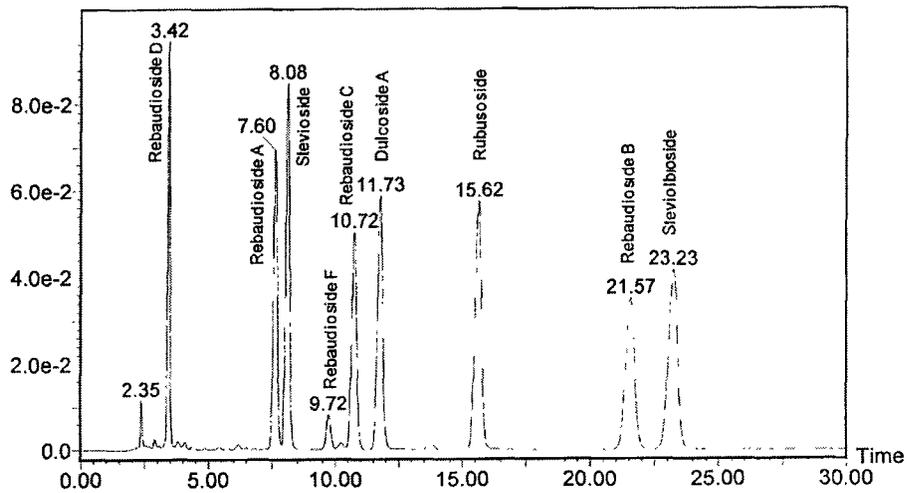


Figure. Chromatogram of mixture of nine steviol glycosides standard solution

Column: Capcell pak C₁₈ MG II

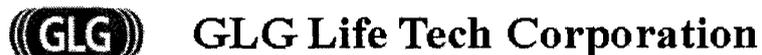
Concentration: 0.5 mg/ml each except rebaudioside F (about 0.1 mg/ml)

APPENDIX B

Manufacturing Information for Production of High Purity Steviol Glycosides ($\geq 97\%$)

- B-1 Process Flow Diagram for Primary Stevia Extract (Stevioside-Rich)**
- B-2 Process Flow Diagram for Primary Stevia Extract (Rebaudioside A-Rich)**
- B-3 Process Flow Diagram for STV 95**
- B-4 Process Flow Diagram for RA 95**
- B-5 Process Flow Diagram for Preparation of Steviol Glycosides Blends**

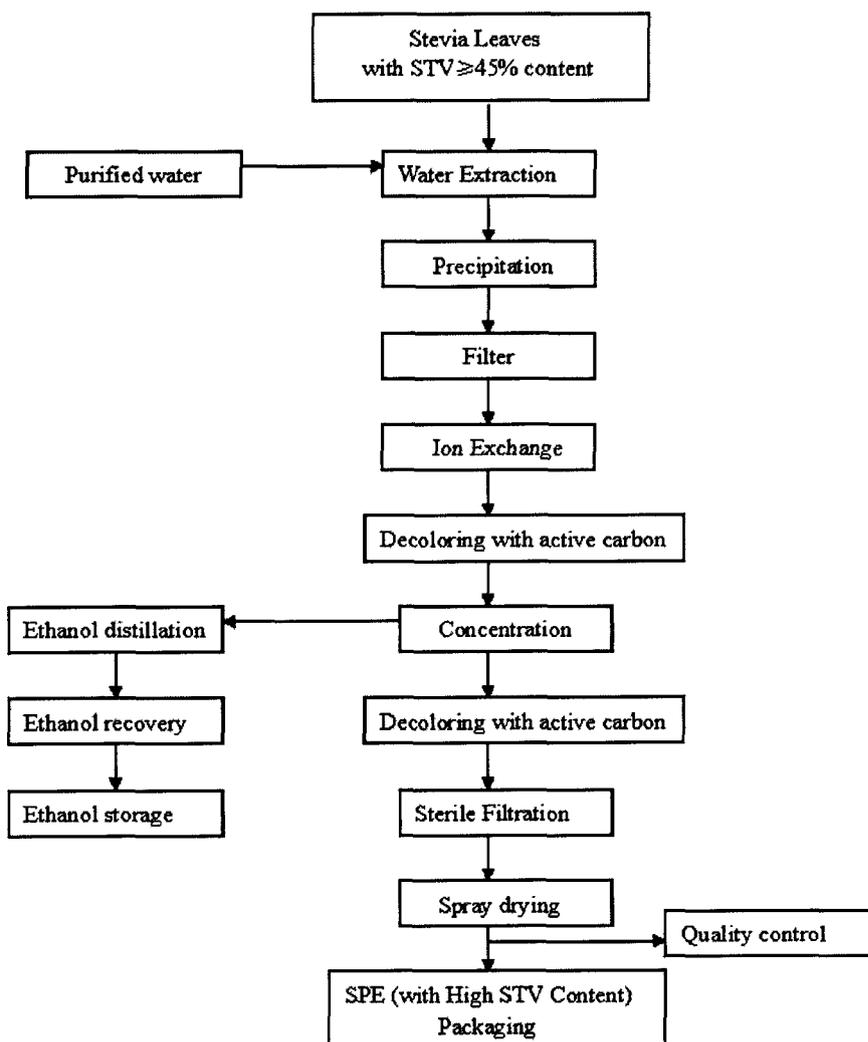
Appendix B-1



File No GLG-QA-STD-086
Reviewed by: Zhang Lei - QC Manager
Approved by: Kevin Li - Vice President

Stevia Primary Extract (with High STV Content)

Process Flow Chart



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

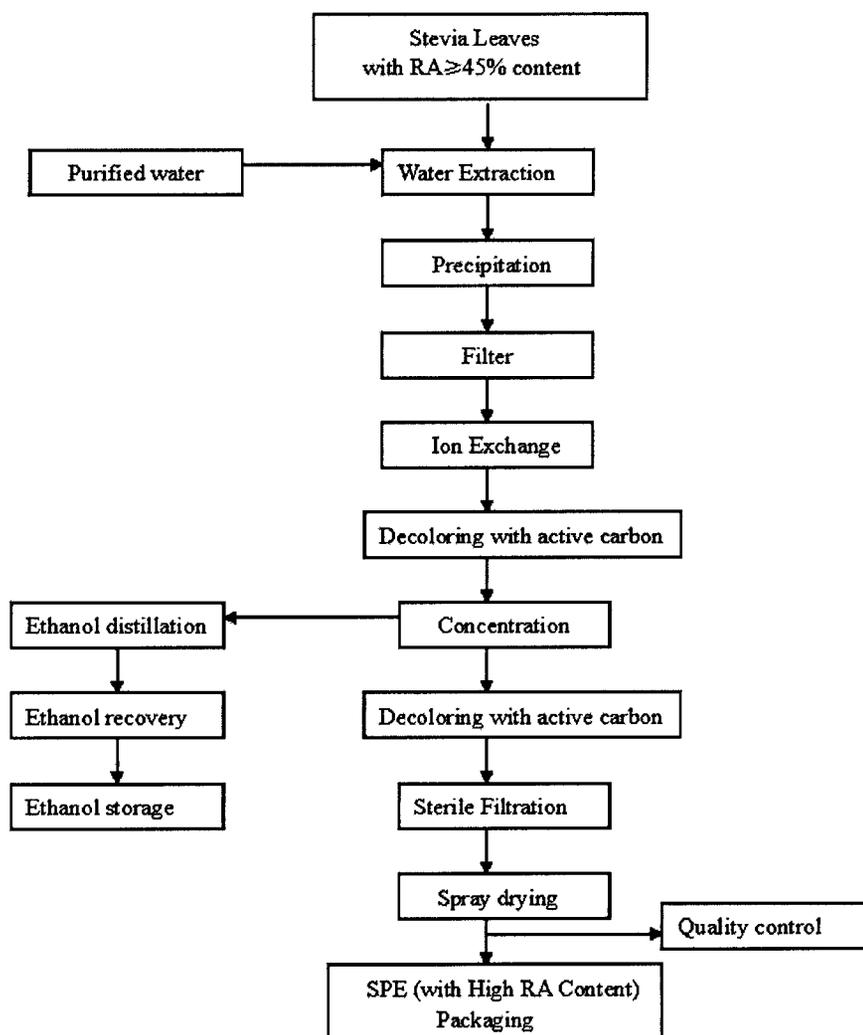
Appendix B-2



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

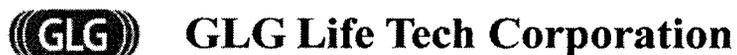
Stevia Primary Extract (with High RA Content)

Process Flow Chart



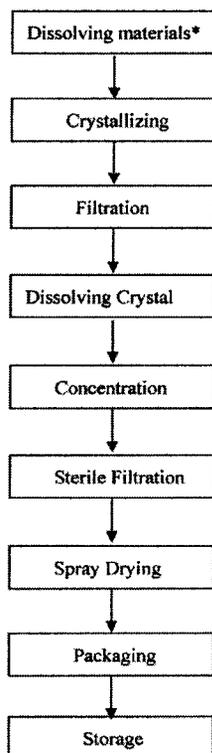
999 Canada Place, 519 World Trade Centre * Vancouver, B.C. * Canada * V6C 3E1
Phone: 1.604.641.1368 * Fax: 1.604.844.2830 * Email: sales@glglifetech.com * Web: glglifetech.com

Appendix B-3



File No. GLG-QA-ST1D-090
Reviewed by: Zhang Lei - QC Manager
Approved by: Kevin Li - Vice President

Pure STV95 Process Flow Chart



*Materials: Stevia Primary Extract with TSG $\geq 87\%$ including STV $\geq 45\%$ or Co-product with a high content of STV $\geq 45\%$

Specialty Solvents to be used in above STV95 process are Ethanol and/or Methanol

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

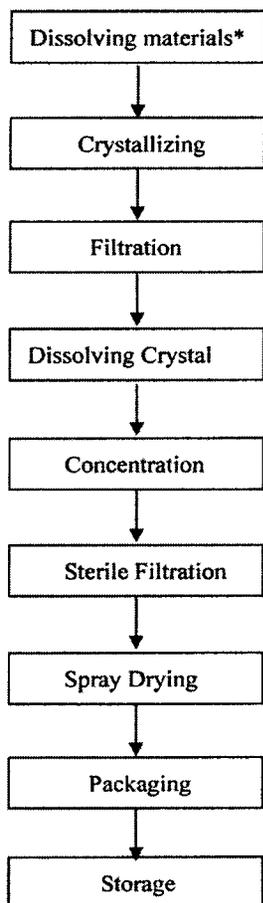
Appendix B-4



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

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

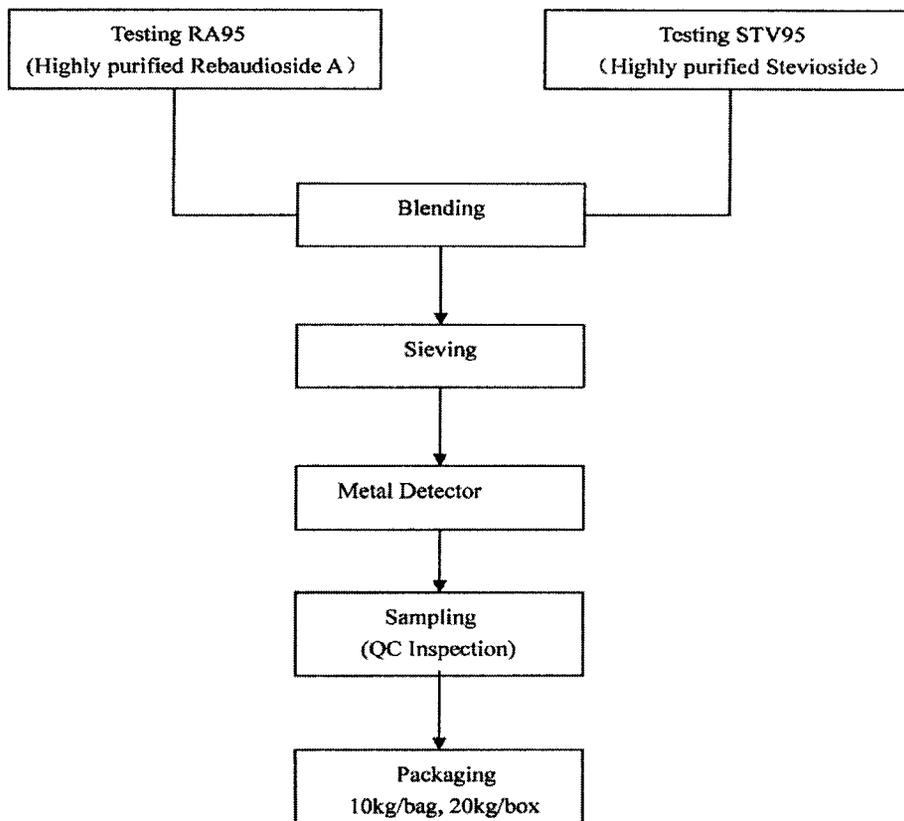
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Appendix B-5



File No. GLG-QA-STD-091
Reviewed by: Zhang Lei - QC Manager
Approved by: Kevin Li – Vice President

Flow Chart – Blending for BlendSure 7.5



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

APPENDIX C

Specifications & Specific Analyses of Multiple Production Lots for GLG's Purified Steviol Glycosides Products

**C-1 Specification Sheets for GLG's BlendSure™ 6.0, BlendSure™7.5,
& BlendSure™8.0**

C-2 Certificates of Analysis for GLG's BlendSure™ 6.0

C-3 Certificates of Analysis for GLG's BlendSure™ 7.5

C-4 Analytical Report BlendSure™ 6.0 for Steviol Glycosides Content

C-5 Certificate of Analyses for Pesticide Residues

Appendix C-1-1

Product Specification Sheet BlendSure™ 6.0



GLG Life Tech Corporation
 www.glglifetech.com
 File No.: GLG10BLS6.0-1002-000
 Reviewed by: Li Yang, QA Manager
 Approved by: Kevin Li, VP Technology
 2010

Product Description:

BlendSure™ 6.0 is a blend of highly purified stevia extracts primarily containing the glycosides rebaudioside A and stevioside (STV) from the *Stevia rebaudiana* Bertoni plant leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages

Shelf Life: 2 years

Physical and Organoleptic Standards

| CHARACTERISTIC | SPECIFICATION | METHOD |
|----------------|------------------------------------|--------------------------|
| Appearance | White/off-white hygroscopic powder | Organoleptic AS IS |
| Flavor | Sweet | Organoleptic AS IS |
| Aroma | Sweet | Organoleptic AS IS |
| Particle Size | 80-100 mesh | Ro Tap 25g for 5 minutes |

Specification

| CHARACTERISTIC | SPECIFICATION | LABEL CLAIM | METHOD |
|--------------------------|---------------|-------------|-------------------|
| Rebaudioside A | ≥ 57% | ≥ 57% | JECFA HPLC |
| Stevioside (STV) | ≥ 38% | ≥ 38% | JECFA HPLC |
| Total Steviol Glycosides | ≥ 97% | ≥ 97% | JECFA HPLC |
| Total Metals | ≤ 10 ppm | None | AFS |
| -Arsenic | ≤ 1.0 ppm | None | AFS |
| -Lead | ≤ 1.0 ppm | None | AFS |
| Solvents, total | ≤ 5200 ppm | None | GC |
| -Ethanol | ≤ 5000 ppm | None | GC |
| -Methanol | ≤ 200 ppm | None | GC |
| Loss on Drying | ≤ 4.0 | None | 105°C, 2 hrs |
| pH | 4.5-7.0 | None | 1 in 100 solution |
| Residue on Ignition | ≤ 1.0% | None | FCC 6 |

Microbiological Standards:

| CHARACTERISTIC | LIMIT | UNITS | METHOD |
|------------------------------|----------|--------|---------------------|
| Total Plate Count | < 1,000 | cfu/g | FDA-BAM current ed. |
| Yeast & Mold | < 100 | cfu/g | FDA-BAM current ed. |
| <i>E. coli</i> | Negative | **** | FDA-BAM current ed. |
| <i>Staphylococcus aureus</i> | Negative | **** | FDA-BAM current ed. |
| <i>Salmonella</i> (/25g) | Negative | (/25g) | FDA-BAM current ed. |

Product Specification Sheet BlendSure™ 6.0 (Continued)



GLG Life Tech Corporation
www.glglifetech.com
File No.: GLG10BLS6.0-L002-000
2010

Storage and Handling

Transport of the product shall be under such conditions that will prevent contamination. The product shall be stored in a sealed container in a cool, dry place.

Packaging

The product shall be shipped in packaging that is suitable for inland and ocean transportation. It shall be contained in a suitable inner bag (e.g. plastic). The inner bag shall be contained in an appropriate outer container (e.g. suitable cardboard box) and the outer container should have a conspicuous label on the side of the outer container. The outer container label shall be legible, indelible and permanent and indicate the proper name of the product, lot number, purchaser name and country of origin.

Product Guarantee

This product was produced in a plant that conforms to Good Manufacturing Practices and meets state and federal regulations. This product has critical control points to protect against the inclusion of metal or other extraneous material in the product. GLG Life Tech Corporation warrants that the lead contained in the product occurs naturally and is ≤ 1 ppm. The product meets the requirements listed in this specification sheet unless otherwise stated by GLG Life Tech Corporation. A certificate of analysis is supplied with each lot of BlendSure™ 6.0 and shall include the name and location of the production facility.

Appendix C-1-2

Product Specification Sheet

BlendSure™ 7.5



GLG Life Tech Corporation
 www.glglifetech.com
 File No.: GLG10BLS7.5-Q002-002
 Reviewed by: Li Yang, QA Manager
 Approved by: Kevin Li, VP Technology
 2010

Product Description:

BlendSure™ 7.5 is a blend of highly purified stevia extracts primarily containing the glycosides rebaudioside A (RA) and stevioside (STV) from the Stevia rebaudiana Bertoni plant leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages

Shelf Life: 2 years



Physical and Organoleptic Standards

| CHARACTERISTIC | SPECIFICATION | METHOD |
|----------------|------------------------------------|--------------------------|
| Appearance | White/off-white hygroscopic powder | Organoleptic AS IS |
| Flavor | Sweet | Organoleptic AS IS |
| Aroma | Sweet | Organoleptic AS IS |
| Particle Size | 80-100 mesh | Ro Tap 25g for 5 minutes |

Specification

| CHARACTERISTIC | SPECIFICATION | LABEL CLAIM | METHOD |
|--------------------------|---------------|-------------|-------------------|
| Rebaudioside A (RA) | ≥ 71% | ≥ 71% | JECFA HPLC |
| Stevioside (STV) | ≥ 24% | ≥ 24% | JECFA HPLC |
| Total Steviol Glycosides | ≥ 97% | ≥ 97% | JECFA HPLC |
| Total Metals | ≤ 10 ppm | None | AFS |
| -Arsenic | ≤ 1.0 ppm | None | AFS |
| -Lead | ≤ 1.0 ppm | None | AFS |
| Solvents, total | ≤ 5200 ppm | None | GC |
| -Ethanol | ≤ 5000 ppm | None | GC |
| -Methanol | ≤ 200 ppm | None | GC |
| Loss on Drying | ≤ 4.0 | None | 105°C, 2 hrs |
| pH | 4.5-7.0 | None | 1 in 100 solution |
| Residue on Ignition | ≤ 1.0% | None | FCC 6 |

Microbiological Standards:

| CHARACTERISTIC | LIMIT | UNITS | METHOD |
|------------------------------|----------|--------|---------------------|
| Total Plate Count | < 1,000 | cfu/g | FDA-BAM current ed. |
| Yeast & Mold | < 100 | cfu/g | FDA-BAM current ed. |
| <i>E. coli</i> | Negative | **** | FDA-BAM current ed. |
| <i>Staphylococcus aureus</i> | Negative | **** | FDA-BAM current ed. |
| <i>Salmonella</i> (/25g) | Negative | (/25g) | FDA-BAM current ed. |

Product Specification Sheet BlendSure™ 7.5 (Continued)



GLG Life Tech Corporation
www.glglifetech.com
File No.: GLG10BLS7.5-Q002-002
2010



Storage and Handling

Transport of the product shall be under such conditions that will prevent contamination. The product shall be stored in a sealed container in a cool, dry place.

Packaging

The product shall be shipped in packaging that is suitable for inland and ocean transportation. It shall be contained in a suitable inner bag (e.g. plastic). The inner bag shall be contained in an appropriate outer container (e.g. suitable cardboard box) and the outer container should have a conspicuous label on the side of the outer container. The outer container label shall be legible, indelible and permanent and indicate the proper name of the product, lot number, purchaser name and country of origin.

Product Guarantee

This product was produced in a plant that conforms to Good Manufacturing Practices and meets state and federal regulations. This product has critical control points to protect against the inclusion of metal or other extraneous material in the product. GLG Life Tech Corporation warrants that the lead contained in the product occurs naturally and is ≤ 1 ppm. The product meets the requirements listed in this specification sheet unless otherwise stated by GLG Life Tech Corporation. A certificate of analysis is supplied with each lot of BlendSure™ 7.5 and shall include the name and location of the production facility.

APPENDIX C-1-3

Product Specification Sheet
BlendSure™ 8.0



GLG Life Tech Corporation
 www.glglifetech.com
 File No.: GLG10BL58.0-N002-000
 Reviewed by: Li Yang, QA Manager
 Approved by: Kevin Li, VP Technology
 2010

Product Description:

BlendSure™ 8.0 is a blend of highly purified stevia extracts primarily containing the glycosides rebaudioside A and stevioside (STV) from the Stevia rebaudiana Bertoni plant leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages

Shelf Life: 2 years

Physical and Organoleptic Standards

| CHARACTERISTIC | SPECIFICATION | METHOD |
|----------------|------------------------------------|--------------------------|
| Appearance | White/off-white hygroscopic powder | Organoleptic AS IS |
| Flavor | Sweet | Organoleptic AS IS |
| Aroma | Sweet | Organoleptic AS IS |
| Particle Size | 80-100 mesh | Ro Tap 25g for 5 minutes |

Specification

| CHARACTERISTIC | SPECIFICATION | LABEL CLAIM | METHOD |
|--------------------------|---------------|-------------|-------------------|
| Rebaudioside A | ≥ 76% | ≥ 76% | JECFA HPLC |
| Stevioside (STV) | ≥ 19% | ≥ 19% | JECFA HPLC |
| Total Steviol Glycosides | ≥ 97% | ≥ 97% | JECFA HPLC |
| Total Metals | ≤ 10 ppm | None | AFS |
| -Arsenic | ≤ 1.0 ppm | None | AFS |
| -Lead | ≤ 1.0 ppm | None | AFS |
| Solvents, total | ≤ 5200 ppm | None | GC |
| -Ethanol | ≤ 5000 ppm | None | GC |
| -Methanol | ≤ 200 ppm | None | GC |
| Loss on Drying | ≤ 4.0 | None | 105°C, 2 hrs |
| pH | 4.5-7.0 | None | 1 in 100 solution |
| Residue on Ignition | ≤ 1.0% | None | FCC 6 |

Microbiological Standards:

| CHARACTERISTIC | LIMIT | UNITS | METHOD |
|------------------------------|----------|--------|---------------------|
| Total Plate Count | < 1,000 | cfu/g | FDA-BAM current ed. |
| Yeast & Mold | < 100 | cfu/g | FDA-BAM current ed. |
| <i>E. coli</i> | Negative | **** | FDA-BAM current ed. |
| <i>Staphylococcus aureus</i> | Negative | **** | FDA-BAM current ed. |
| <i>Salmonella</i> (/25g) | Negative | (/25g) | FDA-BAM current ed. |

Product Specification Sheet BlendSure™ 8.0 (Continued)



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File No.: GLG10BLS8 0-N002-000
2010

Storage and Handling

Transport of the product shall be under such conditions that will prevent contamination. The product shall be stored in a sealed container in a cool, dry place.

Packaging

The product shall be shipped in packaging that is suitable for inland and ocean transportation. It shall be contained in a suitable inner bag (e.g. plastic). The inner bag shall be contained in an appropriate outer container (e.g. suitable cardboard box) and the outer container should have a conspicuous label on the side of the outer container. The outer container label shall be legible, indelible and permanent and indicate the proper name of the product, lot number, purchaser name and country of origin.

Product Guarantee

This product was produced in a plant that conforms to Good Manufacturing Practices and meets state and federal regulations. This product has critical control points to protect against the inclusion of metal or other extraneous material in the product. GLG Life Tech Corporation warrants that the lead contained in the product occurs naturally and is ≤ 1 ppm. The product meets the requirements listed in this specification sheet unless otherwise stated by GLG Life Tech Corporation. A certificate of analysis is supplied with each lot of BlendSure™ 8.0 and shall include the name and location of the production facility.

Appendix C-2-1



Certificate of Analysis



Research and Development
 GLG Life Tech Corporation
 www.glglifetech.com
 GLG-QA-COA-11

Product: Rebpure™ SS60 **Manufacturing Date:** Mar.3th,2010
Lot Number: GLG-SS60-1003001 **Country of Origin:** China
Shelf Life: 2 years

Product Description: Rebpure™ SS60 is 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: +86.532.55553339
 Qingdao Export Processing Zone, 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: Mar.7th,2010

| INSPECTION ITEM | SPECIFICATION | RESULT | METHOD |
|-----------------------------|---------------|--------------|------------|
| Appearance | White powder | White powder | Visual |
| Total Steviol Glycosides | ≥ 97% | 97.45% | JECFA HPLC |
| Stevioside STY | ≥ 38% | 39.03% | JECFA HPLC |
| Rebaudioside A | ≥ 57% | 57.76% | JECFA HPLC |
| Loss on Drying | ≤ 4.0% | 2.60% | USP |
| pH | 4.5 - 7.0 | 4.96 | USP |
| Residue on Ignition | < 1.0% | 0.06% | USP |
| Lead (Pb) | < 1 ppm | 0.10 ppm | AFS |
| Arsenic (As) | < 1 ppm | 0.03ppm | 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) (Lab Manager) **Date:** 2010.3.7
Checked by: (QC Manager) **Date:** 2010.3.7
Approved by: (QA Manager) **Date:** 2010.3.7

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Appendix C-2-2



Certificate of Analysis



Research and Development
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 www.glglifetech.com
 GLG-QA-COA-11

Product: Rebpure™ SS60
 Lot Number: GLG-SS60-1003002

Manufacturing Date: Mar.4th,2010
 Country of Origin: China
 Shelf Life: 2 years

Product Description: Rebpure™ SS60 is from Stevia rebaudiana Bertoni leaf. It is a white hydroscopic powder that is used as a high potency sweetener for food and beverages

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 Fax: +86.532.55566968

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

Date of Analysis: Mar.9th,2010

| INSPECTION ITEM | SPECIFICATION | RESULT | METHOD |
|-----------------------------|---------------|--------------|------------|
| Appearance | White powder | White powder | Visual |
| Total Steviol Glycosides | ≥ 97% | 98.21% | JECFA HPLC |
| Stevioside STV | ≥ 38% | 39.04% | JECFA HPLC |
| Rebaudioside A | ≥ 57% | 57.62% | JECFA HPLC |
| Loss on Drying | ≤ 4.0% | 2.60% | USP |
| pH | 4.5 - 7.0 | 4.93 | USP |
| Residue on Ignition | < 1.0% | 0.06% | 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) (Lab Manager) Date: 2010.3.9
 Checked by: (b) (6) (QC Manager) Date: 2010.3.9
 Approved by: (b) (6) (QA Manager) Date: 2010.3.9

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Appendix C-2-4



Certificate of Analysis



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 GLG-QA-COA-11

Product: Rebpure™ SS60
Lot Number: GLG-SS60-1003004
Manufacturing Date: Mar.6th,2010
Country of Origin: China
Shelf Life: 2 years

Product Description: Rebpure™ SS60 is 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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 Web: www.glglifetech.com

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

Date of Analysis: Mar.11th,2010

| INSPECTION ITEM | SPECIFICATION | RESULT | METHOD |
|-----------------------------|---------------|--------------|------------|
| Appearance | White powder | White powder | Visual |
| Total Steviol Glycosides | ≥ 97% | 98.61% | JECFA HPLC |
| Stevioside STV | ≥ 38% | 38.74% | JECFA HPLC |
| Rebaudioside A | ≥ 57% | 57.80% | JECFA HPLC |
| Loss on Drying | ≤ 4.0% | 3.00% | USP |
| pH | 4.5 - 7.0 | 5.01 | USP |
| Residue on Ignition | < 1.0% | 0.05% | USP |
| Lead (Pb) | < 1 ppm | 0.05 ppm | AFS |
| Arsenic (As) | < 1 ppm | 0.01ppm | AFS |
| Residual Solvents – Ethanol | ≤ 0.5% | 0.14% | 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) (Lab Manager) **Date:** 2010.3.11

Checked by: (QC Manager) **Date:** 2010.3.11

Approved by: (QA Manager) **Date:** 2010.3.11

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Appendix C-2-5



Certificate of Analysis



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 GLG-QA-COA-11

Product: Rebpure™ SS60
Lot Number: GLG-SS60-1003005
Manufacturing Date: Mar. 7th, 2010
Country of Origin: China
Shelf Life: 2 years

Product Description: Rebpure™ SS60 is 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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 Fax: +86.532.55566968

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

Date of Analysis: Mar. 12th, 2010

| INSPECTION ITEM | SPECIFICATION | RESULT | METHOD |
|-----------------------------|---------------|--------------|------------|
| Appearance | White powder | White powder | Visual |
| Total Steviol Glycosides | ≥ 97% | 98.69% | JECFA HPLC |
| Stevioside STV | ≥ 38% | 38.60% | JECFA HPLC |
| Rebaudioside A | ≥ 57% | 58.30% | JECFA HPLC |
| Loss on Drying | ≤ 4.0% | 2.40% | USP |
| pH | 4.5 - 7.0 | 4.96 | USP |
| Residue on Ignition | < 1.0% | 0.05% | USP |
| Lead (Pb) | < 1 ppm | 0.07ppm | 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) (Lab Manager) **Date:** 2010. 3. 12
Checked by: (QC Manager) **Date:** 2010. 3. 12
Approved by: (QA Manager) **Date:** 2010. 3. 12

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Appendix C-3-1

Certificate of Analysis



Research and Development
 GLG Life Tech Corporation
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 GLG-QA-COA-14

Product: BlendSure 7.5
Lot Number: 201005001
Manufacturing Date: May 2nd, 2010
Country of Origin: China
Shelf Life: 2 years

Product Description: BlendSure 7.5 is from Stevia rebaudiana Bertoni leaf. It is a white hydroscopic powder that is used as a high potency sweetener for food and beverages

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 Fax: +86.532.55566958

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

Date of Analysis: May 7th, 2010

| INSPECTION ITEM | SPECIFICATION | RESULT | METHOD |
|-----------------------------|---------------|--------------|------------|
| Appearance | White powder | White powder | Visual |
| Total Steviol Glycosides | ≥ 97% | 97.23% | JECFA HPLC |
| Stevioside STV | ≥ 24% | 24.12% | JECFA HPLC |
| Rebaudioside A | ≥ 71% | 71.46% | JECFA HPLC |
| Loss on Drying | ≤ 4.0% | 2.48% | USP |
| pH | 4.5 - 7.0 | 4.98 | USP |
| Residue on Ignition | < 1.0% | 0.08% | USP |
| Lead (Pb) | < 1 ppm | 0.10 ppm | AFS |
| Arsenic (As) | < 1 ppm | 0.08ppm | AFS |
| Residual Solvents – Ethanol | ≤ 0.5% | 0.24% | USP |
| – Methanol | ≤ 0.02% | 0.01% | USP |
| Total Plate Count | ≤ 1,000 cfu/g | < 10cfu/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:** 07/05/2010
Checked by: (b) (6) **Date:** 07/05/2010
Approved by: (b) (6) Quality Manager **Date:** 07/05/2010

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Appendix C-3-2

Certificate of Analysis



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 GLG-QA-COA-14

Product: BlendSure 7.5
Lot Number: 201005004
Manufacturing Date: May 4th, 2010
Country of Origin: China
Shelf Life: 2 years

Product Description: BlendSure 7.5 is 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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 Fax: +86.532.55566968

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

Date of Analysis: May 9th, 2010

| INSPECTION ITEM | SPECIFICATION | RESULT | METHOD |
|-----------------------------|---------------|--------------|------------|
| Appearance | White powder | White powder | Visual |
| Total Steviol Glycosides | ≥ 97% | 97.51% | JECFA HPLC |
| Stevioside STV | ≥ 24% | 24.33% | JECFA HPLC |
| Rebaudioside A | ≥ 71% | 71.68% | JECFA HPLC |
| Loss on Drying | ≤ 4.0% | 2.48% | USP |
| pH | 4.5 - 7.0 | 5.00 | USP |
| Residue on Ignition | < 1.0% | 0.06% | USP |
| Lead (Pb) | < 1 ppm | 0.10 ppm | AFS |
| Arsenic (As) | < 1 ppm | 0.07ppm | AFS |
| Residual Solvents – Ethanol | ≤ 0.5% | 0.16% | USP |
| – Methanol | ≤ 0.02% | 0.01% | USP |
| Total Plate Count | ≤ 1,000 cfu/g | < 10cfu/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:** 09/05/2010
Checked by: (b) (6) **Date:** 09/05/2010
Approved by: (b) (6) (Quality Manager) **Date:** 09/05/2010

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Appendix C-3-3

Certificate of Analysis



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 GLG-QA-COA-14

Product: BlendSure 7.5
Lot Number: 201005006
Manufacturing Date: May 5th, 2010
Country of Origin: China
Shelf Life: 2 years

Product Description: BlendSure 7.5 is 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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 Fax: +86.532.55566968

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Date of Analysis: May 10th, 2010

| INSPECTION ITEM | SPECIFICATION | RESULT | METHOD |
|-----------------------------|---------------|--------------|------------|
| Appearance | White powder | White powder | Visual |
| Total Steviol Glycosides | ≥ 97% | 97.32% | JECFA HPLC |
| Stevioside STV | ≥ 24% | 24.53% | JECFA HPLC |
| Rebaudioside A | ≥ 71% | 71.32% | JECFA HPLC |
| Loss on Drying | ≤ 4.0% | 2.52% | USP |
| pH | 4.5 - 7.0 | 4.99 | USP |
| Residue on Ignition | < 1.0% | 0.08% | USP |
| Lead (Pb) | < 1 ppm | 0.10 ppm | AFS |
| Arsenic (As) | < 1 ppm | 0.06ppm | AFS |
| Residual Solvents – Ethanol | ≤ 0.5% | 0.20% | USP |
| – Methanol | ≤ 0.02% | 0.01% | USP |
| Total Plate Count | ≤ 1,000 cfu/g | < 10cfu/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: 10/05/2010

Checked by: (b) (6) Date: 10/05/2010

Approved by: (Quality Manager) Date: 10/05/2010

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Appendix C-3-4

Certificate of Analysis



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 GLG-OA-COA-14

Product: BlendSure 7.5
Lot Number: 201005009
Manufacturing Date: May 7th, 2010
Country of Origin: China
Shelf Life: 2 years

Product Description: BlendSure 7.5 is from Stevia rebaudiana Bertoni leaf. It is a white hydroscopic powder that is used as a high potency sweetener for food and beverages.

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

Date of Analysis: May 12th, 2010

| INSPECTION ITEM | SPECIFICATION | RESULT | METHOD |
|-----------------------------|---------------|--------------|------------|
| Appearance | White powder | White powder | Visual |
| Total Steviol Glycosides | ≥ 97% | 97.42% | JECFA HPLC |
| Stevioside STV | ≥ 24% | 24.43% | JECFA HPLC |
| Rebaudioside A | ≥ 71% | 71.26% | JECFA HPLC |
| Loss on Drying | ≤ 4.0% | 2.40% | USP |
| pH | 4.5 - 7.0 | 4.96 | USP |
| Residue on Ignition | < 1.0% | 0.09% | USP |
| Lead (Pb) | < 1 ppm | 0.10 ppm | AFS |
| Arsenic (As) | < 1 ppm | 0.08ppm | AFS |
| Residual Solvents – Ethanol | ≤ 0.5% | 0.22% | USP |
| – Methanol | ≤ 0.02% | 0.01% | USP |
| Total Plate Count | ≤ 1,000 cfu/g | < 10cfu/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:** 12/05/2010
Checked by: **Date:** 12/05/2010
Approved by: (Quality Manager) **Date:** 12/05/2010

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Appendix C-3-5

Certificate of Analysis



Research and Development
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 GLG-QA-COA-14

Product: BlendSure 7.5
Lot Number: 2010050013
Manufacturing Date: May 9th, 2010
Country of Origin: China
Shelf Life: 2 years

Product Description: BlendSure 7.6 is 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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Qingdao Runhao Rebiana high Tech Co., Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation

Date of Analysis: May 14th, 2010

| INSPECTION ITEM | SPECIFICATION | RESULT | METHOD |
|-----------------------------|---------------|--------------|------------|
| Appearance | White powder | White powder | Visual |
| Total Steviol Glycosides | ≥ 97% | 97.23% | JECFA HPLC |
| Stevioside STV | ≥ 24% | 24.47% | JECFA HPLC |
| Rebaudioside A | ≥ 71% | 71.19% | JECFA HPLC |
| Loss on Drying | ≤ 4.0% | 2.48% | USP |
| pH | 4.5 - 7.0 | 4.98 | USP |
| Residue on Ignition | < 1.0% | 0.08% | USP |
| Lead (Pb) | < 1 ppm | 0.09ppm | AFS |
| Arsenic (As) | < 1 ppm | 0.08ppm | AFS |
| Residual Solvents – Ethanol | ≤ 0.5% | 0.19% | USP |
| – Methanol | ≤ 0.02% | 0.01% | USP |
| Total Plate Count | ≤ 1,000 cfu/g | < 10cfu/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:** 14/05/2010
Checked by: (b) (6) **Date:** 14/05/2010
Approved by: (b) (6) Quality Manager) **Date:** 14/05/2010

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

Appendix C-4-Part 1



Eurofins Scientific, Inc.
1365 Redwood Way
Petaluma, Ca 94951

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

Prepared by: (b) (6)
Jules Skamarack
Eurofins Scientific, Inc.

Reviewed by: (b) (6)
Mariel Esquiverra
Eurofins Scientific, Inc.

Approved by: _____
James Kempland
GLG Life Tech Corporation

Date Issued: July, 2010

000099



I. Study Identification

1. Study Title:

Method Verification of the Determination of Stevioside and Rebaudioside A by High Performance Liquid Chromatography (HPLC).

2. Study Objective:

The objective of this study is to verify the assay for stevioside and rebaudioside A in the GLG supplied Stevia leaf extracts.

3. Study Coordinator/Performing Laboratory:
Jules Skamarack, Eurofins Scientific, Inc.

4. Study Monitor(s): Mariel Esguerra

5. Test Materials:
Stevia rebaudiana Leaf extracts

- (1) BlendSure 60, Powder, Lot #GLG-SS60-1003001, Serving = 100g, Eurofins sample number 740-2010-0000**1960**
- (2) BlendSure 60, Powder, Lot #GLG-SS60-1003002, Serving = 100g, Eurofins sample number 740-2010-0000**1961**
- (3) BlendSure 60, Powder, Lot #GLG-SS60-1003003, Serving = 100g, Eurofins sample number 740-2010-0000**1962**
- (4) BlendSure 60, Powder, Lot #GLG-SS60-1003004, Serving = 100g, Eurofins sample number 740-2010-0000**1963**
- (5) BlendSure 60, Powder, Lot #GLG-SS60-1003005, Serving = 100g, Eurofins sample number 740-2010-0000**1964**

6. Test Reagents:

(1) Acetonitrile, HPLC Grade
Fisher P/N A998-4, VWR P/N JT9017-3

(2) Rebaudioside A, Lot. F01077 from USP C.A.S # 58543-16-1
Calibration standard

(3) Rebaudioside A Cerilliant 1mg/ml in solution. Solution Lot #
FN051809-01, C.A.S # 58543-16-1 Accuracy check

(4) Positive control sample identified as Eurofins sample # 04-1172
controlled for rebaudioside A and relative retention time determinations.

(5) Phosphoric Acid, Fischer Chemical Company P/N A260



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

II. Study Description

1. Scope:

This is applicable to the determination of rebaudioside A and stevioside in 5 raw material samples.

2. Test Materials:

- (1) BlendSure 60, Powder, Lot #GLG-SS60-1003001, Serving = 100g, Eurofins sample number 740-2010-0000**1960**
- (2) BlendSure 60, Powder, Lot #GLG-SS60-1003002, Serving = 100g, Eurofins sample number 740-2010-0000**1961**
- (3) BlendSure 60, Powder, Lot #GLG-SS60-1003003, Serving = 100g, Eurofins sample number 740-2010-0000**1962**
- (4) BlendSure 60, Powder, Lot #GLG-SS60-1003004, Serving = 100g, Eurofins sample number 740-2010-0000**1963**
- (5) BlendSure 60, Powder, Lot #GLG-SS60-1003005, Serving = 100g, Eurofins sample number 740-2010-0000**1964**

3. Reference Standards: Separate Standards (stevioside and rebaudioside A)

A. Stock standards.

1. The standard preparation for rebaudioside A was dried at 105 degrees Centigrade for two hours as directed by JECFA.

2. On a microbalance, accurately weigh 10.0 ± 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.



B. Calibration standards (USP rebaudioside A). The range of quantitation will roughly be between 0.5 mg/mL and 2.0 mg/mL in solution. A three point curve is used for routine quantitation. However for the purposes of this study a 5 point curve was used. The sample test concentration will be at approximately 1 mg/ml steviol glycosides, based on the expected test sample concentration. To accommodate this, inject the stock standard to include approximately a 1 mg/ml standard as the midpoint of calibration. The stock standard will then be injected as follows to create a 5 point calibration curve (actual concentration is adjusted for standard purity and is listed below for rebaudioside A):

| Injection Volume (mls) | Realized Concentration (mg/ml) |
|------------------------|--------------------------------|
| 5 | 2.092071 |
| 4 | 1.67366 |
| 3 | 1.2552426 |
| 2 | 0.8368284 |
| 1 | 0.4184142 |

4. Verification Study:

A. Primary method:

Method attached.

B. Linearity:

1. Create a five point calibration curve by making dilutions from the stock standard.
 1. Calculate response factors. RSD between levels must be $\leq 5\%$.
 2. Correlation coefficient must be > 0.999 .

2. Results, rebaudioside A;

1. Response factors RSD between levels was found to be **0.923 (amount) and passed the criteria.**
2. Correlation coefficient was found to be **0.99999 and passed the criteria.**

D. 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 # 201-05-171AA-0101.D and -0102D.)**
2. Positive control sample analysis:
 - a. Analyze the positive control sample where the measured concentration of rebaudioside A should be with-in 2 standard deviations of the mean. The mean result is established at 22.5 %(w/w) and the standard deviation is 3.152.



1. Result: Measured concentration result is 22.1 % (w/w) which passed the criteria. (file # 2010-05-17\1DI-4401.D)

3. Demonstrate separation of the two major peaks, stevioside and rebaudioside A in the positive control:

1. Separation was demonstrated with actual retention times of 4.634 minutes (stevioside) and 6.286 minutes (rebaudioside A) (file # 2010-05-17\1DI-4401.D)

E. System Suitability:

1. A 1.255 mg/ml rebaudioside A standard solution is injected 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 are less than 2%.

a. Results: Average retention time 6.316 minutes with a RSD of 0.032. The actual deviation between the longest and shortest retention time is 0.005 minutes. All criteria pass.

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 0.816.

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 5290. All pass the criteria.

4. USP tailing factor was determined for the rebaudioside A standard peak at the 1.225 mg/ml injection. The tailing factor should be not more than (NMT) 2.0.

a. Results: The tailing factor passed the criteria (USP) at 0.816.

5. Column Efficiency, Not less than (NLT) 5000 theoretical plate count, using the Rebaudioside A standard peak at the 1.225 mg/ml injection..

a. Results: All theoretical plate count calculations were greater than 5000 with the halfwidth method calculating at 8586. All pass the criteria.

000103



F. Accuracy:

Accuracy was examined by applying the analytical procedure to an analyte of known purity. For this purpose a Cerilliant rebaudioside A, a standard of known purity was used at a single concentration point near the middle range of the calibration. The results for this point was calculated based on the USP rebaudioside A standard.

1. Acceptance Criteria: For a typical analysis we would expect the accuracy to be with-in 2 percent of the original value. The Cerilliant standard theoretically presents a good measure of accuracy since it is calibrated in solution and not subject to variances in moisture from storage, shipping and analytical conditions. The Cerilliant standard result calculated out at 110.49 percent recovery based on the USP standard. Two conclusions can be reached from this analysis and the history of testing these samples. Either the USP purity is not properly represented, or the procedure of drying the standard from JECFA (as outlined in the method at 105 degrees Centigrade for 2 hours), is not sufficient to drive moisture off of the standard and samples, and must be determined in an alternative fashion. Additional analysis can be performed to determine if Karl Fischer or other analysis of the samples and standards could alleviate this issue. This issue is likely responsible in part or entirely for the high purity values assigned to the results (specifically stevioside) based on JECFA analysis as related in the results table below.

G. Repeatability and Precision:

1. For each test material, perform 3 replicate sample preparations. The acceptance criteria for the RSD for each set of 3 analyses must be $\leq 5\%$ for the total steviol glycoside value.

a. Results: RSDs for all samples pass the criteria.

| Sample 1960 | 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 | 42.107844 | 42.049396 | 41.933751 | 42.03033 | 0.2107969 |
| Rebaudioside A | 60.902311 | 60.73433 | 60.416487 | 60.684376 | 0.4065861 |
| Sample 1961 | 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 | 41.405359 | 41.041287 | 41.152789 | 41.199812 | 0.4527679 |
| Rebaudioside A | 61.76084 | 61.748898 | 61.339269 | 61.616336 | 0.3895413 |
| Sample 1962 | 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 | 43.023822 | 42.956175 | 43.033038 | 43.004345 | 0.0975952 |
| Rebaudioside A | 59.36618 | 60.014276 | 59.492215 | 59.624224 | 0.5763148 |

000104



| Sample 1963 | 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 | 42.331327 | 41.998659 | 42.037966 | 42.122717 | 0.4313975 |
| Rebaudioside A | 60.528017 | 60.571224 | 60.28183 | 60.45969 | 0.2574969 |
| Sample 1964 | 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 | 40.59418 | 40.85542 | 40.724651 | 40.72475 | 0.3207387 |
| Rebaudioside A | 62.119178 | 61.672563 | 61.753996 | 61.848579 | 0.3845787 |

H. 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. 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 measured: linearity, selectivity, system suitability, accuracy, repeatability and precision. Criteria for each parameter were 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. Based on the selected criteria for passing linearity, the method is acceptable for analysis and quantitation with-in the range of the method.

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 rebaudioside A in the positive control. 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.

Accuracy is performed to indicate that the primary reference material in use is accurately portrayed by the manufacturer. In this instance two reference materials were compared USP (primary reference) and Cerilliant (confirmatory reference).



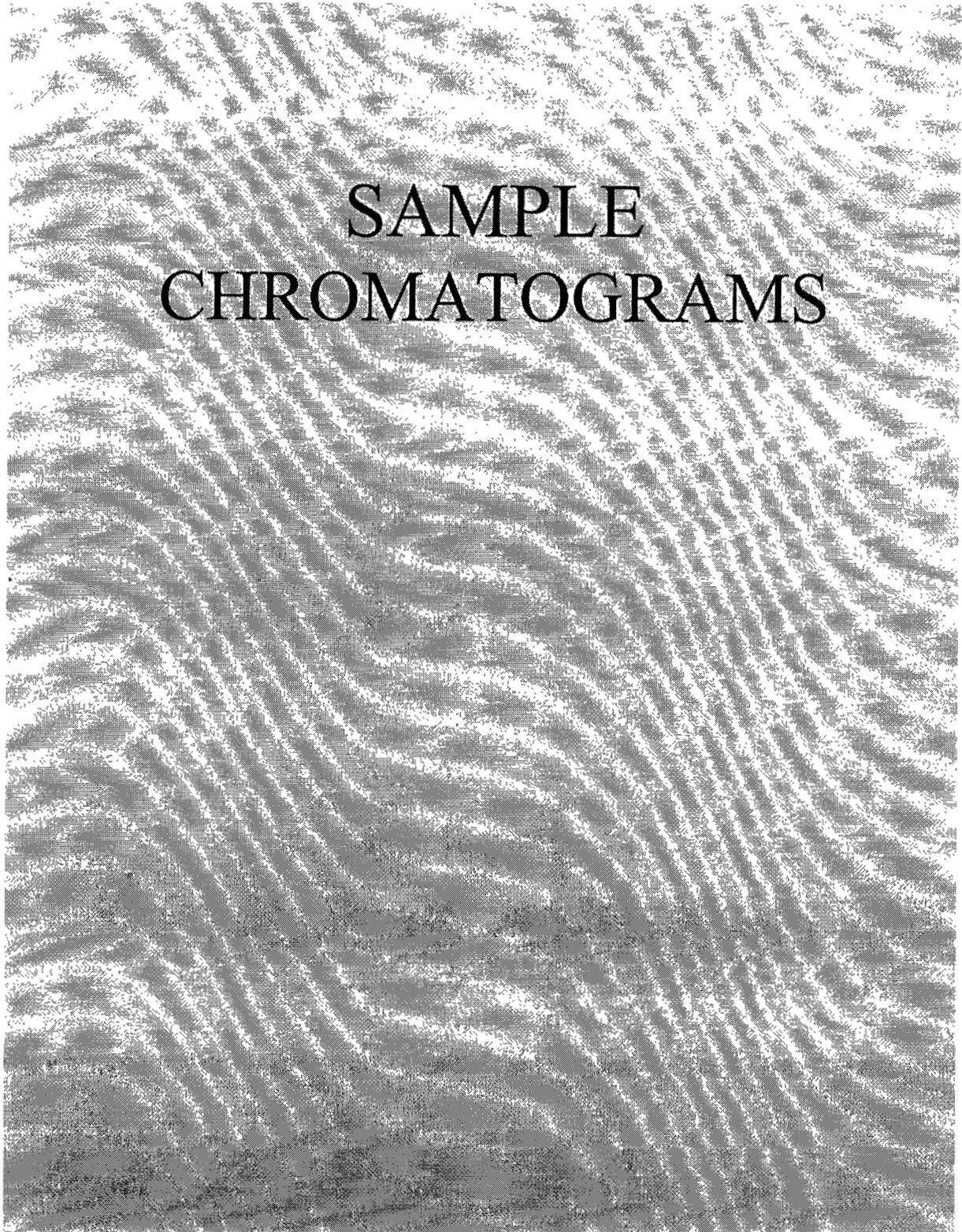
Accuracy is performed to indicate that the primary reference material in use is accurately portrayed by the manufacturer. In this instance two reference materials were compared USP (primary reference) and Cerilliant (confirmatory reference).

Repeatability and precision are 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.

Results of this study further indicate that the KK149 is appropriate and verified for this test material.

000106

Appendix C-4-Part 2

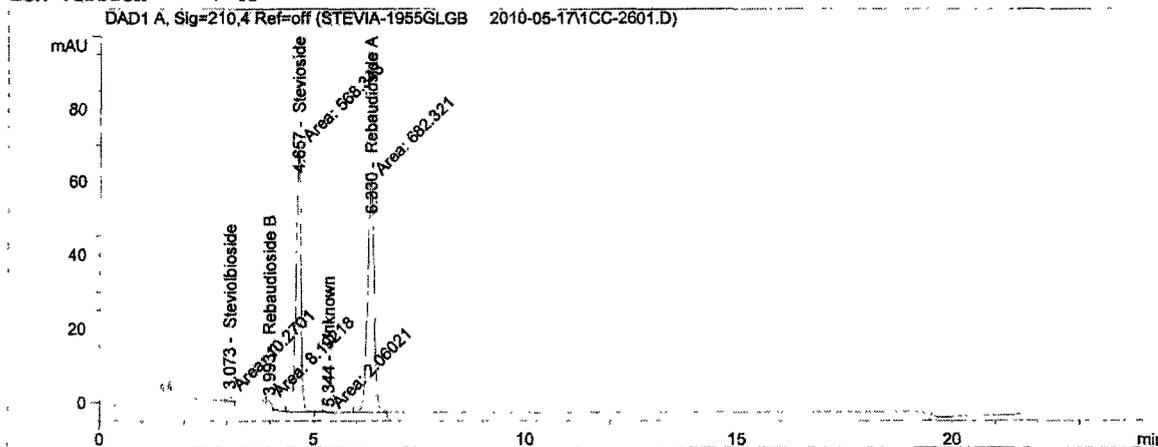


Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CC-2601.D
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Acq. Instrument : HPLC 10                   Location  : P1-C-03
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                                           Inj Volume: 5.0 µl
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Last changed   : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/19/2010 10:32:06 AM by Mariel Esguerra
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : Mariel Esguerra
ECM Path      : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSIZip
ECM Version    : 43
  
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ESTD Percent Report

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Sorted By      : Signal
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Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 1.00475 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
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Signal 1: DAD1 A, Sig=210,4 Ref=off

| RetTime [min] | Type | Area [mAU*s] | Amt/Area | Amount % | Grp | Name |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.073 | MM | 10.27013 | 5.91938e-4 | 0.605054 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.993 | MM | 8.19218 | 7.44407e-4 | 0.606948 | | Rebaudioside B |
| 4.657 | MM | 568.34308 | 7.44407e-4 | 42.107844 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.344 | MM | 2.06021 | 8.96876e-4 | 0.183902 | | Unknown |
| 6.330 | MM | 682.32092 | 8.96815e-4 | 60.902311 | | Rebaudioside A |

Totals : 104.406059

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CC-2601.D
Sample Name: 10-1960A

2 Warnings or Errors :

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

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*** End of Report ***

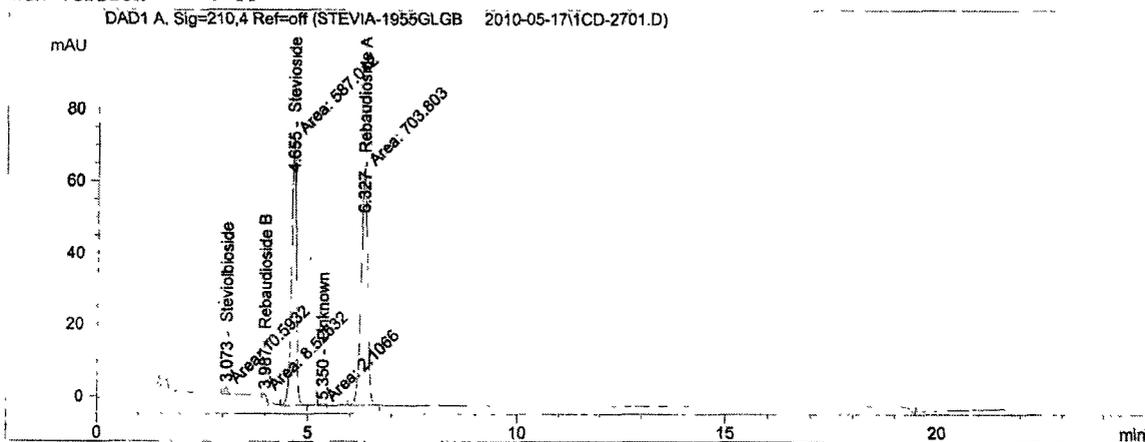
GRAS Assessment – GLG Life Tech Ltd.
 High Purity Steviol Glycosides
 Page 108

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CD-2701.D
 Sample Name: 10-1960B

```

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Acq. Instrument : HPLC 10                   Location  : P1-C-04
Injection Date  : 5/18/2010 2:06:32 PM      Inj       : 1
                                           Inj Volume: 5.0 µl
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Last changed    : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/19/2010 10:32:06 AM by Mariel Esguerra
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSizip
ECM Version     : 44
  
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ESTD Percent Report

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Dilution:      : 1.0000
Sample Amount: : 1.03925 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
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Signal 1: DAD1 A, Sig=210,4 Ref=off

| RetTime [min] | Type | Area [mAU*s] | Amt/Area | Amount % | Grp | Name |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.073 | MM | 10.59321 | 5.91938e-4 | 0.603370 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.987 | MM | 8,52532 | 7.44407e-4 | 0.610663 | | Rebaudioside B |
| 4.655 | MM | 587.04224 | 7.44407e-4 | 42.049396 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.350 | MM | 2.10660 | 8.96876e-4 | 0.181800 | | Unknown |
| 6.327 | MM | 703.80310 | 8.96815e-4 | 60.734330 | | Rebaudioside A |

Totals : 104.179558

HPLC 10 5/19/2010 11:57:36 AM Mariel Esguerra

Page 1 of 2

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CD-2701.D
Sample Name: 10-1960B

2 Warnings or Errors :

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

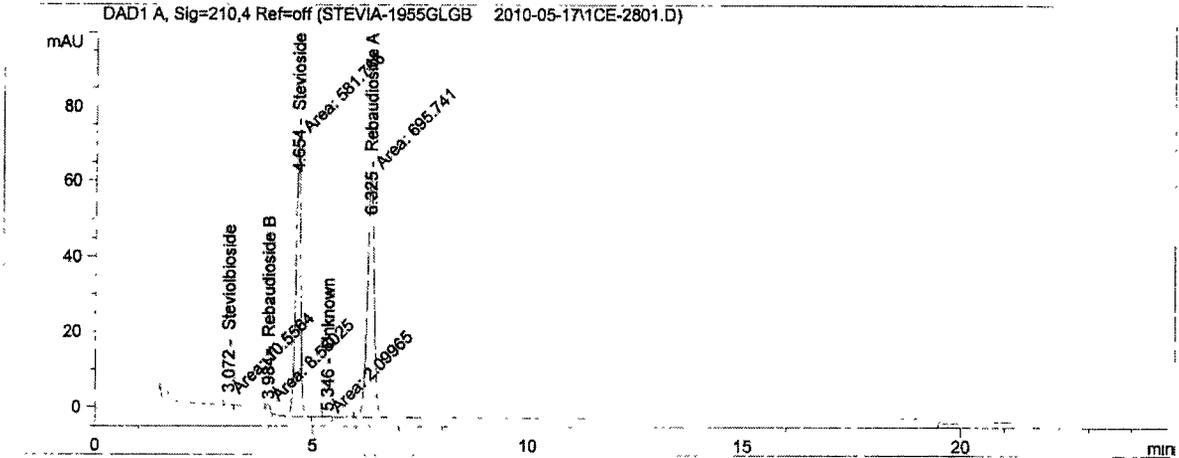
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 Sample Name: 10-1960C

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                                           Inj Volume: 5.0 µl
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Last changed    : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/19/2010 10:32:06 AM by Mariel Esguerra
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSizip
ECM Version     : 44
  
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ESTD Percent Report

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Sorted By           : Signal
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Multiplier          : 1.0000
Dilution            : 1.0000
Sample Amount       : 1.03275 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
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Signal 1: DAD1 A, Sig=210,4 Ref=off

| RetTime [min] | Type | Area [mAU*s] | Amt/Area | Amount % | Grp | Name |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.072 | MM | 10.55636 | 5.91938e-4 | 0.605056 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.984 | MM | 8.58025 | 7.44407e-4 | 0.618465 | | Rebaudioside B |
| 4.654 | MM | 581.76617 | 7.44407e-4 | 41.933751 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.346 | MM | 2.09965 | 8.96876e-4 | 0.182341 | | Unknown |
| 6.325 | MM | 695.74097 | 8.96815e-4 | 60.416487 | | Rebaudioside A |

Totals : 103.756100

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CE-2801.D
Sample Name: 10-1960C

2 Warnings or Errors :

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

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*** End of Report ***

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CF-3001.D
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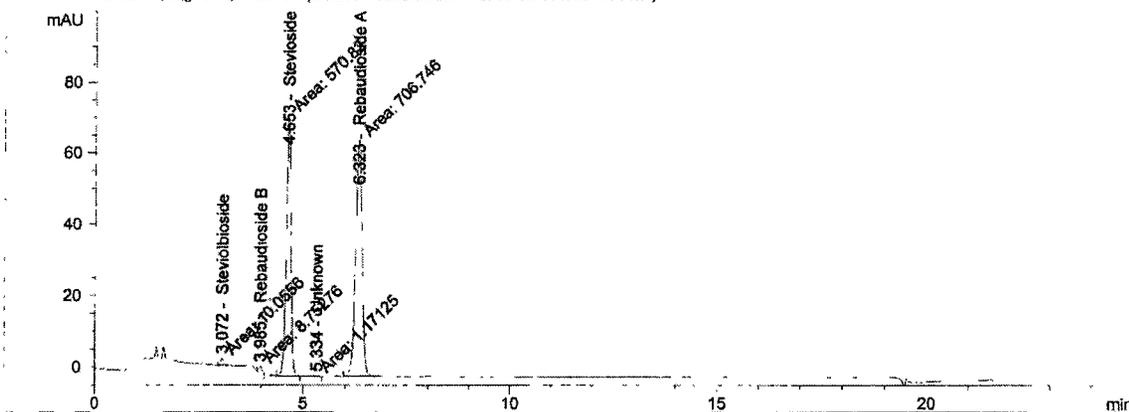
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Injection Date  : 5/18/2010 4:05.40 PM      Inj       : 1
                                           Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/19/2010 10:32:06 AM by Mariel Esguerra
Method Info    : Steviol Glycosides by HPLC (modified JECFA)
  
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ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSIzip
ECM Version     : 38
  
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DAD1 A, Sig=210,4 Ref=off (STEVIA-1955GLGB 2010-05-17\1CF-3001.D)



ESTD Percent Report

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Dilution:      : 1.0000
Sample Amount: : 1.02625 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
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Signal 1: DAD1 A, Sig=210,4 Ref=off

| RetTime [min] | Type | Area [mAU*s] | Amt/Area | Amount % | Grp | Name |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.072 | MM | 10.05563 | 5.91938e-4 | 0.580006 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.985 | MM | 8.75276 | 7.44407e-4 | 0.634896 | | Rebaudioside B |
| 4.653 | MM | 570.82013 | 7.44407e-4 | 41.405359 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.334 | MM | 1.17125 | 8.96876e-4 | 0.102360 | | Unknown |
| 6.323 | FM | 706.74585 | 8.96815e-4 | 61.760840 | | Rebaudioside A |

Totals : 104.483461

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CF-3001.D
Sample Name: 10-1961A

2 Warnings or Errors :

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Warning : Calibrated compound(s) not found

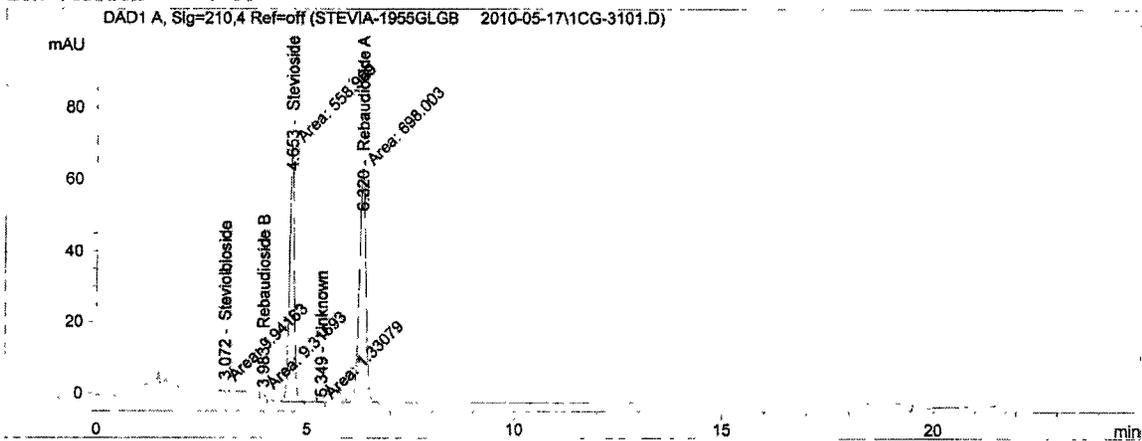
=====
*** End of Report ***

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CG-3101.D
 Sample Name: 10-1961B

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line :   31
Acq. Instrument : HPLC 10                   Location  : P1-C-07
Injection Date  : 5/18/2010 4:45:24 PM      Inj       :    1
                                           Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/19/2010 10:32:06 AM by Mariel Esguerra
Method Info    : Steviol Glycosides by HPLC (modified JECEFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : Mariel Esguerra
ECM Path       : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSIzip
ECM Version    : 44
  
```



ESTD Percent Report

```

Sorted By           : Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier          : 1.0000
Dilution            : 1.0000
Sample Amount       : 1.01375 [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 |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.072 | MM | 9.94163 | 5.91938e-4 | 0.580501 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.983 | MM | 9.31693 | 7.44407e-4 | 0.684152 | | Rebaudioside B |
| 4.653 | MM | 558.90936 | 7.44407e-4 | 41.041287 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.349 | MM | 1.33079 | 8.96876e-4 | 0.117736 | | Unknown |
| 6.320 | MM | 698.00250 | 8.96815e-4 | 61.748898 | | Rebaudioside A |

Totals : 104.172573

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CG-3101.D
Sample Name: 10-1961B

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Warning : Calibrated compound(s) not found

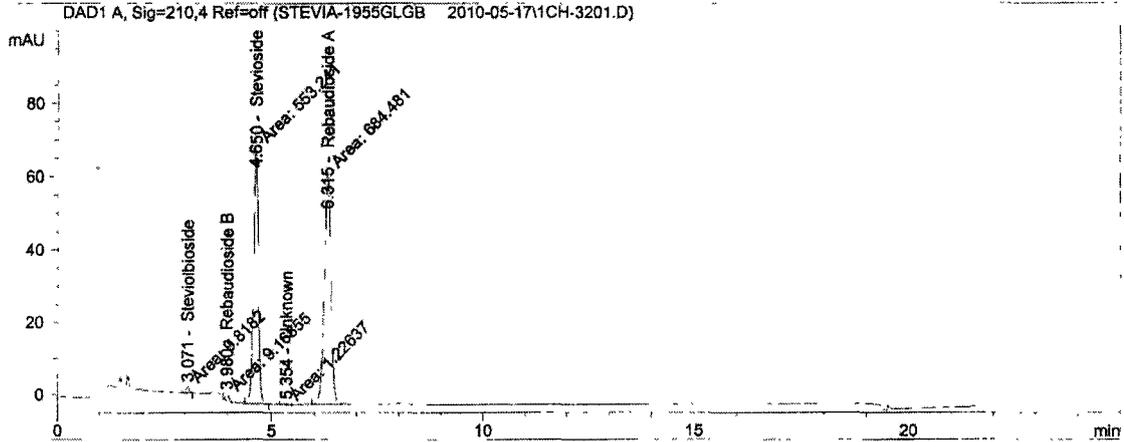
=====
*** End of Report ***

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CH-3201.D
 Sample Name: 10-1961C

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line : 32
Acq. Instrument : HPLC 10                   Location  : P1-C-08
Injection Date  : 5/18/2010 5:25:07 PM      Inj       : 1
                                           Inj Volume: 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/19/2010 12:00:22 PM by Mariel Esguerra
                 (modified after loading)
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSizip
ECM Version     : 45 (modified after loading)
  
```



ESTD Percent Report

```

Sorted By           : Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier          : 1.0000
Dilution            : 1.0000
Sample Amount       : 1.00075 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs
  
```

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

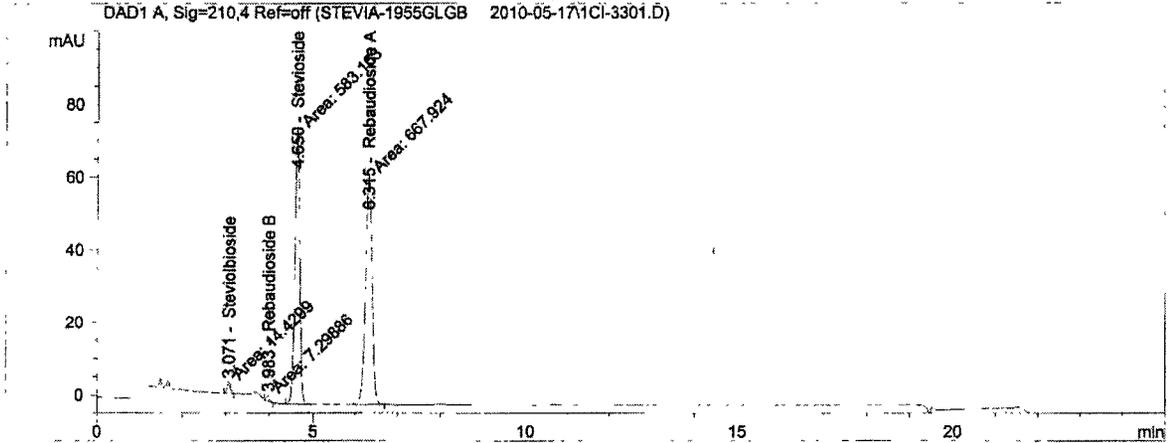
| RetTime [min] | Type | Area [mAU*s] | Amt/Area | Amount % | Grp | Name |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.071 | MM | 9.81820 | 5.91938e-4 | 0.580741 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.980 | MM | 9.16855 | 7.44407e-4 | 0.682002 | | Rebaudioside B |
| 4.650 | MM | 553.24109 | 7.44407e-4 | 41.152789 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.354 | MM | 1.22637 | 8.96876e-4 | 0.109908 | | Unknown |
| 6.315 | MM | 684.48053 | 8.96815e-4 | 61.339269 | | Rebaudioside A |

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1CI-3301.D
 Sample Name: 10-1962A

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line : 33
Acq. Instrument : HPLC 10                   Location  : P1-C-09
Injection Date  : 5/18/2010 6:04:50 PM      Inj       : 1
                                           Inj Volume: 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/19/2010 12:05:10 PM by Mariel Esguerra
                 (modified after loading)
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path       : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSIzip
ECM Version     : 46 (modified after loading)
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 1.00900 [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 |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.071 | MM | 14.42991 | 5.91938e-4 | 0.846543 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.983 | MM | 7.29886 | 7.44407e-4 | 0.538486 | | Rebaudioside B |
| 4.650 | MM | 583.16266 | 7.44407e-4 | 43.023822 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.318 | | - | - | - | | Unknown |
| 6.315 | MM | 667.92419 | 8.96815e-4 | 59.366180 | | Rebaudioside A |

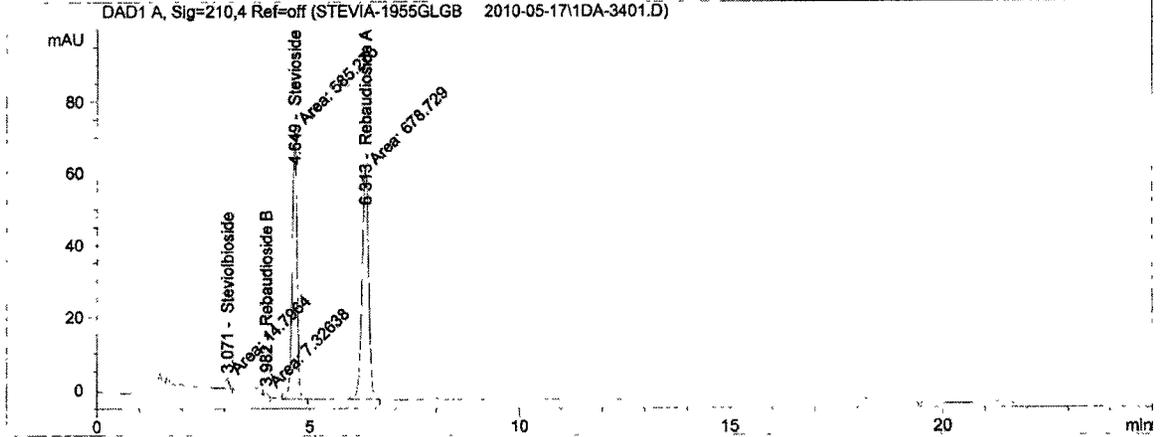
Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DA-3401.D
 Sample Name: 10-1962B

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line :   34
Acq. Instrument : HPLC 10                  Location  : P1-D-01
Injection Date  : 5/18/2010 6:44:32 PM      Inj       :    1
                                           Inj Volume: 5.0 µl

Acq. Method     : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/19/2010 12:06:00 PM by Mariel Esguerra
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSIzip
ECM Version     : 47
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 1.01425 [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 |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.071 | MM | 14.79640 | 5.91938e-4 | 0.863549 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.982 | MM | 7.32638 | 7.44407e-4 | 0.537718 | | Rebaudioside B |
| 4.649 | MM | 585.27527 | 7.44407e-4 | 42.956175 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.318 | | - | - | - | | Unknown |
| 6.313 | MM | 678.72913 | 8.96815e-4 | 60.014276 | | Rebaudioside A |

Totals : 104.371718

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DA-3401.D
Sample Name: 10-1962B

2 Warnings or Errors :

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

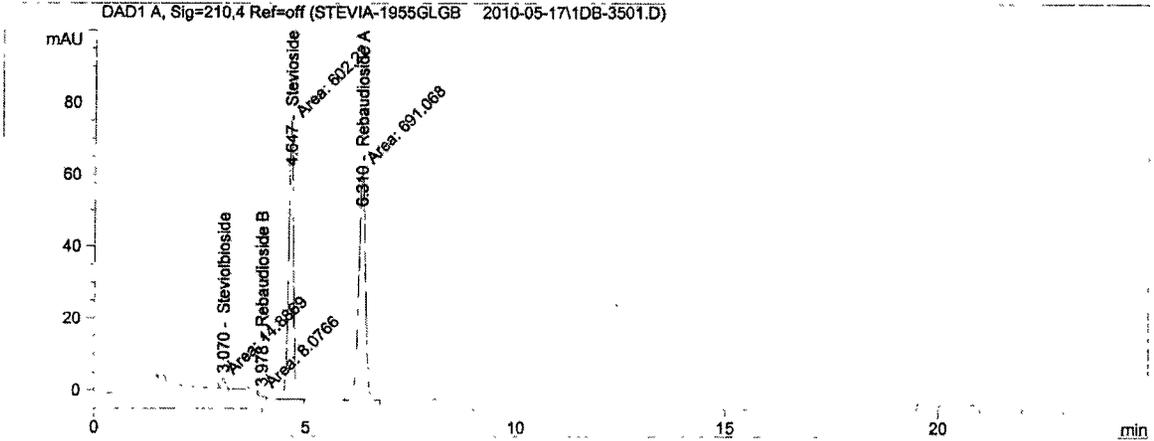
=====
*** End of Report ***

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DB-3501.D
 Sample Name: 10-1962C

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line : 35
Acq. Instrument : HPLC 10                   Location  : P1-D-02
Injection Date  : 5/18/2010 7:24:13 PM      Inj       : 1
                                           Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/19/2010 12:06:00 PM by Mariel Esguerra
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : Mariel Esguerra
ECM Path      : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSIzip
ECM Version    : 48
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 1.04175 [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 |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.070 | MM | 14.88685 | 5.91938e-4 | 0.845893 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.978 | MM | 8.07660 | 7.44407e-4 | 0.577133 | | Rebaudioside B |
| 4.647 | MM | 602.21985 | 7.44407e-4 | 43.033038 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.318 | | - | - | - | | Unknown |
| 6.310 | MM | 691.06763 | 8.96815e-4 | 59.492215 | | Rebaudioside A |

Totals : 103.948279

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DB-3501.D
Sample Name: 10-1962C

2 Warnings or Errors :

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

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

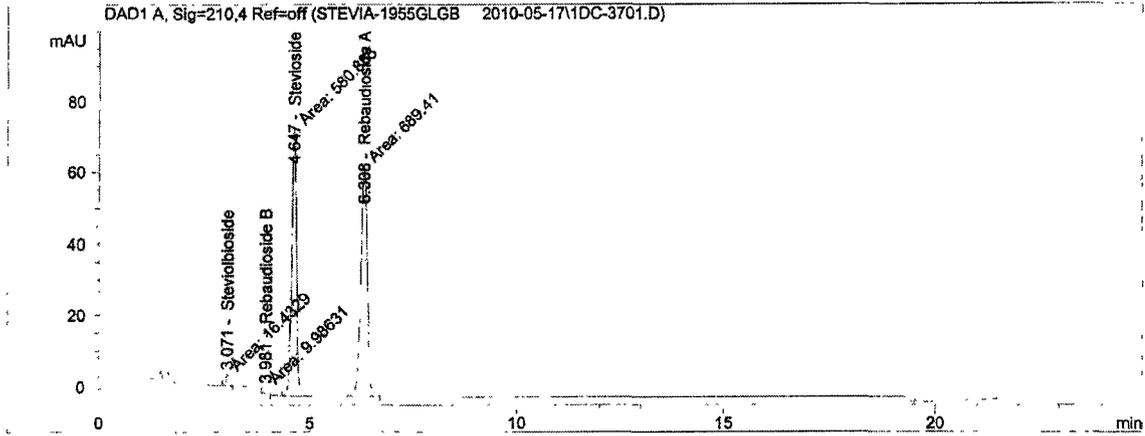
GRAS Assessment – GLG Life Tech Ltd.
 High Purity Steviol Glycosides
 Page 124

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DC-3701.D
 Sample Name: 10-1963A

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line : 37
Acq. Instrument : HPLC 10                  Location  : P1-D-03
Injection Date  : 5/18/2010 8:43:39 PM      Inj       : 1
                                           Inj Volume: 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/19/2010 12:06:00 PM by Mariel Esguerra
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSizip
ECM Version     : 49
  
```



=====
 ESTD Percent Report
 =====

```

Sorted By      : Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier:    : 1.0000
Dilution:     : 1.0000
Sample Amount: : 1.02150 [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 |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.071 | MM | 16.43291 | 5.91938e-4 | 0.952253 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.981 | MM | 9.98631 | 7.44407e-4 | 0.727741 | | Rebaudioside B |
| 4.647 | MM | 580.88452 | 7.44407e-4 | 42.331327 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.318 | | - | - | - | | Unknown |
| 6.308 | MM | 689.40967 | 8.96815e-4 | 60.526017 | | Rebaudioside A |

Totals : 104.537339

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DC-3701.D
Sample Name: 10-1963A

2 Warnings or Errors :

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

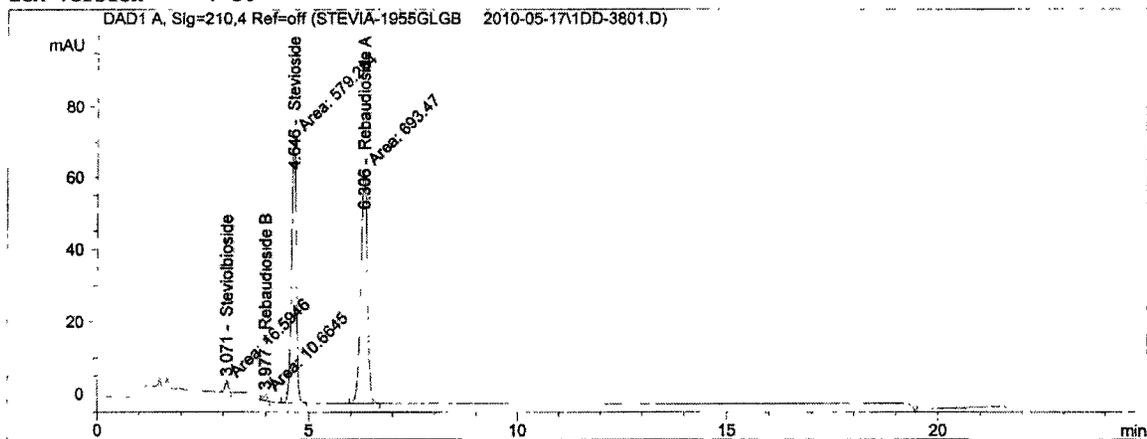
=====
*** End of Report ***

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DD-3801.D
 Sample Name: 10-1963B

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line :   38
Acq. Instrument : HPLC 10                  Location  : P1-D-04
Injection Date  : 5/18/2010 9:23:22 PM     Inj       :    1
                                           Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/19/2010 12:06:00 PM by Mariel Esguerra
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05sqli/cmwg
ECM Operator   : Mariel Esguerra
ECM Path       : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSIzip
ECM Version    : 50
  
```



ESTD Percent Report

```

Sorted By           : Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier          : 1.0000
Dilution            : 1.0000
Sample Amount       : 1.02675 [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 |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.071 | MM | 16.59457 | 5.91938e-4 | 0.956704 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.977 | MM | 10.66451 | 7.44407e-4 | 0.773191 | | Rebaudioside B |
| 4.646 | MM | 579.28430 | 7.44407e-4 | 41.998859 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.318 | | - | - | - | | Unknown |
| 6.306 | MM | 693.47046 | 8.96815e-4 | 60.571224 | | Rebaudioside A |

Totals : 104.299979

HPLC 10 5/19/2010 12:14:34 PM Mariel Esguerra

Page 1 of 2

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DD-3801.D
Sample Name: 10-1963B

2 Warnings or Errors :

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

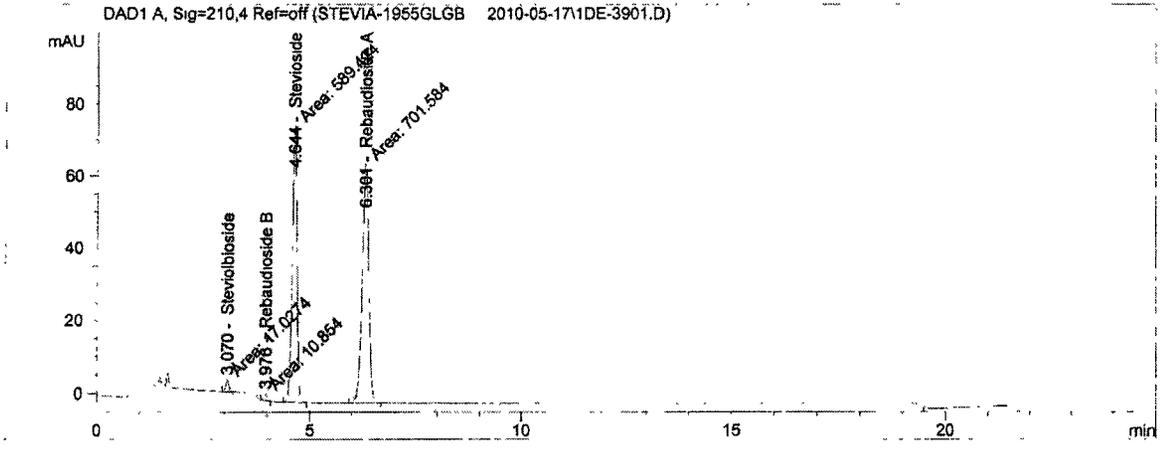
=====
*** End of Report ***

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DE-3901.D
 Sample Name: 10-1963C

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line : 39
Acq. Instrument : HPLC 10                   Location  : P1-D-05
Injection Date  : 5/18/2010 10:03:06 PM    Inj       : 1
                                           Inj Volume: 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/19/2010 12:06:00 PM by Mariel Esguerra
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSIzip
ECM Version     : 51
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier:    : 1.0000
Dilution:      : 1.0000
Sample Amount: : 1.04375 [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 |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.070 | MM | 17.02744 | 5.91938e-4 | 0.965671 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.976 | MM | 10.85398 | 7.44407e-4 | 0.774111 | | Rebaudioside B |
| 4.644 | MM | 589.42389 | 7.44407e-4 | 42.037966 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.318 | | - | - | - | | Unknown |
| 6.301 | MM | 701.58423 | 8.96815e-4 | 60.281830 | | Rebaudioside A |

Totals : 104.059577

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DE-3901.D
Sample Name: 10-1963C

2 Warnings or Errors :

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

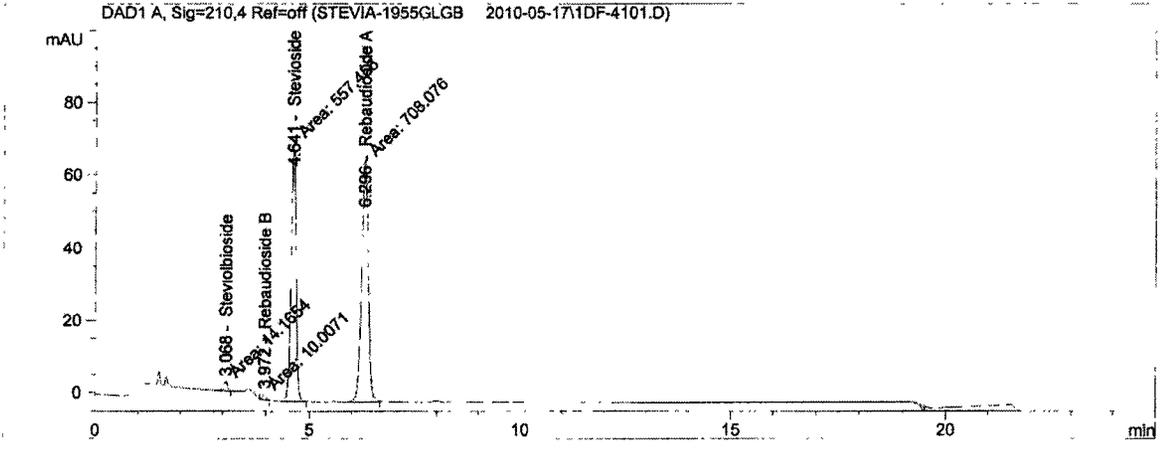
=====
*** End of Report ***

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DF-4101.D
 Sample Name: 10-1964A

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line :   41
Acq. Instrument : HPLC 10                  Location  : P1-D-06
Injection Date  : 5/18/2010 11:22:30 PM    Inj       :    1
                                           Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/19/2010 12:06:00 PM by Mariel Esguerra
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : Mariel Esguerra
ECM Path       : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSIzip
ECM Version    : 52
  
```



=====
 ESTD Percent Report
 =====

```

Sorted By      :      Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier:    :      1.0000
Dilution:      :      1.0000
Sample Amount: :      1.02225 [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 |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.068 | MM | 14.16542 | 5.91938e-4 | 0.820254 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.972 | MM | 10.00710 | 7.44407e-4 | 0.728721 | | Rebaudioside B |
| 4.641 | MM | 557.45581 | 7.44407e-4 | 40.594180 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.318 | | - | - | - | | Unknown |
| 6.296 | MM | 708.07574 | 8.96815e-4 | 62.119178 | | Rebaudioside A |

Totals : 104.262334

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGE 2010-05-17\1DF-4101.D
Sample Name: 10-1964A

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Warning : Calibrated compound(s) not found

=====

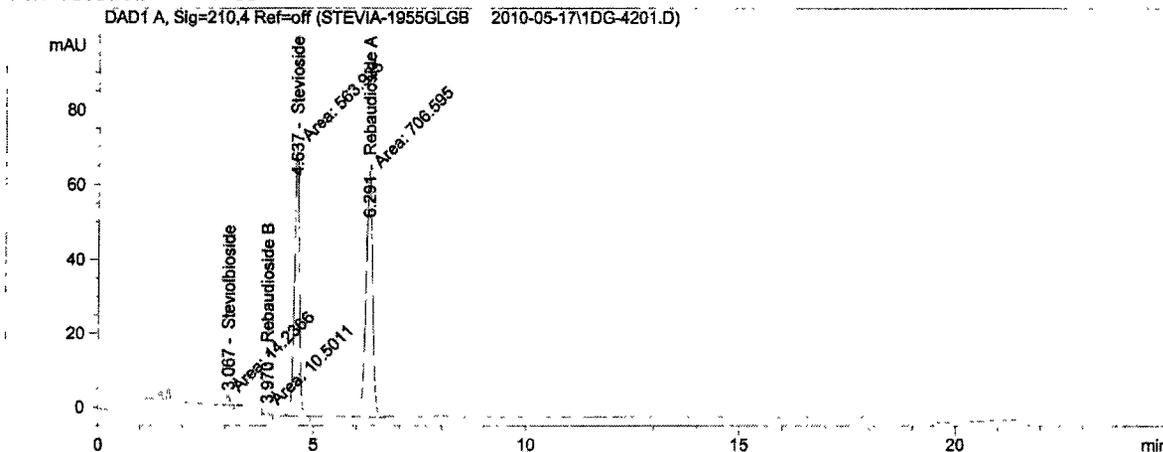
*** End of Report ***

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DG-4201.D
 Sample Name: 10-1964B

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line :   42
Acq. Instrument : HPLC 10                   Location  : P1-D-07
Injection Date  : 5/19/2010 12:02:14 AM     Inj       :    1
                                           Inj Volume: 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed    : 5/19/2010 12:06:00 PM by Mariel Esguerra
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSizip
ECM Version     : 53
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier:    : 1.0000
Dilution:     : 1.0000
Sample Amount: : 1.02750 [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 |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.067 | MM | 14.23660 | 5.91938e-4 | 0.820164 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.970 | MM | 10.50108 | 7.44407e-4 | 0.760786 | | Rebaudioside B |
| 4.637 | MM | 563.92462 | 7.44407e-4 | 40.855420 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.318 | | - | - | - | | Unknown |
| 6.291 | MM | 706.59528 | 8.96815e-4 | 61.672563 | | Rebaudioside A |

Totals : 104.108933

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DG-4201.D
Sample Name: 10-1964B

2 Warnings or Errors :

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

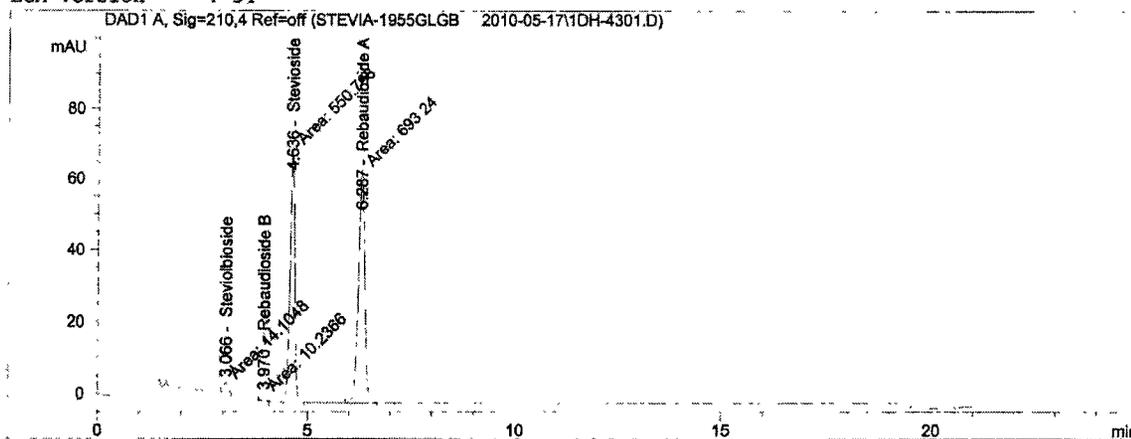
=====
*** End of Report ***

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DH-4301.D
 Sample Name: 10-1964C

```

=====
Acq. Operator   : Mariel Esguerra           Seq. Line : 43
Acq. Instrument : HPLC 10                   Location  : P1-D-08
Injection Date  : 5/19/2010 12:41:57 AM     Inj       : 1
                                           Inj Volume: 5.0 µl
Acq. Method    : C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/17/2010 5:06:53 PM by Mariel Esguerra
Analysis Method: C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\STEVIA.M
Last changed   : 5/19/2010 12:06:00 PM by Mariel Esguerra
Method Info    : Steviol Glycosides by HPLC (modified JECFA)

ECM Server     : http://us05sqlc/ecmwg
ECM Operator   : Mariel Esguerra
ECM Path       : Petaluma\LC\HPLC-10\Data\STEVIA-1955GLGB 2010-05-17.SC.SSIzip
ECM Version    : 54
  
```



ESTD Percent Report

```

Sorted By      : Signal
Calib. Data Modified : 5/19/2010 10:21:24 AM
Multiplier:    : 1.0000
Dilution:     : 1.0000
Sample Amount: : 1.00675 [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 |
|---------------|------|--------------|------------|-----------|-----|----------------|
| 3.066 | MM | 14.10480 | 5.91938e-4 | 0.829319 | | Steviolbioside |
| 3.754 | | - | - | - | | Dulcoside A |
| 3.970 | MM | 10.23660 | 7.44407e-4 | 0.756911 | | Rebaudioside B |
| 4.636 | MM | 550.76782 | 7.44407e-4 | 40.724651 | | Stevioside |
| 5.114 | | - | - | - | | Rebaudioside C |
| 5.318 | | - | - | - | | Unknown |
| 6.287 | MM | 693.23999 | 8.96815e-4 | 61.753996 | | Rebaudioside A |

Totals : 104.064876

Data File C:\CHEM32\10\DATA\STEVIA-1955GLGB 2010-05-17\1DH-4301.D
Sample Name: 10-1964C

2 Warnings or Errors :

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

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

Appendix C-5



Eurofins Scientific Inc., Petaluma
1365 Redwood Way
Petaluma, CA 94954
US

Tel:+1 707 792 7300
Fax:+1 707 792 7309

05/20/2010

James Kempland
GLG Life Tech
Suite 519 World Trade Center
999 Canada Place
Vancouver, BC V6C 3E2
CANADA

CERTIFICATE OF ANALYSIS

AR-10-KK-003108-02

Batch#EUCAPE-00009450

This analytical report supersedes AR-10-KK-003108-01.

Sample Identification:

Sample #: 740-2010-00001960
Description: BlendSure 60, Powder, Lot #GLG-SS60-1003001, Serving = 100g
Condition: Powder in silver heat sealed pouch received at room temperature.
Date Received: 03/18/2010

Method:

QA01B: Pesticides - Luke II Carbamates
QA01A: Pesticides - Luke II Organophosphorus
QA019: Pesticides - Luke II Organonitrogen
QA017: Pesticides - Luke II Organochlorine
QA018: Pesticides - Luke II Pyrethroids
QA256: Ethanol, Residual
QA367: Methanol

Date Completed:

04/09/2010
04/09/2010
04/09/2010
04/09/2010
04/09/2010
04/09/2010
04/09/2010

Results:

Sample #740-2010-00001960

| Test | Result | Units |
|--------------------|--------|-------|
| Aldicarb sulfone | <0.02 | mg/kg |
| Aldicarb sulfoxide | <0.02 | mg/kg |
| Aldicarb | <0.02 | mg/kg |
| Carbaryl | <0.02 | mg/kg |
| Carbofuran | <0.02 | mg/kg |
| Carbofuran 3-OH | <0.02 | mg/kg |
| Methiocarb | <0.02 | mg/kg |
| Methomyl | <0.02 | mg/kg |
| o-Phenylphenol | <0.02 | mg/kg |
| Oxamyl | <0.02 | mg/kg |
| Propoxur | <0.02 | mg/kg |
| Thiodicarb | <0.02 | mg/kg |
| Acephate | <0.02 | mg/kg |
| Azinphos-methyl | <0.02 | mg/kg |
| Bensulide | <0.02 | mg/kg |
| Sulprofos | <0.02 | mg/kg |



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Sample #740-2010-00001960

| Test | Result | Units |
|-----------------------|--------|-------|
| Carbophenothion | <0.02 | mg/kg |
| Chlorfenvinphos | <0.02 | mg/kg |
| Chlorpyrifos | <0.02 | mg/kg |
| Chlorpyrifos-methyl | <0.02 | mg/kg |
| Ciodrin (Crotoxyphos) | <0.02 | mg/kg |
| Courmaphos | <0.02 | mg/kg |
| DEF | <0.02 | mg/kg |
| Demeton group | <0.02 | mg/kg |
| Diazinon | <0.02 | mg/kg |
| Dibrom (Naled) | <0.02 | mg/kg |
| Dicrotophos | <0.02 | mg/kg |
| Dimethoate | <0.02 | mg/kg |
| Disulfoton | <0.02 | mg/kg |
| EPN | <0.02 | mg/kg |
| Ethion | <0.02 | mg/kg |
| Ethoprop | <0.02 | mg/kg |
| Fenamiphos | <0.02 | mg/kg |
| Fenitrothion | <0.02 | mg/kg |
| Fenthion | <0.02 | mg/kg |
| Fonofos | <0.02 | mg/kg |
| Isofenphos | <0.02 | mg/kg |
| Malathion | <0.02 | mg/kg |
| Metasystox-R | <0.02 | mg/kg |
| Methamidophos | <0.02 | mg/kg |
| Methidathion | <0.02 | mg/kg |
| Methyl Parathion | <0.02 | mg/kg |
| Mevinphos | <0.02 | mg/kg |
| Omethoate | <0.02 | mg/kg |
| Parathion | <0.02 | mg/kg |
| Phorate | <0.02 | mg/kg |
| Phosalone | <0.02 | mg/kg |
| Phosmet | <0.02 | mg/kg |
| Phosphamidon | <0.02 | mg/kg |
| Profenofos | <0.02 | mg/kg |
| Propetamphos | <0.02 | mg/kg |
| Ronnel | <0.02 | mg/kg |
| Tetrachlorvinphos | <0.02 | mg/kg |
| Thionazin | <0.02 | mg/kg |
| Acetamiprid | <0.02 | mg/kg |
| Atrazine | <0.02 | mg/kg |
| Azoxystrobin | <0.02 | mg/kg |
| Benthiocarb | <0.02 | mg/kg |
| Cyanazine | <0.02 | mg/kg |
| Cyprodinil | <0.02 | mg/kg |



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Sample #740-2010-00001960

| Test | Result | Units |
|---------------------|--------|-------|
| Cyromazine | <0.02 | mg/kg |
| Dimethomorph | <0.02 | mg/kg |
| Diphenylamine | <0.02 | mg/kg |
| Fenamidone | <0.02 | mg/kg |
| Fludioxonil | <0.02 | mg/kg |
| Hexazinone | <0.02 | mg/kg |
| Imazalil | <0.02 | mg/kg |
| Kresoxim-methyl | <0.02 | mg/kg |
| Metalaxyl | <0.02 | mg/kg |
| Metolachlor | <0.02 | mg/kg |
| Molinate | <0.02 | mg/kg |
| Prometon | <0.02 | mg/kg |
| Prometryn | <0.02 | mg/kg |
| Propamocarb | <0.02 | mg/kg |
| Pymetrozine | <0.02 | mg/kg |
| Pyraclostrobin | <0.02 | mg/kg |
| Pyriproxyfen | <0.02 | mg/kg |
| Simazine | <0.02 | mg/kg |
| Tebuconazole | <0.02 | mg/kg |
| Terbacil | <0.02 | mg/kg |
| Thiabendazole | <0.02 | mg/kg |
| Alachlor | <0.02 | mg/kg |
| Aldrin | <0.01 | mg/kg |
| Benfluralin | <0.02 | mg/kg |
| Bifenox | <0.03 | mg/kg |
| Cyanazine | <0.02 | mg/kg |
| Boscalid | <0.02 | mg/kg |
| Bromacil | <0.02 | mg/kg |
| Captafol | <0.01 | mg/kg |
| Captan | <0.02 | mg/kg |
| Chlorobenzilate | <0.04 | mg/kg |
| Chlordane (total) | <0.01 | mg/kg |
| Chlorfenapyr | <0.01 | mg/kg |
| Chlorothalonil | <0.02 | mg/kg |
| Dacthal (Chlorthal) | <0.02 | mg/kg |
| DDD | <0.01 | mg/kg |
| DDE | <0.01 | mg/kg |
| DDT | <0.01 | mg/kg |
| Dichlobenil | <0.01 | mg/kg |
| Dichlone | <0.05 | mg/kg |
| Dicofol | <0.02 | mg/kg |
| Dieldrin | <0.01 | mg/kg |
| Endosulfan, alpha- | <0.01 | mg/kg |
| Endosulfan beta | <0.01 | mg/kg |



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Sample #740-2010-00001960

| Test | Result | Units |
|--------------------------------|--------|-------|
| Endosulfan-sulfate | <0.01 | mg/kg |
| Endrin | <0.01 | mg/kg |
| Ethalfuralin | <0.01 | mg/kg |
| Fenhexamid | <0.02 | mg/kg |
| Folpet | <0.02 | mg/kg |
| HCB (Hexachlorbenzene) | <0.01 | mg/kg |
| Heptachlor epoxide | <0.01 | mg/kg |
| Heptachlor | <0.01 | mg/kg |
| Indoxacarb | <0.02 | mg/kg |
| Iprodione | <0.02 | mg/kg |
| Lindane (gamma-HCH) | <0.01 | mg/kg |
| Limuron | <0.1 | mg/kg |
| Methoxychlor | <0.02 | mg/kg |
| Metribuzin | <0.01 | mg/kg |
| Mirex | <0.01 | mg/kg |
| Myclobutanil | <0.01 | mg/kg |
| Oxadiazon | <0.02 | mg/kg |
| Oxyfluorfen | <0.01 | mg/kg |
| Pendimethalin | <0.01 | mg/kg |
| Pentachloroaniline | <0.01 | mg/kg |
| Pentachloronitrobenzene (PCNB) | <0.01 | mg/kg |
| Perthane | <0.01 | mg/kg |
| Polychlorinated Biphenyls | <0.01 | mg/kg |
| Procymidone | <0.01 | mg/kg |
| Profluralin | <0.02 | mg/kg |
| Pronamide | <0.02 | mg/kg |
| Propanil | <0.01 | mg/kg |
| Tetradifon | <0.02 | mg/kg |
| Toxaphene (camphechlor) | <0.01 | mg/kg |
| Tridimephon | <0.02 | mg/kg |
| Trifloxystrobin | <0.01 | mg/kg |
| Triflumizole | <0.01 | mg/kg |
| Trifluralin | <0.02 | mg/kg |
| Vegadex | <0.02 | mg/kg |
| Vinclozolin | <0.02 | mg/kg |
| Pyrethrins (sum of 6) | <0.02 | mg/kg |
| Bifenthrin | <0.02 | mg/kg |
| Cyfluthrin | <0.02 | mg/kg |
| Cypermethrin | <0.02 | mg/kg |
| Deltamethrin | <0.02 | mg/kg |
| Esfenvalerate | <0.02 | mg/kg |
| Fenpropathrin | <0.02 | mg/kg |
| Fluvalinate | <0.02 | mg/kg |
| Cyhalothrin lambda | <0.02 | mg/kg |



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CANADA

Sample #740-2010-00001960

| Test | Result | Units |
|------------------------------------|--------|-------|
| Tralomethrin | <0.02 | mg/kg |
| Permethrin | <0.02 | mg/kg |
| Dicloran | <0.01 | mg/kg |
| Fipronil | <0.02 | mg/kg |
| a, β, δ-BHC (Benzene hexachloride) | <0.01 | mg/kg |
| Ethanol, Residual | 30 | mg/kg |
| Methanol | <50 | µg/g |
| Pirimiphos-methyl | <0.02 | mg/kg |

Results relate only to the items tested.
Estimation of uncertainty of measurement is available upon request.

(b) (6)

Dani Ignacio
QC Supervisor Analytical Lab

Page 5 of 5

SUBMISSION END

000143

Belay, Negash

From: Bob McQuate [mcquate@gras-associates.com]
Sent: Friday, February 04, 2011 4:22 PM
To: Belay, Negash
Subject: Clalrification Letters for GRN 348 and GRN 349
Attachments: FDA STV Ltr 02 04 11.doc; FDA SG Blends Ltr 02 04 11.doc

Dear Negash,

As we discussed earlier today, I have confirmed with GLG Life Tech that the specifications and analytical test results in fact do reflect the dry weight basis as you suspected. Consequently, we are providing you with the attached two letters of clarification.

I hope you will return to good health soon.

Best regards,

Bob

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com



20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
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February 4, 2011

Dr. Negash Belay
Food and Drug Administration
Center for Food Safety & Applied Nutrition
Division of Biotechnology and GRAS Notice Review
5100 Paint Branch Parkway
College Park, MD 20740

Re: GRN 349

Dear Dr. Belay:

In response to your telephone inquiry earlier today, I wish to confirm that the specifications for rebaudioside A, stevioside and total steviol glycosides shown in Table 2 of GRN 349 are on a dry weight basis. Correspondingly, the specifications and analytical test results for rebaudioside A, stevioside and total steviol glycosides appearing in the appendices also reflect a dry weight basis.

I trust that this feedback adequately responds to your inquiry.

We look forward to your continued review and feedback on the subject GRAS notification.

Sincerely,

(b) (6)

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
541-678-5522
mcquate@gras-associates.com

Belay, Negash

From: Bob McQuate [mcquate@gras-associates.com]
Sent: Monday, May 02, 2011 2:24 PM
To: Belay, Negash
Cc: 'Brian Meadows'; 'Richard Kraska'
Subject: Responses to Requests for Clarification of Content in GRN 348 & GRN 349
Attachments: FDA Clarification Ltr STV 05 02 11.pdf; FDA Clarification Ltr Blends 05 02 11.pdf

Negash,

Attached you will find our responses to your requests for clarification of selected items as contained in GRNs 348 and 349.

Thank you for your facilitation of the safety reviews.

Sincerely,

Bob
Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com



20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com

May 2, 2011

Negash Belay, Ph.D.
Division of Biotechnology and GRAS Notice Review, HFS-255
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification 349

Dear Dr. Belay:

On April 29, 2011, you called to request clarification of selected items presented in GRAS notification 349 regarding the production of high purity steviol glycosides from stevia leaves. In particular, you requested clarification regarding information found in the first box of the production flow chart in Appendix B-1 on page 75 where the starting material is identified as stevia leaves with a content of not less than 45% stevioside. In addition, you asked about the entry in the first box of the production flow chart in Appendix B-2 on page 76 that identifies the starting material for this portion of the production as stevia leaves with a content of not less than 45% rebaudioside A.

We have referred these questions to GLG Life Tech Corporation, and they in fact have confirmed that the information as presented in GRN 349 is correct.

We hope that this clarification will enable the agency to complete its safety review of GRN 349.

Sincerely,

(b) (6)

Robert S. McQuate, Ph.D.

Cc: Brian Meadows, GLG Life Tech Corp.
Richard C. Kraska, GRAS Associates, LLC, Chair, Expert Panel