ORIGINAL SUBMISSION

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

April 11, 2014

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

Attention: Dr. Paulette Gaynor

Re: GRAS Notification – High Purity Rebaudioside M (≥ 95%)

Dear Dr. Gaynor:

On behalf of GLG Life Tech Corporation of Vancouver, British Columbia, Canada, we are submitting for FDA review Form 3667 and the enclosed CD containing a GRAS notification for High Purity Rebaudioside M (≥ 95%). The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

We also wish to advise you that the CD provided for agency review is free of viruses.

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

Enclosure: GRAS Notification for GLG Life Tech Corporation – High Purity Rebaudioside M (≥ 95%)

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

GRN 000512

April 11, 2014

RECEIVED
APR 14 2014
OFFICE OF
FOOD ADDITIVE SAFETY

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5100 Paint Branch Parkway
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Enclosure: GRAS Notification for GLG Life Tech Corporation – High Purity Rebaudioside M (≥ 95%)

Form Approved: OMB No. 0910-0342; Expiration Date: 02/29/2016
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

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

PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No._____ ☐ Supplement to GRN No. _____

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

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

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

PART II – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position
Brian R. Meadows	President & CFO

Company *(if applicable)*
GLG Life Tech Corporation

Mailing Address *(number and street)*
1050 West Pender Street, Suite 2168

City	State or Province	Zip Code/Postal Code	Country
Vancouver	British Columbia	V6E 3S7	Canada

Telephone Number	Fax Number	E-Mail Address
604-669-2602 (ext 105)	604-662-8858	brian.meadows@glglifetech.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position
Robert S McQuate	CEO

Company *(if applicable)*
GRAS Associates, LLC

Mailing Address *(number and street)*
20482 Jacklight Lane

City	State or Province	Zip Code/Postal Code	Country
Bend	Oregon	97702-3074	United States of America

Telephone Number	Fax Number	E-Mail Address
541-678-5522	541-678-5522 call first	mcquate@gras-associates.com

PART III – GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

High Purity Rebaudioside M (minimum purity 95%)

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

☐ Electronic Submission Gateway

☐ Paper

☒ Electronic files on physical media with paper signature page

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

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

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

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

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

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

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

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

☐ Yes *(Proceed to Item 8)*

☒ No *(Proceed to Part IV)*

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

☐ Yes, see attached Designation of Confidential Information

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

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

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

PART IV – INTENDED USE

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

Intend to use as table top sweetener and general purpose non-nutritive sweetener for incorporation into foods other than infant formulas and meat and poultry products.

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

☐ Yes ☒ No

PART V – IDENTITY

1. Information about the Identity of the Substance

	Name of Substance[1]	Registry Used *(CAS, EC)*	Registry No.[2]	Biological Source *(if applicable)*	Substance Category *(FOR FDA USE ONLY)*
1	High purity Rebaudioside M	CAS	1220616-44-3	Stevia rebaudiana Bertoni	
2					
3					

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

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

2. Description

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

High purity rebaudioside M extracted from the leaves of Stevia rebaudiana Bertoni and subsequently purified to meet the detailed specifications provided on page 20 within Table 2.
Chemical structure provided on page 15 in Figure 2.
Molelcular weight: Rebaudioside M - 1291.29 daltons . See page 14.
Chemical formulas: Rebaudioside M - C56H90O33 as found on page 14.

3. Synonyms

Provide as available or relevant:

1	Rebaudioside M --> 13-[(O-ß-D-Glucopyranosyl-(1-2)-O-[ß-D-glucosylpyranosyl-(1-3)]-ß-D-glucosylpyranosyl)oxy]-kaur-16-en-18-oic acid (4-)-O-ß-D-glucosylpyranosyl-(1-2)-O-[ß-D-glucosylpyranosyl-(1-3)]-ß-D-glycosylpyranosyl ester - from page 14.
2	
3	

Add Continuation Page

PART VI – OTHER ELEMENTS IN YOUR GRAS NOTICE

(check list to help ensure your submission is complete – check all that apply)

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

Other Information

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

☒ Yes ☐ No

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

☒ Yes ☐ No

PART VII – SIGNATURE

1. The undersigned is informing FDA that GLG Life Tech Corporation *(name of notifier)*

has concluded that the intended use*(s)* of High Purity Rebaudioside M (minimum purity 95%) *(name of notified substance)*

described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

2. ☒ GLG Life Tech Corporation *(name of notifier)* agrees to make the data and information that are the basis for the determination of GRAS status available to FDA if FDA asks to see them.

GLG Life Tech Corporation *(name of notifier)* agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so.

1050 West Pender Street, Suite 2168 Vancouver, British Columbia CANADA V6E 3S7
(address of notifier or other location)

GLG Life Tech Corporation *(name of notifier)* agrees to send these data and information to FDA if FDA asks to do so.

OR

☐ The complete record that supports the determination of GRAS status is available to FDA in the submitted notice and in GRP No. ----------
(GRAS Affirmation Petition No.)

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
(b) (6)	Robert S McQuate	04/11/2014

PART VIII – LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Multiple appendices---Appendices A through L---with supporting safety information attached.	

OMB Statement: Public reporting burden for this collection of information is estimated to average 150 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services,Food and Drug Administration, Office of Chief Information Officer, 1350 Piccard Drive, Room 400, Rockville, MD 20850. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

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GRAS ASSESSMENT

of

Rebpure™ RM95

Rebaudioside M

Food Usage Conditions for General Recognition of Safety

for

GLG Life Tech Corporation

Suite 2168-1050 West Pender St.
Vancouver, BC V6E 3S7
Canada

Evaluation by

GRAS Expert Panel
Richard C. Kraska, Ph.D., DABT
Robert S. McQuate, Ph.D.
Robert W. Kapp, Jr., Ph.D., Fellow ATS, ERT (UK)

April 11, 2014

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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 Corporation ("GLG") has determined that its high purity rebaudioside M (≥ 95%) product, referred to as Rebpure™ RM95, and which meets the specifications described below, is Generally Recognized As Safe (GRAS) in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the following sections. The evaluation accurately reflects the intended conditions of food use for the designated stevia-derived sweetener.

Signed:

(b) (6)

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

Date: April 11, 2014

B. Name & Address of Notifier

GLG Life Tech Corporation
1050 West Pender St., Suite 2168
Vancouver, BC V6E 3S7 Canada

As the notifier, GLG Life Tech Corporation accepts responsibility for the GRAS determination that has been made for its high purity rebaudioside M product, as described in the subject notification; consequently, the rebaudioside M preparations having purities no less than 95% rebaudioside M, which meet the conditions described herein, are exempt from premarket approval requirements for food ingredients.

C. Common Name & Identity of Notified Substance

High purity rebaudioside M, abbreviated as Reb M or reb M, is the common name for the notified substance; also see Section III.A.

[1] See 62 FR 18938, 17 April 1997. Accessible at www.gpo.gov/fdsys/pkg/FR-1997-04-17/pdf/97-9706.pdf.

D. Conditions of Intended Use in Food

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

E. Basis for GRAS Determination

Pursuant to 21 CFR 170.30, GLG's high purity rebaudioside M (≥ 95%) preparation, extracted from the leaves of *Stevia rebaudiana* Bertoni, has been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

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

II. INTRODUCTION

A. Objective

At the request of GLG, GRAS Associates, LLC ("GA") has undertaken an independent safety evaluation of GLG's high purity rebaudioside M ("Reb M") product. The Reb M preparation is extracted from the leaves of *Stevia rebaudiana* Bertoni and is purified to yield a rebaudioside M ≥ 95% product with a total steviol glycoside content of ≥ 97%. The purpose of the evaluation is to ascertain whether the intended food uses of rebaudioside M as a general purpose non-nutritive sweetener as described in Section IV.A are generally recognized as safe, i.e., GRAS, under the intended conditions of use.

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; history of use of stevia in food; and compositional details, specifications, and method of preparation of the subject high purity rebaudioside M. 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 results from human fecal

homogenate testing. GLG was also asked to supply past and present human food use information. Knowing how much steviol glycosides---including Reb M---have been safely consumed, i.e., the use levels, is critical in extrapolating to safe exposures for highly purified component 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.

In addition to the product specifications, chemical properties, manufacturing, and safety related information, GLG also provided some consumption/exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through February 26, 2014. A GRAS assessment based primarily on the composite safety information, i.e., based on scientific procedures, was undertaken. Those references that were deemed pertinent to the objective at hand are listed in Section VIII.

C. Summary of Regulatory History of Rebaudioside M, Stevia & Stevia-Derived Sweeteners

Stevia-derived sweeteners are permitted as food additives in South America and in several countries in Asia, including China, Japan, and Korea. In recent years, these sweeteners have received food usage approvals in Mexico, Australia, New Zealand, Switzerland, France, Peru, Uruguay, Colombia, Senegal, Russia, Malaysia, Turkey, Taiwan, Thailand, Israel, Canada, and Hong Kong (EFSA, 2010; NutraIngredients, 2010; Health Canada, 2012). In the US, steviol glycosides have been used as a dietary supplement since 1995 (Geuns, 2003).

Recently, GRN 473 submitted to FDA by PureCircle, regarding purified steviol glycosides with rebaudioside M (Reb X[2]) as the principal component, received a "no questions" letter from FDA. The subject material of GRN 473 is purified to ≥95% steviol glycosides, with a rebaudioside M content of > 50%. Rebaudioside A, rebaudioside B, and rebaudioside D are also present in the material. PureCircle estimated the material to be 200 times sweeter than sucrose, and they calculated the daily exposure to be 1.11 mg/kg body weight/day for adults and 1.22 mg/kg body weight/day for children. On December 2, 2013, FDA stated, "the agency has no questions at this time regarding PureCircle's notice that [purified steviol glycosides with rebaudioside M as the principal component] is GRAS under the intended conditions of use" (PureCircle, 2013a).

Based on available information from FDA's GRAS Notice Inventory[3] website as of April 6, 2014, the agency has issued 31 "no questions" letters on GRAS notices on rebaudioside A, rebaudioside D, rebaudioside M, or steviol glycosides, including those undergoing enzyme treatment. A summary of these filings is presented in Table 1.

The Joint Expert Committee on Food Additives (JECFA) 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

[2] GRN 473 was originally filed as Rebaudioside X. The FDA "no questions" letter clarified the nomencalutre of the subject ingredient as Rebaudioside M.

[3] Accessible at: http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing. FDA's GRAS Inventory website, which was last updated on January 15, 2014, includes GRN 493, submitted by GLG Life Tech Corporation for high purity steviol glycosides, was filed December 23, 2013, and it is presently under review by FDA.

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 more recently completed clinical studies with steviol glycosides (WHO, 2008). In 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009).

Table 1. FDA's GRAS Notice Inventory on Rebaudioside & Steviol Glycosides Preparations[a, b]

COMPANY	FDA GRAS IDENTIFIER	MATERIAL IDENTITY	INTENDED FOOD USES
1. Merisant	GRN 252	High-Purity Reb A ≥95%	Variety of food categories & table top sweetener
2. Cargill Inc.	GRN 253	High-Purity Reb A ≥97%	General-purpose sweetener, excluding meat & poultry products
3. McNeil Nutritionals LLC	GRN 275	Purified Steviol Glycosides – Reb A Principal Component	Table top sweetener
4. Blue California	GRN 278	High-Purity Reb A ≥97%	General-purpose & table top sweetener
5. Sweet Green Fields LLC	GRN 282	High-Purity Reb A ≥97%	General-purpose sweetener, excluding meat & poultry products
6. Wisdom Natural Brands	GRN 287	Purified Steviol Glycosides >95% - Reb A and Stevioside Principal Component	General-purpose sweetener, excluding meat, poultry products & infant formulas
7. Sunwin USA LLC & WILD Flavors	GRN 303	High-Purity Reb A ≥95%/ ≥98%	General-purpose sweetener, excluding meat, poultry products & infant formulas
8. Sunwin USA LLC & WILD Flavors	GRN 304	Purified Steviol Glycosides >95% - Reb A and Stevioside Principal Component	General-purpose sweetener, excluding meat, poultry products & infant formulas
9. Pyure Brands, LLC	GRN 318	High-Purity Reb A 95%/ 98%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
10. PureCircle USA Inc	GRN 323	Purified Steviol Glycosides – Reb A Principal Component	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
11. GLG Life Tech Ltd[c]	GRN 329	High-Purity Reb A ≥97%	General-purpose sweetener, excluding meat & poultry products
12. NOW Foods	GRN 337	Enzyme Modified Steviol Glycosides Preparation (EMSGP)	General-purpose sweetener in foods, excluding meat & poultry products, at levels determined by good manufacturing practices
13. GLG Life Tech Ltd[c]	GRN 348	High-Purity Stevioside ≥95%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
14. GLG Life Tech Ltd[c]	GRN 349	High-Purity Steviol Glycosides ≥97%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
15. Guilin Layn Natural Ingredients, Corp.	GRN 354	High-Purity Reb A ≥97%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
16. BrazTek International Inc.	GRN 365	Purified Reb A	General-purpose sweetener, excluding meat & poultry products

COMPANY	FDA GRAS IDENTIFIER	MATERIAL IDENTITY	INTENDED FOOD USES
17. Sinochem Qingdao Co. Ltd.	GRN 367	High-Purity Steviol Glycosides ≥95%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
18. Shanghai Freemen Americas LLC	GRN 369	Purified Reb A	General-purpose sweetener, excluding meat & poultry products
19. Toyo Sugar Refining Co., Ltd. & Nippon Paper Chemicals Co., Ltd.	GRN 375	Enzyme Modified Steviol Glycosides	General-purpose sweetener in foods, excluding meat and poultry products, at levels determined by good manufacturing practices
20. GLG Life Tech Ltd[c]	GRN 380	Purified Reb A	General purpose & table top sweetener, excluding meat & poultry products
21. Chengdu Wagott Pharmaceutical	GRN 388	Purified Reb A	General purpose & table top sweetener, excluding meat & poultry products
22. Chengdu Wagott Pharmaceutical	GRN 389	Steviol Glycosides with Stevioside as the Principal Component	General purpose & table top sweetener, excluding meat & poultry products
23. Daepyung Co., Ltd.	GRN 393	Purified Reb A	General purpose & table top sweetener, excluding meat & poultry products
24. Daepyung Co., Ltd.	GRN 395	Steviol Glycosides with Reb A and Stevioside as the Principal Components	General purpose & table top sweetener, excluding meat & poultry products
25. MiniStar International, Inc.	GRN 418	Purified Reb A	General-purpose sweetener, excluding meat, poultry products & infant formulas.
26. Daepyung Co., Ltd.	GRN 448	Enzyme Modified Steviol Glycosides	General-purpose sweetener, excluding meat, poultry products & infant formulas.
27. Daepyung Co., Ltd.	GRN 452	Enzyme Modified Steviol Glycosides	General-purpose sweetener, excluding meat, poultry products & infant formulas.
28. PureCircle USA, Inc.	GRN 456	High-Purity Reb D ≥95%	General-purpose sweetener, excluding meat, poultry products & infant formulas.
29. Almendra, Ltd.	GRN 461	High-Purity Reb A ≥97%	General-purpose sweetener, excluding meat, poultry products & infant formulas.
30. Qufu Xiangzhou Stevia Products Co., Ltd.	GRN 467	High-Purity Reb A ≥98%	General-purpose sweetener, excluding meat, poultry products & infant formulas.
31. PureCircle USA, Inc.	GRN 473	Purified Steviol Glycosides – Reb M (Reb X) Principal Component	General-purpose sweetener, excluding meat, poultry products & infant formulas.

[a] This table was derived, in part, from McQuate (2011). [b] GRN 493, addressing high purity steviol glycosides, was submitted by GLG Life Tech Corporation and filed by FDA on December 23, 2013, and is presently under review by FDA. [c] The name of this company is now GLG Life Tech Corporation.

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

Also in 2008, the Food Standards Australia New Zealand (FSANZ) completed its evaluation of an application for use of steviol glycosides in foods. 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). In December 2010, FSANZ recommended accepting the increased usage levels as requested since no public health and safety issues were identified (FSANZ, 2010). Subsequently, FSANZ approved an increase in the maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages, and flavored soy beverages up to 200 mg/kg, and in plain soy beverages up to 100 mg/kg (FSANZ, 2011).

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

On September 18, 2009, based on a review of the international regulation of *Stevia rebaudiana* and the clinical evidence for safety and efficacy, the Natural Health Products Directorate, Health Canada (2009) 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 of 4 mg steviol/kg bw established by JECFA (WHO, 2008).

In light of JECFA's 2008 findings, and in response to a June 2008 request by the European Commission for European Food Safety Authority (EFSA) to deliver a scientific opinion on the safety of steviol glycosides as a sweetener for use in the food categories specified in the dossiers from 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/bw/day, which is similar to JECFA's determination.[4] In addition, on May 25, 2011, EFSA published a determination that the daily dietary intake for use of rebaudioside A as a flavoring substance in a variety of foods would be less than the ADI for steviol glycosides (EFSA, 2011a).

The international community continues to exhibit much interest in the food uses of steviol glycosides, with additional advances reported in early July 2011. The Codex Alimentarius Commission has adopted proposed maximum use levels for steviol glycosides in all major food and beverage categories, and this action is expected to favorably influence authorizations of stevia uses in India, Indonesia, Thailand, and the Philippines (FoodNavigator, 2011). An article published

[4] From a historical perspective, it is noted that the UK's Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food on September 24, 1998 rejected an application for use of steviol glycosides as a sweetener in herbal teas because "the applicant had not provided all of the information necessary to enable an assessment to be made." See http://archive.food.gov.uk/maff/archive/food/novel/980924.htm. In 1999, the Scientific Committee on Food for the European Commission concluded that "there are no satisfactory data to support the safe use of these stevia plants and leaves" (European Commission, 1999a). In another opinion also dated June 17, 1999, the Committee reiterated "its earlier opinion that stevioside is not acceptable as a sweetener on the presently available data" (European Commission, 1999b).

online by FoodNavigator (2013) states the following: "with approvals now in Vietnam, the Philippines, Malaysia, Singapore and Thailand, Indonesia is the only [Southeast Asian nation] where stevia hasn't been given the rubber stamp." Furthermore, the International Alliance of Dietary/Food Supplement Associations (IADSA) reported that the Codex Alimentarius Commission agreed to adopt the use of steviol glycosides for addition to chewable food supplements as had been requested by IADSA (NewHope360, 2011).

The appropriate European regulatory bodies, including the joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA), have now agreed that steviol glycosides are safe for all populations to consume and are a suitable sweetening option for diabetics. Effective December 2, 2011, the EU approved their use as food additives (EU, 2011).

On September 10, 2012, the South African Department of Health issued an amendment to labeling regulations indicating: "in the case of the sweetener steviol glycosides, it shall be described as 'Steviol Glycosides' or 'Steviol Extract.'" On the same date, steviol glycosides were added to the List of Permissible Sweeteners (Republic of South Africa Department of Health, 2012a, b).

The Food Safety and Standards Authority of India (FSSAI) convened on September 20, 2012, and approved the use of steviol glycosides as a non-nutritive sweetener in a variety of foods. The FSSAI specified that: the steviol glycosides must meet the specifications and purity as established by JECFA; table top sweetener tablets may contain 7 mg of steviol equivalents per 100 mg carrier/filler, as well as established maximum use levels specific to 11 distinct food categories including dairy, beverage, and chewing gum applications (FSSAI, 2012)

On November 30, 2012, Health Canada published its final clearance for use of steviol glycosides as a sweetener in foods (Health Canada, 2012).

Since Februrary 10, 2014, multiple food registrations have been granted by FDA Philippines to stand-alone steviol glycosides sweeteners or foods containing steviol glycosides as ingredients (Republic of the Philippines, Food and Drug Administration, 2014).

Finally, steviol glycosides are listed under INS number 960 in the Food Additives Permitted Under the Singapore Food Regulations document prepared by the Agri-Food & Veterinary Authority (AVA) of Singapore, and this information can be accessed on their website as of September 24, 2013 (AVA, 2013).

D. FDA Regulatory Framework

In order to be incorporated into conventional foods, food ingredients 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]

[5] See 21 CFR 170.3(i)(3).

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

III. CHEMISTRY & MANUFACTURE OF REBAUDIOSIDE M

A. Common or Usual Name

High purity rebaudioside M is the common or usual name of the non-nutritive sweetener derived from *Stevia rebaudiana* Bertoni that is the subject of the GRAS evaluation. Rebaudioside M is synonymous with rebaudioside X (Reb X). The compositional features of the subject high purity steviol glycosides (≥ 97%), primarily containing rebaudioside M ≥ 95%, are described in more detail in this section. Rebpure™ RM95 is the term used by GLG in referring to the notified substance. In the scientific literature, steviol glycosides have been referred to as stevia, stevioside, steviol glycosides, and stevia glycoside. JECFA adopted the term, steviol glycosides, for the family of steviol derivatives with sweetness properties that are derived from the stevia plant. Presently, the term, stevia, is used more narrowly to describe the plant or crude extracts of the plant, while Reb M –like stevioside–is the common name for another one of the specific glycosides that is extracted from stevia leaves.

B. Chemistry of Steviol Glycosides

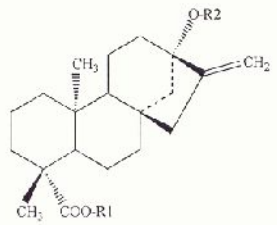
At its 51st meeting, JECFA reviewed the safety related information on steviol glycosides, including the identity and chemistry of these compounds. The following chemistry related description of steviol glycosides is taken from the original JECFA monograph (WHO, 2000).

> 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).

[6] See 62 FR 18938 (17 April 1997) which is accessible at www.gpo.gov/fdsys/pkg/FR-1997-04-17/pdf/97-9706.pdf.

In the 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.

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) | β-Glc(3→1)
5	Rebaudioside B	58543-17-2	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
6	Rebaudioside C (dulcoside B)	63550-99-2	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
7	Rebaudioside D	63279-13-0	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
8	Rebaudioside E	63279-14-1	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
9	Rebaudioside F	438045-89-7	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
10	Rubusoside	63849-39-4	β-Glc	β-Glc
11	dulcoside A	64432-06-0	β-Glc	β-Glc-α-Rha(2→1)

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

In a number of reviews by different authors (Kinghorn and Soejarto, 1989; Kinghorn, 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 Lavielle (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 Reb B, were obtained from methanol extracts of stevia leaves, along with the major sweet principle constituent, stevioside, and a minor constituent steviolbioside, 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). In addition, 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, Reb D, and Reb E. It was reported that Reb A and Reb 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). Later, dulcoside B and Reb C were shown to be structurally identical.

More recently, Chaturvedula et al. reported isolating the low-level steviol glycoside, rebaudioside M, from commercially available *Stevia rebaudiana* Bertoni extracts (Chaturvedula et al., 2013).

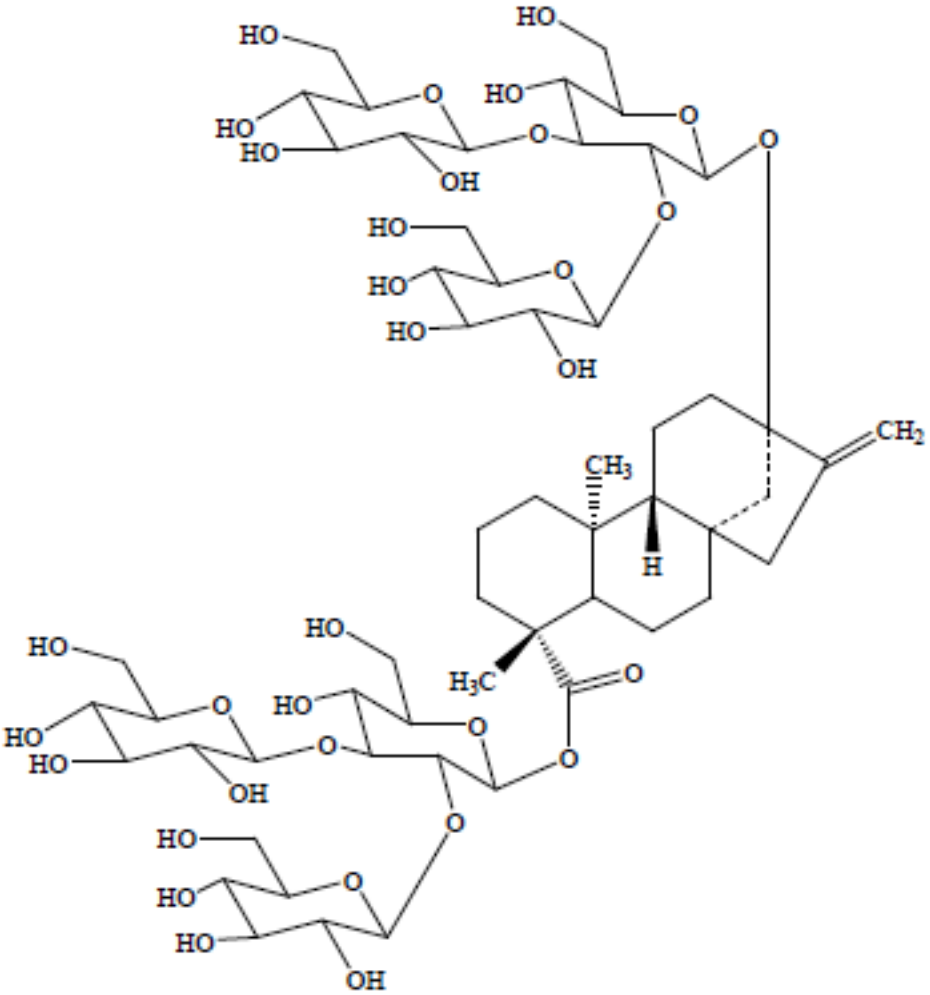
1. Chemistry of Rebaudioside M

Rebaudioside M is a minor naturally occurring steviol glycoside obtained from the leaves of *Stevia rebaudiana* Bertoni, which is reported to be 160-500 times sweeter than sugar. Similar to the other steviol glycosides, Reb M is an *ent*-kaurane diterpene glycoside with a steviol backbone. Unlike the other steviol glycosides, Reb M has two 2-O-ß-D-glucopyranosyl-3-O-ß-D-glucopyranosyl-ß-D-glucopyranosyl units, an ether at position C-13 and an ester at position C-19 (Chaturvedula et al., 2013; Prakash et al., 2014).

Chemical name:	13-[(O-ß-D-Glucopyranosyl-(1-2)-O-[ß-D-glucosylpyranosyl-(1-3)]-ß-D-glucosylpyranosyl)oxy]-kaur-16-en-18-oic acid (4-)-O-ß-D-glucosylpyranosyl-(1-2)-O-[ß-D-glucosylpyranosyl-(1-3)]-ß-D-glycosylpyranosyl ester.
Synonyms:	Rebaudioside M, Reb M, Rebaudioside X, Reb X
Chemical formula:	$C_{56}H_{90}O_{33}$
Molecular weight:	1291.29 daltons
CAS Number:	1220616-44-3

The chemical structure of rebaudioside M is presented in Figure 2.

Figure 2. Chemical Structure of Rebaudioside M[a]



[a] From Chaturvedula et al., 2013.

C. Accepted Identity Specifications for Food Grade Steviol Glycosides

In addition to the manufacturing process, the compositions of *Stevia rebaudiana* Bertoni extracts depend upon the composition of the harvested leaves, which, in turn, is influenced by soil, climate, etc. (FAO, 2007b). As discussed in Section III.E.1., 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), 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 Reb D and Reb F, the other five glycosides are found at substantially lower levels in the preparations of steviol glycosides---and recognized by JECFA---are Reb C, dulcoside A, rubusoside, steviolbioside, and Reb B. Recently, there has been an increased interest in other low-level steviol glycosides, including Reb M.

D. Manufacturing Processes

Manufacturing processes for stevia-derived sweeteners have been described in the published scientific and patent literature. These processes are summarized below, along with GLG's manufacturing process for high purity RM95, which is also specifically discussed in Section III.D.2.

1. Scientific & Patent Literature

In general, steviol glycosides are typically obtained by extracting leaves of *Stevia rebaudiana* Bertoni with hot water or alcohols (ethanol or methanol). This extract is a dark particulate solution containing all the active principles, plus leaf pigments, soluble polysaccharides, and other impurities. Some processes remove the "grease" from the leaves before extraction by employing solvents such as chloroform or hexane (Kinghorn, 2002). 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 CO_2, and extract clarification with zeolite have been employed.

At the 68th JECFA meeting, steviol glycosides were defined as the products obtained from the leaves of *Stevia rebaudiana* Bertoni. As described by JECFA, the typical manufacturing process 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 then washed with methanol to release the steviol glycosides, and the product is recrystallized with methanol. Ion-exchange resins may be used in the purification process. The final product is commonly spray-dried.

2. GLG's Manufacturing Process for Purified Rebaudioside M

The source of GLG's high purity rebaudioside M preparation is the leaves of the *Stevia rebadiana* (Bertoni) plant. The manufacturing process employed by GLG is fairly typical and similar to the process used in the industry for the production of stevia-derived sweeteners that are prepared in accordance with current Good Manufacturing Practices regulations (cGMP). The ethanol and/or methanol used in the purification process comply with FCC's 8 Edition specifications for these solvents. The ion exchange resins used in the manufacturing process comply with 21 CFR 173.65. Specifications and certificates of analysis are provided in Appendix A.

GLG has developed a state-of-the-art process for extracting steviol glycosides from leaves of select varieties of stevia plants. In brief, steviol glycosides are obtained by water extraction. Ferric chloride and calcium hydroxide are added to the extract solution to facilitate precipitation. The extraction solution is passed through plate filtration, and then two columns packed with anion and cation ion-exchange resins, followed by passage through several columns packed with macropore adsorption resins. The steviol glycosides are separately eluted with ethanol. The adsorbed solutions are decolored with active carbon and concentrated with film evaporators. The concentrated extract is decolored with active carbon and filtered. The resulting concentrates are spray dried separately to obtain the primary stevia extracts. Extracts from the last columns have enriched rebaudioside M content.

The enriched rebaudioside M stevia extracts undergo additional purification steps to obtain the high purity rebaudioside M. The stevia extract is dissolved in ethanol and/or methanol, crystallized, and filtered. The crystallization and drying process is repeated one or several more times using ethanol and/or methanol to obtain high purity Reb M, yielding total steviol glycosides content ≥97% and Reb M ≥95%. The rebaudioside M crystals are separated by plate filtration and spray dried to obtain the dry powder product. The manufacturing process is summarized in a flow chart provided as Figure 3.

The resulting high purity steviol glycosides powder is then tested for specification compliance. The analytical testing methods, representative HPLC chromatograms, and certificates of analysis of five representative lots of the high purity RM95 preparation are detailed in Appendix B, Appendix C, and Appendix D, respectively. Results from pesticide analyses of representative high purity RM95 samples are provided in Appendix E. The content of rebaudioside M in the final high purity RM95 product in all cases is ≥ 95%.

E. Product Specifications & Supporting Methods

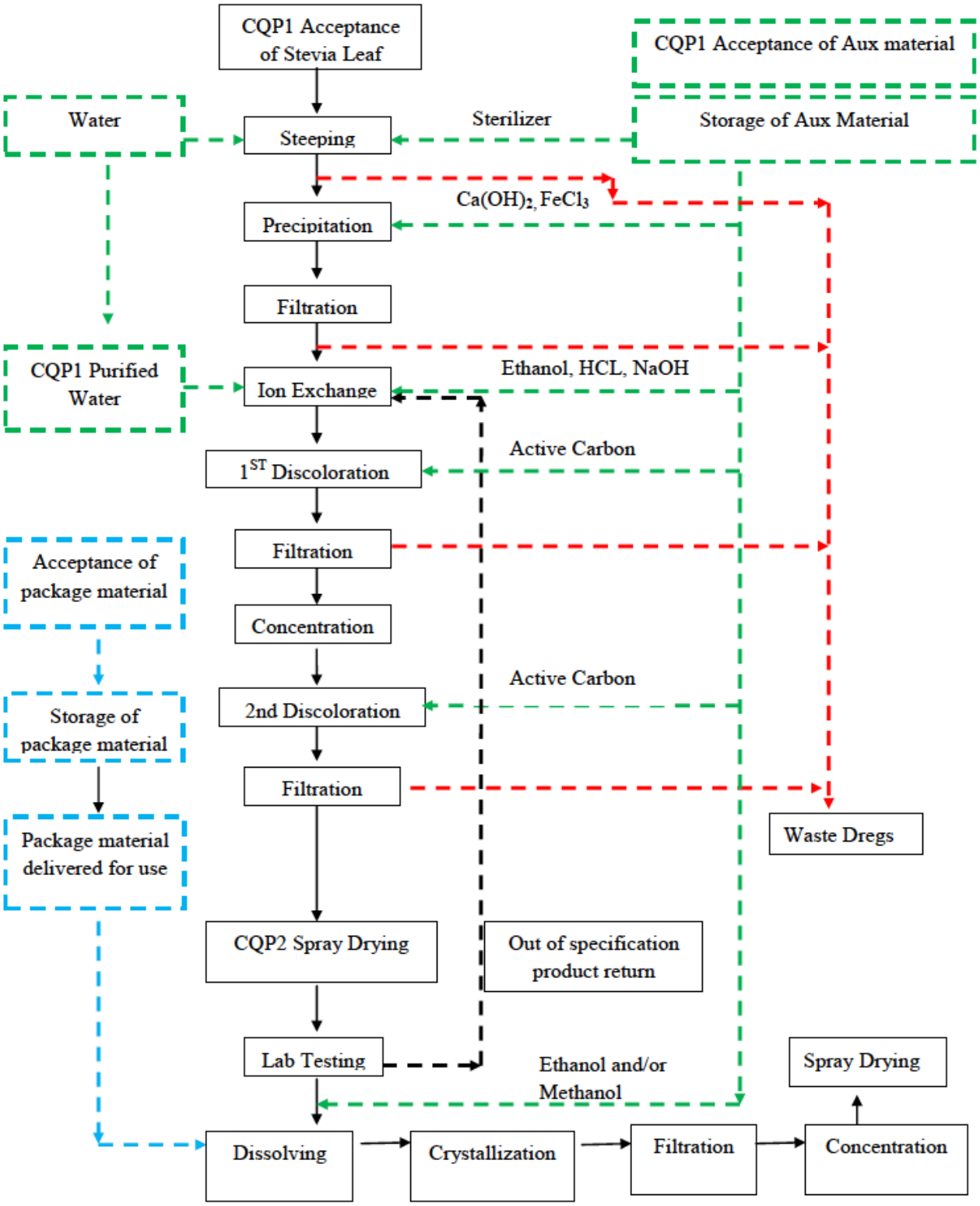
1. JECFA Specifications for Steviol Glycosides

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

As reported in Section II.C, JECFA has been intimately involved over the past several years in the safety considerations of the steviol glycosides, and their deliberations have explicitly addressed requisite specifications for total steviol glycosides and component steviol glycosides. In summary, JECFA requires a minimum steviol glycosides composition of no less than 95% based on stevioside, Reb A, Reb C. dulcoside A, rubusoside, steviolbioside, Reb B, Reb D and Reb F.

Furthermore, steviol glycosides are described as a white to yellow powder, odorless to having a slight characteristic odor, and exhibiting a sweetness that is 200-300 times greater than sucrose. The ingredient must consist of a minimum of 95% of nine specific steviol glycosides. The steviol glycosides are freely soluble in water and ethanol, and the 1 in 100 solutions exhibit pH values between 4.5 and 7.0. The product should not have more than 1% ash, with no more than a 6% loss on drying at 105°C for 2 hours. Any residual methanol levels should not exceed 200 ppm, and ethanol residues should not exceed 5,000 ppm. Arsenic levels should not exceed 1 ppm as determined by the atomic absorption hydride technique. Lead levels should not exceed 1 ppm.

Figure 3. GLG Production Process for High Purity Rebaudioside M



2. Specifications for GLG's High Purity Rebaudioside M With Supporting Methods

No established regulatory specifications were identified for food grade rebaudioside M. However, in GRN 473, PureCircle based their specifications for their rebaudioside M product on those determined by JECFA and the FCC. The specifications established by PureCircle are detailed in Table 2. A "no questions" letter was issued by FDA (PureCircle, 2013a).

GLG has adopted similar product specifications for its high purity rebaudioside M preparation that meet or exceed JECFA recommendations (FAO, 2010), while also complying with Food Chemicals Codex (FCC, 2010) specifications for rebaudioside A as a consumable human food substance. Five product batches of Rebpure™ RM95 provided by GLG are compared to the specifications provided by JECFA and FCC specifications in Table 2. Results of analyses performed by GLG demonstrate that the five production batches of Reb M ≥95% meet the designated specifications.

Table 2. Specifications for GLG Rebaudioside M Preparations

Physical & Chemical Para-meters	JECFA[a] Specifica-tions Steviol Glycosides	FCC[b] Specifications Rebaudioside A	PureCircle[c] Specifications Rebaudioside M	GLG Specifications Rebaudioside M (RM95)	Results of Batch Numbers				
					RM95-20131002	RM95-20131008	RM95-20131012	RM95-20131016	RM95-20131020
Appearance Form	Powder	Crystal, granule or powder	Powder	Powder	NS	NS	NS	NS	NS
Appearance Color	White to light Yellow	White to off-white	White to off-white	White to off-white	NS	NS	NS	NS	NS
Solubility[d]	Freely soluble in water	Freely soluble in water:ethanol (50:50)	Sparingly Soluble	Sparingly Soluble	NS	NS	NS	NS	NS
Purity (HPLC Area) %	NS	≥ 95	≥ 50% (Reb M)	≥ 95% (Reb M)	96.3%	96.4%	96.2%	96.4%	96.3%
Residual Ethanol	NMT 5000 mg/kg	NMT 0.5%	<0.3%	≤ 5,000 ppm	360 ppm	289 ppm	465 ppm	392 ppm	421 ppm
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	<0.02%	≤ 200 ppm	78 ppm	56 ppm	82 ppm	59 ppm	71 ppm
Loss on Drying (%)	NMT 6.0%	NMT 6.0%	≤ 6 %	< 4.0%	2.8%	3.2%	3.3%	2.9%	2.8%
pH, 1% Solution	4.5-7.0	4.5-7.0	4.5-7.0	4.5-7.0	5.18	5.11	5.14	5.10	5.15
Total Ash (%)	NMT 1%	NMT 1%	<1 %	< 1.0%	0.08%	0.06%	0.09%	0.06%	0.08%
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	<1 ppm	< 1.0 ppm	0.05 ppm	0.03 ppm	0.01 ppm	0.03 ppm	0.01 ppm
Lead	NMT 1 mg/kg	NMT 1 mg/kg	<1 ppm	< 1.0 ppm	0.01 ppm	0.02 ppm	0.02 ppm	0.01 ppm	0.01 ppm
Mercury (ppm)	NS	NS	<1 ppm	< 1.0 ppm	0.02 ppm	0.01 ppm	0.01 ppm	0.03 ppm	0.01 ppm
Cadmium (ppm)	NS	NS	<1 ppm	< 1.0 ppm	0.01 ppm	0.02 ppm	0.01 ppm	0.02 ppm	0.02 ppm
Total Plate Count (cfu/g, max)	NA	NA	<1,000	< 1,000	< 10	< 10	< 10	< 10	< 10
Yeast & Mold (cfu/g, max)	NA	NA	ND	< 100	< 10	< 10	< 10	< 10	< 10
Salmonella spp	NA	NA	Absent in 25 g	Negative in 25 g	Negative	Negative	Negative	Negative	Negative
Staphyl-ococus aureus	NA	NA	ND	Negative	Negative	Negative	Negative	Negative	Negative
***E. coli* (mpn/g)**	NA	NA	ND	Negative	Negative	Negative	Negative	Negative	Negative

[a] Prepared at 73rd JECFA, 2010.
[b] FCC, 2010. Rebaudioside A monograph. Food Chemicals Codex (7th Ed.).
[c] Specifications detailed in GRN 473 submitted by PureCircle (2013a).
NS = not specified; NA = not applicable; NLT = not less than; NMT = not more than; ND = not detected

Details of the analytical methodology employed to determine steviol glycosides are provided in Appendix B, the chromatograms for representative RM95 preparations are provided in Appendix C, and certificates of analysis for five representative lots of RM95 are provided in Appendix D. Pesticide residue screening is periodically conducted on various product lots. Test reports for analysis of pesticide residues in representative lots are located in Appendix E. The collection of these reports demonstrates that the substance is well characterized and meets the established purity criteria.

F. Stability Documentation

1. Stability Data on Steviol Glycosides

Based on its chemical structure compared with other closely related steviol glycosides, rebaudioside M is expected to exhibit comparable chemical stability to other steviol glycosides.

Steviol glycosides have been reported to be stable over the pH range 3-9 and can be heated at 100°C for 1 hour, but, at pH levels greater than 9, they rapidly decompose (Kinghorn, 2002). At pH 10, steviolbioside would be the major decomposition product produced from stevioside by alkaline hydrolysis (Wood et al., 1955). Chang and Cook (1983) investigated the stability of pure stevioside and Reb 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 Reb A, was noted. Exposure to one week of sunlight did not affect stevioside but did result in approximately 20% loss of rebaudioside A. Heating at 60°C for 6 days resulted in 0-6% loss of rebaudioside A.

Merisant (2008) conducted stability testing on rebaudioside A (1) as a powder, (2) as a pure sweetener in solution, and (3) on both cola-type and citrus carbonated beverages. In these investigations, no degradation was detected when the powder was stored at 105˚C for 96 hours. It was concluded that the powder was stable when stored for 26 weeks at 40±2˚C with relative humidity of 75±5%. Both published and unpublished testing results from Merisant revealed that rebaudioside A in carbonated citric acid beverages and phosphoric acid beverages did not significantly degrade during prolonged storage at refrigeration, normal ambient, or elevated ambient temperatures. Minimal loss of rebaudioside A was detected after storage at 60˚C, with considerable degradation noted after 13 hours at 100˚C for carbonated beverage solutions and pure sweetener solutions (Merisant, 2008).

Cargill (2008) also conducted extensive stability testing on rebaudioside A as a powder under various storage conditions and under a range of pHs and temperatures. Additionally, Cargill also investigated rebaudioside A stability in several representative food matrices at room temperature and elevated temperatures. Stability profiles were created for table top sweetener applications, mock beverages including cola, root beer and lemon-lime, thermally processed beverages, yogurt, and white cake. The results of stability testing revealed some degradation products that had not been detected in bulk rebaudioside A. These degradation products were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana* Bertoni. All the degradation products were found to share the same steviol aglycone backbone structure as found in stevioside and rebaudioside A, but they differ by virtue of the glucose moieties present. The results of stability testing revealed that rebaudioside A is stable in various food matrices following

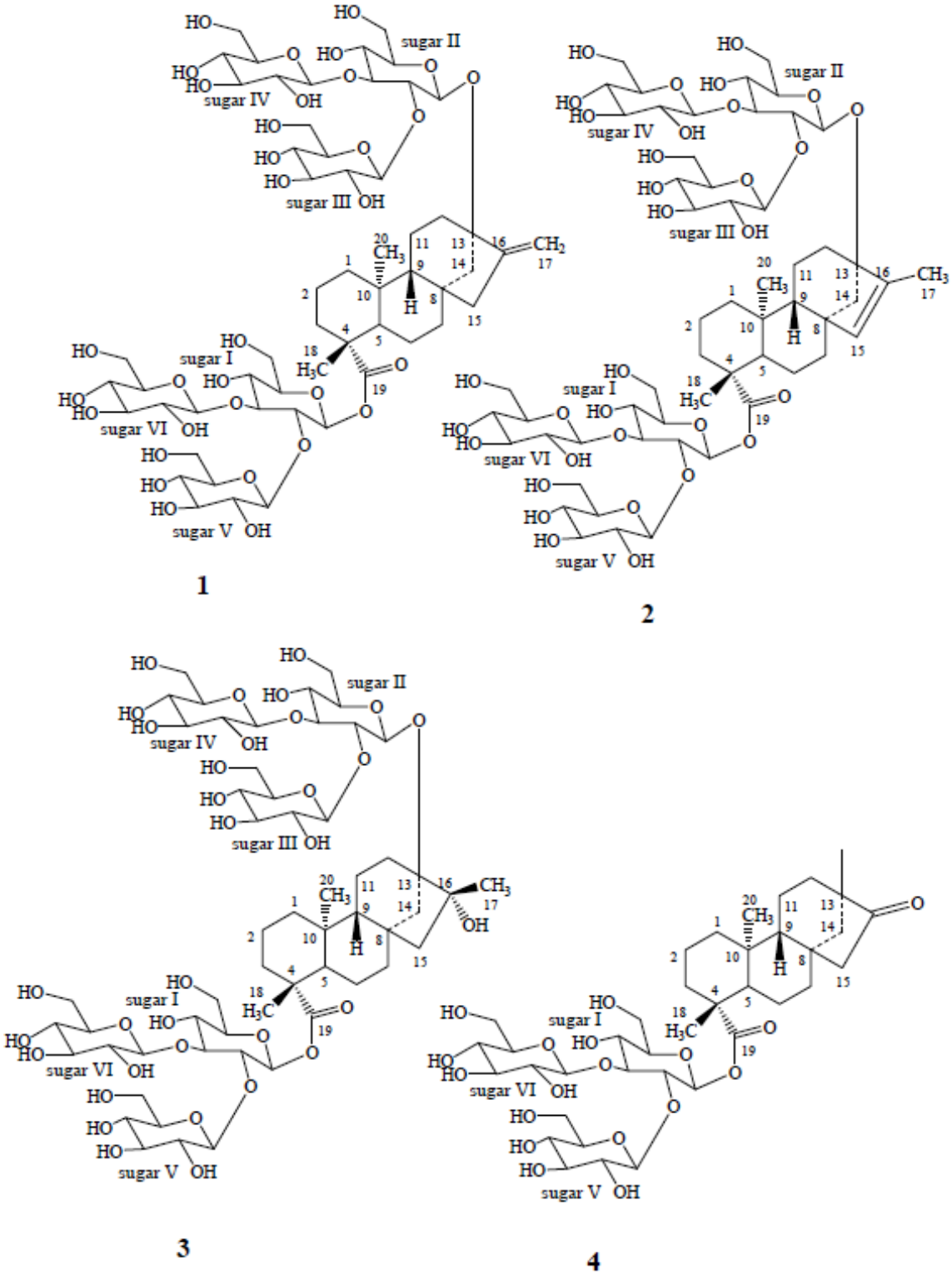
several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, rebaudioside A is more stable in the pH range 4 to 6, and at temperatures from 5˚C to 25˚C (Cargill, 2008). Photostability studies of the dry powder and mock beverages were performed to ascertain rebaudioside A behavior under defined conditions of fluorescent and near UV light exposure. Rebaudioside A was found to be photostable under the defined conditions of analysis (Clos et al., 2008).

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

Chaturvedula et al. studied acid and base hydrolysis of rebaudioside M. The authors found that, under acid hydrolysis, rebaudioside M was converted to isosteviol and that, under base hydrolysis, rebaudioside M was converted to rebaudioside B (Chaturvedula et al., 2013). Similarly, isosteviol and rebaudioside B are known degradation products of rebaudioside A (Merisant, 2008; Sunwin and Wild Flavors, 2010).

In addition, Prakash et al. studied the degradation products of rebaudioside M under acidic conditions (Prakash et al., 2014). Rebaudioside M was treated with a phosphorpic acid solution (pH 2.0, 0.1 M) over the course of 24 hours at a temperature of 80°C. Prakash et al. observed “three minor degradation products,” and their structures are provided in Figure 4.

Figure 4. Acid- & Temperature-Induced Degradation Products of Rebaudioside M[a]



[a] From Prakash et al., 2014.

Storage stability, pH stability, and forced degradation studies were conducted on rebaudioside X, a synonym of Reb M, by PureCircle Ltd. and were reported in GRAS notification 473. The storage stability study resulted in observed degradation of Reb M accompanied by "minimal changes…in the other steviol glycosides detected." PureCircle Ltd. also reported that "the extent and rate of rebaudioside X degradation were shown to be dependent on pH, temperature, and time." In addition, no significant degredation of Reb M was observed at pH values ranging from 3-8 over 24 weeks independent of temperature. Finally, in a forced degredation study, PureCircle Ltd. observed the formation of 8 degredation products, including steviol, isosteviol, and rebaudioside B (PureCircle, 2013a).

2. Stability Data for GLG Rebaudioside M Preparations

Due to the favorable stability profile for products of similar composition as noted in Section III.F.1., GLG conducted a shelf-stability test study on its high purity RM preparation. Over the course of 8 weeks, samples were stored at 25°C ± 5°C at a relative humidity of 60% ± 5% for 0, 2, 4, 6, and 8 weeks. The stability samples were then tested for total steviol glycosides, including rebaudioside M and rebaudioside D, and microbial parameters. A summary of the shelf-stability results is presented in Table 3. A detailed stability report is provided in Appendix G.

Table 3. High Purity Rebaudioside M Storage Stability Data

Lot# GLG-RM95-20131002, Steviol Glycosides, % dry basis							
Duration	Reb M	Reb D	Total Steviol Glycosides	Total plate count	*Salmonella*	*E. coli*	*Staphylococcus*
t=0	96.3	1.04	97.4	＜10 cfu/g	Negative	Negative	Negative
2 weeks	96.2	1.05	97.4	＜10 cfu/g	Negative	Negative	Negative
4 weeks	96.4	1.0	97.4	＜10 cfu/g	Negative	Negative	Negative
6 weeks	96.2	1.03	97.2	＜10 cfu/g	Negative	Negative	Negative
8 weeks	95.9	0.98	96.9	＜10 cfu/g	Negative	Negative	Negative

The stability data in the scientific literature for stevioside, the JECFA report, and the extensive stability testing for the structurally similar rebaudioside A (as presented by Merisant, Cargill, and Sunwin & WILD Flavors) and rebaudioside M (as presented by PureCircle Ltd. in GRN 473), along with GLG's stability testing results, support the position that GLG's high purity rebaudioside M preparations are well-suited for the intended food uses.

G. Sweetness Equivalence of Rebaudioside M

GLG conducted a sweetness equivalence evaluation to compare Reb M to sucrose at various concentrations. The results of this comparison show that Reb M, at a concentration of 0.013% (130 ppm), is estimated to be equivalent to a 5.0% sucrose solution. This suggests a sweetness intensity of approximately 380 times the sweetness of sucrose. The sweetness equivalence report is provided in Appendix F.

IV. INTENDED FOOD USES & ESTIMATED DIETARY INTAKE

A. Intended Uses

The subject GLG high purity Reb M preparation with steviol glycosides (≥ 97%), containing rebaudioside M as the principal component (≥ 95%), is intended to be used as a table top sweetener and general purpose non-nutritive sweetener in various foods other than infant formulas and meat and poultry products The intended use will be as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19).[7] The intended use levels will vary by actual food category, but the actual levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts of GLG's high purity Reb M preparation to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation.[8]

B. Estimated Daily Intake of Rebaudioside M

There have been many scholarly estimates of potential dietary intake replacement of sweeteners, including steviol glycosides, that have been published (FSANZ, 2008; Renwick, 2008; WHO, 2003) or submitted to FDA (Merisant, 2008). These are summarized in Appendix H.

In GRAS notification 301, a simplified estimate was proposed to, and accepted by FDA, based on the estimates of exposure in "sucrose equivalents" (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90th percentile consumer of a sweetener which is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg/kg bw/day for any population subgroup. The estimated sweetness intensity for high purity rebaudioside M is 380-fold that of sucrose (Appendix F). Therefore, the highest 90th percentile consumption by any population subgroup of GLG's Reb M ≥ 95% preparation would consume approximately 2.61 mg/kg bw/day. Based on an estimate that Reb M preparations consist of approximately 23% steviol equivalents,[9] the consumption would be less than 0.61 mg/kg bw/day on a steviol equivalents basis for any population group. These calculations are summarized in Table 4.

7 Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.
8 See 21 CFR 182.1(b)(1).
9 Calculated by the Expert Panel by as percent of molecular weight of steviol to molecular weight of rebaudioside M.

Table 4. Daily Intake of Sweeteners (In Sucrose Equivalents) & Estimated Daily Intakes of Rebaudioside M

Population Group	Intakes of Sweeteners (mg sucrose/kg bw/day)[a]		Calculated Intake of Reb M (mg/kg bw/day)[b]		Calculated Intake of Reb M as Steviol Equivalents (mg/kg bw/day)[c]	
	Low	High	Low	High	Low	High
Healthy Population	255	675	0.67	1.78	0.16	0.42
Diabetic Adults	280	897	0.74	2.36	0.17	0.55
Healthy Children	425	990	1.12	2.61	0.26	0.61
Diabetic Children	672	908	1.77	2.39	0.41	0.56

[a] From Renwick, 2008.
[b] Calculated by dividing the sucrose intake by the average relative sweetness value of 380 for Reb M.
[c] Calculated based on the ratio of molecular weights of Reb M and steviol.

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

For about 25 years, consumers in Japan and Brazil, where stevia has long been approved as a food additive, have been using stevia extracts as non-caloric sweeteners.[10] It was previously reported that 40% of the artificial sweetener market in Japan is stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999). Although there are no reported uses of rebaudioside A as a dietary supplement, use of steviol glycosides as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand, and as a natural health product in Canada. It has wide use in China and Japan in food and in dietary supplements. In 2005, it was estimated that sales of stevia in the US reached $45 million (The Food Institute Report, 2006). More recent reports of consumption figures for stevia reveal pronounced increases in global consumption. Worldwide, Zenith International estimates stevia sales of 3,500 metric tons in 2010, which represents a 27% increase over 2009 figures. The market value is estimated to have increased to $285 million (Zenith, 2011). In 2013, worldwide sales of stevia was reported to reach 4,100 tons which represents a 6.5% increase over 2011 figures, and this corresponds to an overall market value of $304 million. Furthermore, it has been projected that the total market for stevia in 2016 will be 6,250 tons with an associated market value of $490 million (Zenith, 2013).

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

[10] See Raintree Nutrition Tropical Plant Database http://www.rain-tree.com/stevia.htm.

V. SAFETY INVESTIGATIONS FOR STEVIOL GLYCOSIDES

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

GLG's high purity rebaudioside M (≥ 95%) preparation contains not less than 97% total steviol glycosides. Given its structural similarity among rebaudioside A, stevioside, and other steviol glycosides, along with metabolic considerations, the scientific data on stevia and its other components, the scientific data on these components have a direct bearing on the present safety assessment for Reb M. This is further supported by the fact that EFSA (2010) views the results of toxicology studies on either stevioside or rebaudioside A as applicable to the safety assessment of steviol glycosides, as both rebaudioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for both.

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

From the safety perspective, some of the earliest studies on steviol glycosides were of limited value as the actual compositions of materials investigated and their questionable purities undermined drawing firm toxicological conclusions. These early studies reported a decrease in fertility with crude stevia preparations and increased mutagenic activity of the principal metabolite, steviol. Based on these and other questions raised about safety by studies with materials of lesser purity and by studies with unusual protocols in *in vivo* and in *in vitro* systems usually employing high doses or high concentrations of test materials, FDA was reluctant to authorize the use of stevia. These concerns included renal toxicity, effects on glucose metabolism, and inhibition of mitochondrial enzymes. Over the last decade and a half, the safety of steviol glycosides and rebaudioside A in particular have been extensively investigated by employing comprehensive and modern toxicology protocols using scientifically accepted dosing regimens of purified and standardized test substances.

Since the JECFA evaluation (WHO, 2008), more than two dozen GRAS notifications for steviol glycosides or enzyme modified steviol glycosides have been submitted to FDA, all of which were determined to be GRAS based largely on the ADI established by JECFA. To date, 31 of the submitted notifications have had "no questions" letters of response from FDA (see Table 1).

More detailed reviews on the safety of steviol glycosides by expert bodies such as JECFA, FSANZ and EFSA are summarized in Appendix I. A more detailed review on steviol, the principal metabolite of steviol glycosides, can be found in Appendix J with the corresponding detailed reviews for steviol glycosides appearing in Appendices K and L.

B. Safety Data on Rebaudioside M

There is a high presumption of safety of rebaudioside M because it is a naturally occurring steviol glycoside obtained from *Stevia rebaudiana* Bertoni in a manner similar to the other well-recognized steviol glycosides, including rebaudioside A.

As detailed in GRN 473, PureCircle Ltd. studied the metabolism of rebaudioside X by *in vitro* methods (PureCircle Ltd., 2013a) similar to those used in previous studies with enzyme treated stevia extract (Koyama et al., 2003a; NOW Foods, 2010) and Rebaudioside D (PureCircle Ltd, 2013a; Nikiforov et al., 2013). Rebaudioside X was incubated with pooled fecal homogenates over the course of 24 hours at 37°C under anaerobic conditions. After 16 hours, the rebaudioside X was completely hydrolysed to steviol. In a parallel study, rebaudioside A was also completely converted to steviol after 16 hours of incubation. Reb A was metabolized more quickly than Reb X and the observation was attributed to the two additional glucose moieties being present in Reb X (PureCircle Ltd., 2013b).

The results of this study were recently published comparing anaerobic *in vitro* metabolism of rebaudiosides A, B, D, and M (Purkayastha et al., 2014). In all cases, the rebaudiosides were hydrolysed to steviol within 24 hours with the majority of metabolism occurring within the first 8 hours. Metabolism of rebaudiosides took longer at higher concentrations (2.0 mg/mL vs. 0.2 mg/mL). There were no marked differences in rate or extent of hydrolysis observed between male and female fecal homogenates or the individual rebaudiosides (Purkayastha et al., 2014). Results from this study corroborate the presumption of safety of rebaudioside M, given that it is observed to have a similar metabolism to that of Reb A.

VI. 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.”[11]

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.

[11] See 21 CFR 170.3(i).

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."[12]

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:[13]

- 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.

The apparent imprecision of the terms "appreciable," "at the time," and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety in this or any other area (Lu, 1988; Renwick, 1990; Rulis & Levitt, 1997).

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. Discussion on Safety Studies of High Purity Steviol Glycosides

Because of their sweetness characteristics, steviol glycosides have viable uses as a non-nutritive sweetener in foods.[14] Periodic reviews by JECFA over the years indicate the progression of knowledge on the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple

[12] See 21 CFR 170.30(a).

[13] See Footnote 1.

[14] It has also been reported that steviol glycosides may have pharmacological properties, which can be used to treat certain disease conditions such as hypertension and type 2 diabetes. Chatsudthipong and Muanprasat (2009), as well as others, have published reviews where they note that such therapeutic applications have not been firmly established as being due to steviol glycosides. The reviewers point out that the effects occur at higher doses than would be used for sweetening purposes. Furthermore, many effects noted in older studies may have been due to impurities in preparations that do not meet the contemporary purity specifications established by JECFA for use as a sweetener. If oral doses of steviol glycosides impart pharmacological effects, such effects would undoubtedly occur due to actions of the principal metabolite, steviol, but the pharmacological effects of steviol have not been comprehensively investigated. For more a more comprehensive discussion of this subject, see Section 7 of Appendix K.

investigations with *in vivo* and *in vitro* models, which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years, as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic, and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably, the initially reported concerns related to the effects of stevia leaves or crude extracts on fertility were refuted by the well-designed reproductive studies with purified steviol glycosides. All other concerns failed to manifest themselves at the doses employed in the long-term rat studies.

As discussed in Appendix I and elsewhere, at its 51st meeting, JECFA determined that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), to establish a temporary ADI of 0 - 2 mg/kg bw/day with an adequate margin of safety. The committee also critically reviewed the lack of carcinogenic response in well-conducted studies. These studies justified the Committee conclusion that the *in vitro* mutagenic activity of steviol did not present a risk of carcinogenic effects *in vivo* and, therefore, all common steviol glycosides that likely share the same basic metabolic and excretory pathway and that use high purity preparations of various steviol glycosides, are safe as a sugar substitute. Subsequently, the additional clinical data reviewed by JECFA allowed the Committee to establish a permanent ADI of 0 - 4 mg/kg bw/day (based on steviol equivalents). The GRAS Expert Panel critically reviewed the JECFA assessment and agrees with the calculation of the ADI for steviol glycosides.

The Panel has reviewed the findings from human clinical studies. The Panel noted that, regarding the clinical effects reported 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 supplemental data presented to JECFA and also published by Barriocanal et al. (2008) demonstrate the lack of pharmacological effects of steviol glycosides at 11 mg/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 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 sucrose, 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 Appendix H, 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 16 mg/kg bw/day for rebaudioside M, and notes that the estimates of anticipated dietary intake, as contained in Table 4, are below the ADI.

The Panel also noted from a study that DNA damage was seen in a variety of organs as assessed by Comet assay in rats given drinking water containing 4 mg/mL steviol glycosides for up to 45 days (Nunes et al., 2007a). Several experts in the field have since questioned the methodology used in this study (Geuns, 2007; Williams, 2007; Brusick, 2008). The Panel has reviewed the cited publications, along with the responses made by the authors (Nunes et al., 2007b; Nunes et al., 2007c), and concurs with the challenges to the methodology utilized by Nunes et al., 2007a, thereby discounting the validity and importance of this study.

In a recent review, Urban et al. (2013) examined the extensive genotoxicity database on steviol glycosides because some concern has been expressed in two recent publications (Brahmachari et al., 2011 and Tandel, 2011) in which the authors concluded that additional testing is necessary to adequately address the genotoxicity profile. The review aimed to address this matter by evaluating the specific genotoxicity studies of concern, while evaluating the adequacy of the database that includes more recent genotoxicity data not noted in these publications. The results of this literature review showed that the current database of *in vitro* and *in vivo* studies for steviol glycosides is robust, and does not indicate that either stevioside or rebaudioside A are genotoxic. This finding, combined with a paucity of evidence for neoplasm development in rat bioassays, establishes the safety of all steviol glycosides with respect to their genotoxic/carcinogenic potential.

In summary, the Expert Panel agrees with the safety conclusions of the 31 GRAS Expert Panels in the notifications previously submitted to FDA that resulted in "no questions" responses from FDA (as summarized in Table 1), JECFA (WHO, 2006; WHO, 2008), and Renwick (2008) that there are a sufficient number of good quality health and safety studies to support the determination that the intended use of purified preparations of steviol glycosides, including rebaudioside M, when added to food at levels up to full replacement of sucrose on a sweetness equivalency basis, meets FDA's definition of safe. In addition, the Panel has compared the specifications of GLG's high purity rebaudioside M preparation to the composition of the test materials used in all the published studies. The Panel agrees that GLG's high purity rebaudioside M preparation is sufficiently similar to those used in all key studies reviewed by JECFA, and those on rebaudioside A previously reviewed by FDA, and there is no need for further safety studies to be conducted on the GLG Rebpure™ RM95 product. The Panel has also reviewed the expected levels of dietary intake and agrees that there is sufficient information to conclude that the subject rebaudioside M product can be safely used as a table top sweetener and as a general purpose non-nutritive sweetener in various foods other than infant formulas and meat and poultry products.

C. Panel Findings on Safety of Rebaudioside M (≥95%)

Based on fundamental toxicological priniciples, in concert with the supporting safety data on structurally similar steviol glycosides and the safety studies reported herein, Reb M is considered to be safe under the anticipated food use conditions. The major naturally-occurring steviol glycosides are deemed to be safe as discussed more fully in Appendices I, J, K and L based in large measure on the fact that Reb M is metabolized to steviol.

Steviol glycosides are unique compounds in that they have viable uses as non-nutritive sweeteners in foods. The series of reviews by JECFA over several years indicate the progression of knowledge on the toxicology and clinical effects of these compounds. The early toxicology studies were largely performed on crude extracts of stevia. Several initial concerns were noted, including impairment of fertility and mutagenicity. As more studies were performed on purified

glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, reproductive, and chronic studies have been conducted in laboratory animals. The studies were, in general, adequately designed with appropriately high doses and sufficient numbers of animals to maximize the probability of detection of important adverse effects. Notably, the reproductive toxicity studies with purified steviol glycosides refuted the concern over effects on fertility that were initially reported with stevia leaves or crude extracts.

As discussed in Section V.A and Appendix I, JECFA reasoned that there were ample 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 concluded that high purity preparations of various steviol glycosides are safe for use as non-nutritive sweeteners. The additional clinical data subsequently presented allowed JECFA to establish a permanent ADI of 0-4 mg/kg bw/day (based on steviol equivalents).

Regarding pharmacological effects in humans, JECFA called for additional studies in individuals that are neither hypertensive nor diabetic (WHO, 2006) to corroborate the observations that the effects of steviol glycosides of reducing blood glucose or blood pressure (or both) only occur in patients who already have these conditions. Since that time a number of clinical studies have been published. The first of these was presented to JECFA demonstrated the lack of pharmacological effects of steviol glycosides at 11 mg/kg bw/day in normal individuals (Barriocanal et al., 2006, 2008). A similar study conducted with rebaudioside A showed comparable results (Wheeler et al., 2008). The Panel has reviewed the clinical studies and concludes that there should be no effects on reducing blood pressure or adversely effecting glucose metabolism in humans at the doses of Reb M expected from use in food as a non-nutritive sweetener.

The Panel reviewed a recently published *in vitro* metabolism study of rebaudioside M by Purkayastha et al. (2014). The authors demonstrated that the predominant metabolic pathway of ingested Reb M is conversion to steviol in the lower GI tract, as expected for any of the steviol glycosides. This data were presented in GRN 473 with the conclusion that “the results of this study corroborate the findings from previously published studies demonstrating that all steviol glycosides sharing the same steviol backbone are degraded by fecal microbes to steviol in the gastrointestinal tract. Therefore, it was determined that JECFA s [sic] ADI for steviol glycosides would extend to rebaudioside X.” In response to GRN 473, FDA issued a “no questions” letter. The Panel considers these findings corroborative information. Therefore, the Panel agrees that the primary information to support safety is fulfilled by previously published information on steviol glycosides.

The GLG Reb M product identified in the subject notification meets the equivalent of the 95% purity standard comparable to the JECFA specifications for purity of steviol glycosides and FCC specifications for Reb A. Furthermore, Reb M is manufactured by a process that complies with FDA Good Manufacturing Practices regulations, and GLG maintains a rigorous set of chemical and microbiological specifications to assure that safe products are generated. The Panel concludes that the GLG high purity rebaudioside M finished product is a carefully manufactured and safe food grade product.

D. Acceptable Daily Intake for GLG Rebaudioside M (≥95%)

The Panel concludes that it is reasonable to apply the JECFA ADI of 4 mg/kg bw/day for steviol glycosides (expressed on a steviol basis) to Reb M. Therefore, with the steviol equivalence values shown in Table 4, the Panel concludes that, for the general population, the estimated maximum daily intake of Reb M is 2.61 mg/kg bw or 0.61 mg/kg expressed as steviol equivalents. Based upon these calculations, the intake of Reb M safely aligns with the 4 mg/kg bw/day ADI expressed as steviol equivalents as determined by JECFA.

E. Common Knowledge Elements for GRAS Determinations

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 second common knowledge element for a GRAS determination requires that consensus exists within the broader scientific community.

1. Generally Available Information

The majority of the studies reviewed on steviol glycosides and steviol have been published in the scientific literature as summarized in Appendices H, I, and J. Most of the literature relied upon by JECFA has also been published, most importantly the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website.

With regard to the safety documentation, the key pharmacokinetic data establish that steviol glycosides are not absorbed through the GI tract, *per se*; they are converted to steviol by bacteria normally present in the large intestine and the steviol is absorbed but rapidly metabolized and excreted. It has been well-established experimentally from various published studies that the steviol glycoside molecules are not absorbed from the GI tract (Gardana et al., 2003; Koyama et al., 2003a). The action of bacteria in the large intestine is directly supported by the published study that steviol glycosides can be converted to steviol in the large intestine by normal anaerobic GI flora as demonstrated by an *in vitro* study in fecal homogenates (Koyama et al., 2003b; Renwick and Tarka, 2008). The ADI for steviol glycosides has been set largely based on published chronic study in rats (Toyoda et al., 1997) and several published clinical studies that there are no pharmacological effects in humans at doses several fold higher than the ADI (Barriocanal et al., 2006, 2008; Wheeler et al., 2008). The toxicity of the metabolite steviol has been well reviewed in the published literature (Geuns, 2003; WHO, 2006; Urban et al., 2013).

Studies regarding rebaudioside M isolation, structural determination, and metabolism have been published (Chaturvedula et al., 2013; Prakash et al., 2014; Purkayastha et al., 2014) in the literature. In addition, there is a large publically available collection of GRNs regarding steviol glycosides (including rebaudioside M) on FDA's website.

2. Scientific Consensus

The second common knowledge element for a GRAS determination requires that there must be a basis to conclude that consensus exists among qualified scientists about the safety of the substance for its intended use. The Panel maintains that well-qualified scientists would conclude that Reb M is not absorbed from the GI tract, *per se.* By virtue of fundamental principles of pharmacokinetics, the majority of scientists would support this determination, and they would likewise concur that Reb M undergoes a conversion to steviol as is known to be the case with the other naturally occurring steviol glycosides.

Regarding the safety of 10 naturally occurring steviol glycosides, the 2008 JECFA final opinion largely meets the scientific consensus test on its own that steviol glycosides are safe. This is the case because of the well-recognized scientific rigor and broad base of scientific expertise that resides within the prestigious JECFA, which is composed of expert scientists from various regulatory agencies around the world, as well as other scientists chosen because of their specific expertise on various classes of food ingredients. In addition, FDA scientists participate in JECFA deliberations, and EFSA has recently concurred with the JECFA evaluation including the ADI (EFSA, 2010). The JECFA conclusion has been reviewed and validated by other respected regulatory agencies, including FSANZ and the Switzerland Office of Public Health, and, most recently, Health Canada (FSANZ, 2008; Switzerland Office of Public Health; Health Canada, 2012). A number of 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 scientific consensus element has been embellished by the many well-respecteed 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). The authors of a recent review of the genetic toxicology database of steviol glycosides concluded that the available data “establish the safety of all steviol glycosides with respect to their genotoxic/carcinogenic potential” (Urban et al., 2013). We also note that, since December 2008, more than thirty GRAS notifications have been submitted to FDA for stevia-derived sweetener products, and FDA detailed reviews have consistently yielded “no questions” letters.

In summary, a compelling case can be made that scientific consensus exists regarding the safety of Reb M, as well as the other steviol glycosides, when of sufficiently high purity. The central role of conversion to steviol and subsequent elimination with these naturally occurring steviol glycosides extends to the manner in which Reb M molecules are metabolized and eliminated from the body. Due to the similarities in metabolic fate, the safety of Reb M can be established based on studies conducted with non-modified steviol glycosides. While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Panel believes that a wide consensus does exist in the scientific community to support a GRAS conclusion as evidenced by several publications (Carakostas, 2012; Geuns, 2007; Urban et al., 2013; Waddell, 2011; Williams, 2007; Brusick, 2008) that refute safety concerns expressed by a minority of scientists.

VII. CONCLUSIONS[15]

In consideration of the aggregate safety information available on Reb M and the naturally occurring steviol glycosides, the Panel concludes that Reb M is safe for use as a general purpose non-nutritive sweetener in foods other than infant formulas and meat and poultry products. Based on the information that Reb M will display similar pharmacokinetics to the other naturally occurring steviol glycosides, the JECFA ADI for steviol glycosides of 4 mg/kg bw/day (as steviol equivalents) can be applied to Reb M. Based on published dietary exposure data for other approved sweeteners and adjusting for relative sweetness intensity, the intake of rebaudioside M was estimated for healthy non-diabetic children and adults, and diabetic children and adults.

The estimated intakes of Reb M for several population groups summarized in Table 4 are no greater than 3 mg/kg bw/day, which is below the ADI of 4 mg/kg bw expressed as steviol equivalents as established by JECFA. The Panel finds that the dietary levels from anticipated food consumption will not exceed the ADI when Reb M is used as a general non-nutritive sweetener.

The Panel also finds that the 95% purity specification for Reb M is sufficient in view of the accepted JECFA specification for 95% purity for other naturally occurring steviol glycosides. The Panel concludes that Reb M, as manufactured by GLG, is an appropriate food grade ingredient and that adverse pharmacological effects are not likely to occur at this designated ADI level. Furthermore, even high consumers of steviol glycosides are not likely to exceed this specified ADI. Therefore, the Panel concludes that Reb M, when consumed in foods as described within this GRAS notification, is generally recognized as safe (GRAS) within the meaning of the Food, Drug, and Cosmetic Act.

GLG's high purity rebaudioside M (≥ 95%), referred to as Rebpure™ RM95, when produced in accordance with FDA Good Manufacturing Practices requirements and when meeting at a minimum the JECFA purity specifications for steviol glycosides, is Generally Recognized As Safe when consumed as a non-nutritive sweetener in foods other than infant formulas and meat and poultry products within the JECFA ADI of 4 mg/kg bw/day on a steviol equivalent basis which corresponds to 16 mg/kg bw/day as Reb M. 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.

[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. Kapp's *curriculum vitae* can be accessed at http://www.biotox.net. 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.

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

(b) (6)

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

(b) (6)

Robert S. McQuate, Ph.D.

(b) (6)

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

Date: April 11, 2014

VIII. REFERENCES

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APPENDIX A

Specifications & Certificates of Analysis for Production Processing Aids

A-1 GLG Specifications for Ethanol
A-2 GLG Specifications for Methanol
A-3 Certificate of Analysis for GLG Active Carbon

A-1 GLG Specifications for Ethanol

Ethanol Specification

Prepared by GLG QA Department
File No. GLG-QA-PA2016

Ethyl Alcohol (Ethanol)

C_2H_6O Formula wt 46.07

Description

Ethyl Alcohol occurs as a clear, colorless, mobile liquid. It is miscible with water, with ether, and with chloroform.

Function Extraction solvent; carrier solvent.

Physical and Organoleptic Standards

Characteristic	Specification	Method
Appearance	Clear, colorless liquid.	Organoleptic as is
Flavor ,Aroma	Normal	Organoleptic as is

Physical and Chemical Standards
（According to：GB10343-2008/FCC（8th））

Characteristic	Specification
Assay（C_2H_6O，by volume）	≥95.0%
Acidity (as acetic acid)	≤0.003%
Alkalinity(as NH_3)	≤3ppm
Fusel Oil	Passes test
Ketones, Isopropyl Alcohol	Passes test
Lead	≤0.5ppm
Methanol	Passes test
Nonvolatile Residue	≤0.003%
Solubility in water	Passes test
Substances Darkened by Sulfuric Acid	Passes test
Substances Reducing Permanganate	Passes test

A-2 GLG Specifications for Methanol

Methanol Specification

Prepared by GLG QA Department
File No. GLG-QA-PA2018

Methyl Alcohol (Methanol)

CH_3OH Formula wt 32.04

Description

Methyl Alcohol occurs as a clear, colorless, flammable liquid. It is miscible with water, with ethyl alcohol, and with ether.

Function Extraction solvent.

Physical and Organoleptic Standards

Characteristic	Specification	Method
Appearance	clear, colorless liquid.	Organoleptic as is

Physical and Chemical Standards
（According to：GB 29218—2012 /FCC（8th））

Characteristic	Specification
Assay（CH_3OH%）	≥99.5
Acetone and Aldehydes	≤0.003 %
Acidity (as formic acid)	≤0.0015%
Alkalinity(as NH_3)	≤3 ppm
Distillation Range ℃	64.5~65.5
Lead	≤1 ppm
Nonvolatile Residue	≤10 ppm
Readily Carbonizable Substance	Passes test
Solubility in water	Passes test
Substances Reducing Permanganate	Passes test
Water	≤0.1%

A-3 Certificate of Analysis for GLG Active Carbon

GLG

Certificate of Analysis

Product: Active Carbon
Manufacturing Date: May 10th, 2013 **Lot No. 20130506**
Analysis Date: May 21st, 2013 **Shelf Life: two years**
Manufacture: Ning Guo city Hengda Active Carbon Co.,Ltd
Country of Origin: China
According: GB/T13803.3-1999

Inspection Item	Specfication	Results	Method
Adsorptive power ,ml/g	≥110	116	GBT12496.10
Ph	5-7	6.54	Q/GLG-01-2008-06
moisture, %	≤10	5.68	Q/GLG-01-2008-06
Fe, %	≤0.02	0.01	Q/GLG-01-2008-06
Ash, %	≤3	2.12	Q/GLG-01-2008-06
Lead, ppm	≤5	0.16	Q/GLG-01-2008-06

Analyzed by: (b) (6) [redacted] Checked by (b) (6) [redacted] Approved by: (b) (6) [redacted]

21/05/2013

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APPENDIX B

Analytical Method for Steviol Glycosides Quantitation

GLG LIFE TECH CORPORATION	Issue Date:10/12/2013
DETERMINATION OF REBAUDIOSIDE M AND RELATED STEVIOL GLYCOSIDES BY LC	File No: GLG-QA-STD-HPLC-2

PRINCIPLE:

This assay is capable of determining the concentrations of rebaudioside M and related steviol glycosides using an isocratic LC system.

Standards

1. Rebaudioside M Standard; (Chromadex Inc. Irvine, CA USA);
2. Rebaudioside D Standard; (Chromadex Inc. Irvine, CA USA);
3. Stevioside Standard; (Chromadex Inc. Irvine, CA USA);

Solvents and Reagents

- Acetonitrile, HPLC grade (Merck, Germany);
- Water, HPLC grade (Millipore, Germany);
- Ammonium acetate, reagent grade (Merck, Germany);
- Acetic acid, reagent grade (Merck, Germany).

Apparatus

1. Agilent1200 HPLC system equipped with binary pump, auto sampler, thermostatted column compartment and UV detector, (Agilent Technologies, USA);
2. Analytical column, Luna 5µ C18 (2) 100A (Phenomenex, USA)
3. Analytical balance, XS205, (Mettler Toledo, USA);
4. Karl Fischer coulometer, Mettler Toledo DL-39, (Mettler Toledo, USA);
6. Volumetric (class ***A)*** and Laboratory glassware.

ASSAY PROCEDURE

Acetate buffer: Dissolve 0.125 g ammonium acetate in 900 mL of water, adjust pH to 2.6 with glacial acetic acid solution and dilute to 1 L. [NOTE: It may be necessary to adjust the ratio of ammonium acetate to acetic acid. Changing the pH adjusts the retention time of rebaudioside M and related glycosides. Decreasing the pH of the buffer will decrease the retention time of rebaudioside M.]

Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L ammonium acetate buffer (pH 2.6). After that, flush the column with Mobile phase until the retention time of rebaudioside M is less than 8 min (may take up to one day of flushing).]

RebaudiosideM standard solutions: 4500, **4750,** 5250, and 5500 mg/L Rebaudioside A RS in Diluent

Stevioside standard stock solution: 250 mg/L Stevioside RS in Diluent

Stevioside standard solutions: 2.5, 5.0, 10, 25, and 50 mg/L USP Stevioside RS in Diluent: from

Sample solution: 5000 mg/L in Diluent

Chromatographic system

Mode: High-performance liquid chromatography

1/3

Detector: UV (210 nm)
Column: Capcell pak C_{18} 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)
Column temperature: 40°C
Flow rate: 1.0 mL/min
Injection size: 20 µ L
System suitability
Samples: 5250 mg/L RebaudiosideM standard solution, 2.5 mg/L Stevioside standard solution.

Suitability requirements
Detector response: Peak to noise ratio (peak height /baseline noise) is NLT 3 for the stevioside peak from the 2.5 mg/L Stevioside standard solution, where peak height is expressed in mAU, and baseline noise is the maximum deflection of the baseline (mAU) in a blank at the retention time of stevioside over the same baseline peak width in min.
Relative standard deviation: NMT 2.0% for rebaudioside M peak area and retention time from the 5250 mg/L Rebaudioside M standard solution
Column efficiency: NLT 5000 theoretical plates count, *N*, using the rebaudioside B peak from the Sample solution
Retention time: The retention time for the rebaudioside M peak from the 5250 mg/L Rebaudioside M standard solution is less than 8.0 min.
Tailing factor: NMT 2.0 for the rebaudioside B peak from the Sample solution, Stevioside standard solutions, and Sample solution into the chromatograph

*Chromatographic Profile Table*1

Name	ABBR.	Molecular	R_1	R_2	CAS NO	MW	RT(min)
Rebaudioside D	RD	$C_{50}H_{80}O_{28}$	Glcβ(1−2)Glcβ1−	Glcβ(1−2)[Glcβ(1−3)]Glcβ1−	63279-13-0	1129.15	**4.3**
Rebaudioside M (X)	RM/RX	$C_{56}H_{90}O_{33}$	Glcβ(1−2)[Glcβ(1−3)]Glcβ1−	Glcβ(1−2)[Glcβ(1−3)]Glcβ1−	1220616-44-3	1291.29	5.1
Rebaudioside A	RA	$C_{44}H_{70}O_{23}$	Glcβ1−	Glcβ(1−2)[Glcβ(1−3)]Glcβ1−	58543-16-1	967.01	**10.3**

Analysis: Separately inject equal volumes of the Rebaudioside M standard solutions, Stevioside standard solutions, and Sample solution into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [NOTE: The approximate retention times for rebaudioside M and its related steviol glycosides are listed above in *Chromatographic Profile Table*1. If the retention time for rebaudioside M is below 6 min, adjust the ratio of ammonium acetate to acetic acid.]

2 / 3

Prepare a standard curve for rebaudioside M by plotting rebaudioside M peak areas versus concentrations in mg/L, corrected for purity, based on the USP Reference Standard label claim. [NOTE: Peak responses for all other steviol glycosides besides rebaudioside M are used below in the Related Steviol Glycosides impurities test procedure.] From the standard curve, calculate the concentration *(CUI* of rebaudioside *M* in the Sample solution in mg/L. Calculate the percentage of rebaudioside **M** in the portion of the sample taken by the formula:

CU/CSMPX 100%

Cu = Concentration of rebaudioside M in the *Sample solution* determined from the standard curve (mg/L)

CsMp = Concentration of the sample in the Sample solution (mg/L)

RELATEDS TEVIOLG LYCOSIDES

Acetate buffer, Mobile phase, Diluent, Rebaudioside M standard solutions, Stevioside standard stock solution, Stevioside standard solutions, Sample solution, Chromatographic system, and System suitability: Prepare as directed in the *Assay* (above).

Analysis: Proceed as directed above in the *Assay*, but with the following modifications for the standard curve and calculations.

Using the peak area responses from the Stevioside standard solutions, prepare a standard curve for stevioside by plotting stevioside peak areas versus concentrations, in mg/L, corrected for purity, based on the USP Reference Standard label claim. From this standard curve, determine the concentration (mg/L) of stevioside in the Sample solution.

Calculate the percent of stevioside in the sample taken using the following formula:

Cu/Csmp x 100%

Cu = Concentration of stevioside in the Sample solution determined from the standard curve (mg/L)

Csmp = Concentration of the sample in the Sample solution (mg/L)

For the seven other steviol glycoside impurities (rubusoside, dulcoside A, rebaudioside C rebaudioside F rebaudioside D, steviolbioside, and rebaudioside B) use the stevioside standard curve prepared above to calculate the mg/L stevioside equivalents for each. Separately calculate the percents of each analyte (rubusoside, dulcoside A, rebaudioside C, rebaudioside F, rebaudioside D, steviolbioside, and rebaudioside B) in the sample taken using the following formula which takes into account the differences in molecular weights between the analytes and stevioside:

(Cu X M,, /Me) / CsMp X 100%

Cu = concentration of stevioside equivalents in the *Sample solution* determined from the standard curve (mg/L)

M_{rt}, = Molecular weight of the analyte (see *Chromatographic Profile Table* 1, above under Assay)

M_{r2}= Molecular weight of stevioside 804.88

CsMp = Concentration of the sample in the Sample solution (mg/L).

3 / 3

APPENDIX C

HPLC Chromatograms for Rebpure™ RM95

C-1 HPLC Chromatogram for Rebaudioside M Standard

C-2 HPLC Chromatogram for RM95 Batch 20131002

C-3 HPLC Chromatogram for RM95 Batch 20131008

C-4 HPLC Chromatogram for RM95 Batch 20131012

C-5 HPLC Chromatogram for RM95 Batch 20131016

C-6 HPLC Chromatogram for RM95 Batch 20131020

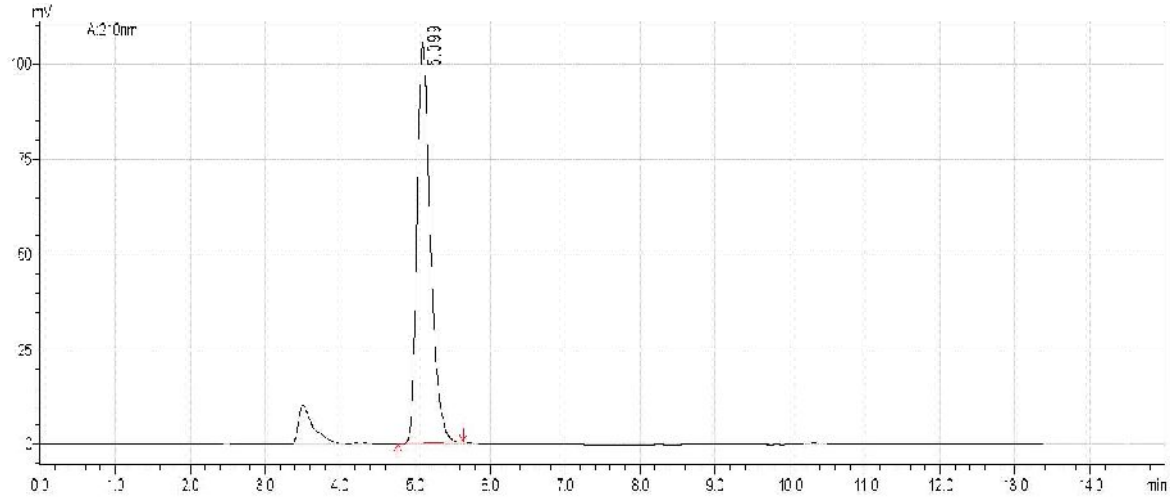
C-1 HPLC Chromatogram for Rebaudioside M Standard

Data File: M:\DATA\2013\1006-1\008-0401.D

Operator : sun hongkai
Instrument : Instrument 3
Injection Date : 2013-10-06 07:40:32
Line : 8
Location : 4
Inj : 1
Inj Volume : 20.0 µl

Acq.Method : D:\CHEM32\1\METHODS\SUGAR-D LC.M
Last Changed : 2013-10-06 07:20:17 sun hongkai
(modified after loading)
Analysis Method : D:\CHEM32\1\METHODS\07_8_ C.M
Last Changed : 2013-10-06 10:25:23 sun hongkai
(modified after loading)

Sample Info : Rebaudioside M Standard(ASP-00018234-010)



External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier and Dilution Factor with ISTDs

Signal 1: VWD1 A, Sig=210 nm

Peak #	Ret Time [min]	Type	Peak Width [min]	Area [mAU*s]	Peak Height [mAU]	Amt/Area %	Name
1	5.099	BB	0.3756	1325.46783	56.77496	100.0000	Reb M
Total :				1325.46783	56.77496		

*** End of report ***

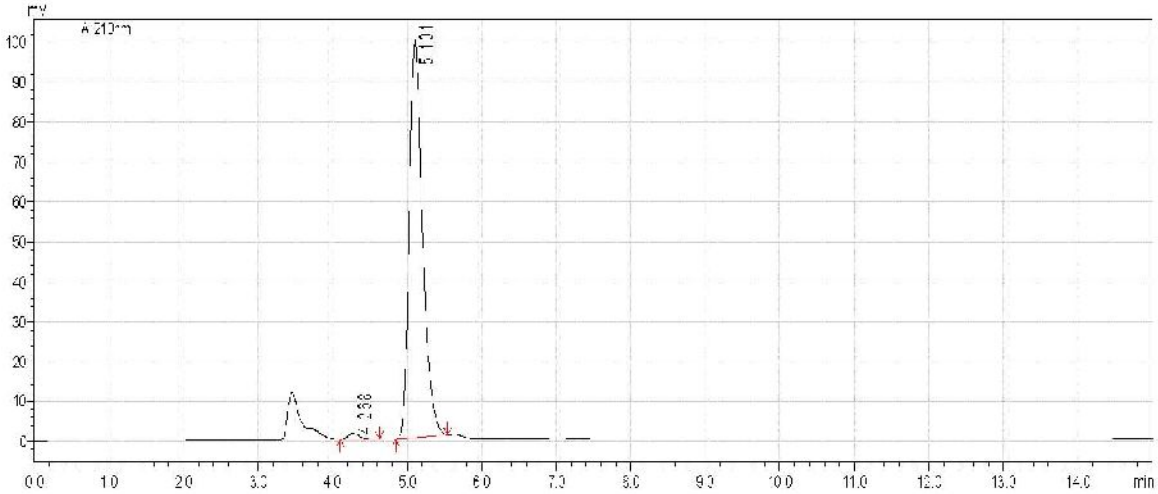
Instrument 1 2013-10-06 10:25:58 sun hongkai

1/ 1

C-2 HPLC Chromatogram for RM95 Batch 20131002

Data File: E:\DATA\2013\1006-1\002-0501.D

Operator	: sun hongkai	Line	: 5
Instrument	: Instrument 3	Location	: 9
Injection Date	: 2013-10-06 18:05:26	Inj	: 1
		Inj volum	: 20.0 µl
Acq.Method	: D:\CHEM32\1\MET HODS\S.OAR-D_IC.M		
Last Changed	: 2013-10-06 11:25:01 sun hongkai (modified after loading)		
Analysis Method	: D:\CHEM32\1\METHODS\C18 IC.M		
Last Changed	: 2013-10-06 16:10:20 sun hongkai (modified after loading)		
Sample Info	: GLG-RM95-20131002		



External Standard Report

Sorted By	:	Signal
Multiplier	:	1.0000
Dilution	:	1.0000

Use Multiplier and Dilution Factor with ISTDs

Signal 1: VWD1 A, Sig=210 nm

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Area %	Name
1	4.268	VV	0.1347	3.21399	4.21660e-1	1.17	Reb D
2	5.101	BB	0.3773	1273.92212	57.74876	98.83	Reb M
Total				1277.13611	61.96540		

*** End of report ***

Instrument 3 2013-10-06 16:20:35 sun hongkai

1/1

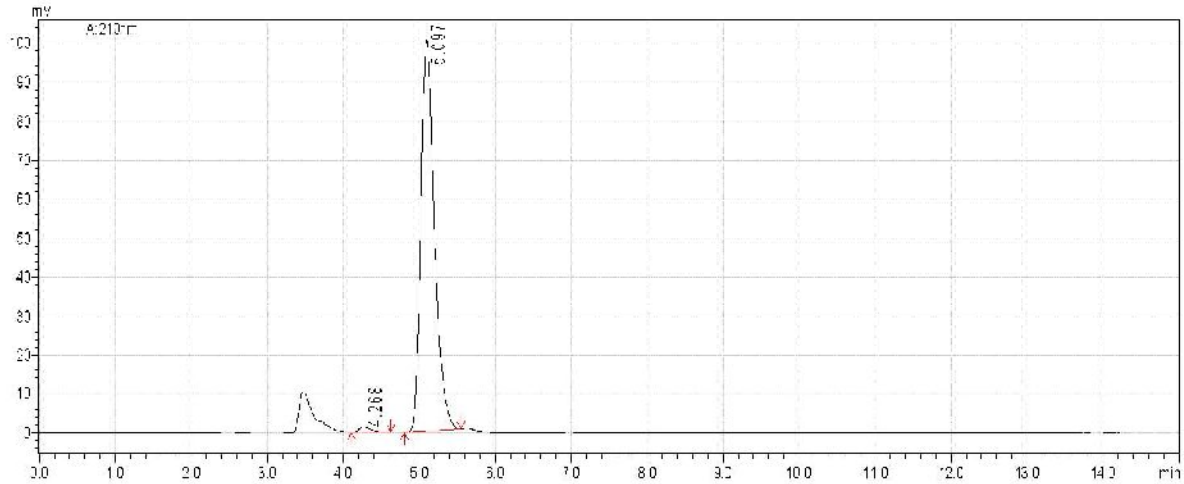
C-3 HPLC Chromatogram for RM95 Batch 20131008

Data File: D:\DATA\2013\1009-1\001-0401.D

Operator : sun hongkai
Instrument : Instrument 3
Injection Date : 2013-10-09 11:32:10
Line : 4
Location : 2
Inj : 1
Inj Volum : 20.0 µl

Acq.Method : D:\CHEM32\1\METHODS\SUGAR-D LC.M
Last Changed : 2013-10-09 11:30:09 sun hongkai
(modified after loading)
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed : 2013-10-09 15:20:25 sun hongkai
(modified after loading)

Sample Info : GLG-RM95-20131008



External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier and Dilution Factor with ISTDs

Signal 1: VWD1 A, Sig=210 nm

Peak #	Ret. Time [min]	Type	Peak Width [min]	Area [mAU*s]	Peak Height [mAU]	Amt/Area %	Name
1	4.268	VV	0.1410	3.33288	1.12060e-1	1.18	Reb D
2	5.097	BB	0.3773	1297.87654	61.78323	98.82	Reb M
Total:				1301.2094	62.90390		

*** End of report ***

Instrument 3 2013-10-09 16:20:35 sun hongkai

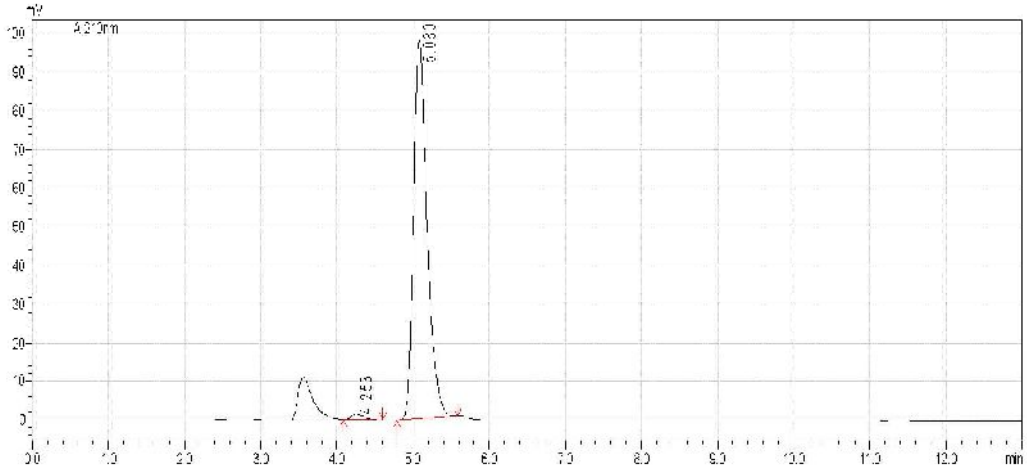
1/1

C-4 HPLC Chromatogram for RM95 Batch 20131012

Data File: D:\DATA\2013\2010-1\008-0207.D

Operator : sun hongkai
Instrument : Instrument 3
Injection Date : 2013-10-10 09:01:15
Acq.Method : D:\CHEM32\1\METHODS\SUGAR-D EC.M
Last Changed : 2013-10-10 10:20:32 sun hongkai
(modified after loading)
Analysis Method : D:\C-RM95\1\METHODS\CF3_EC.M
Last Changed : 2013-10-10 12:36:48 sun hongkai
(modified after loading)
Sample Info : C-C-RM95-20131012

Line : 2
Location : 3
Vial : 1
Inj Volume: 20.0 µl



External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier and Dilution Factor with ISTDs

Signal1: VWD1 A, Sig=210 nm

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height mAU	Amt/Area %	Name
1	4.235	VV	0.1210	0.78288	0.98060e-1	1.14	Reb D
2	5.080	BB	0.3690	1289.7432	89.88871	98.82	Reb M
Total:				1292.5261	90.98480		

*** End of Report ***

Instrument 3 2013-10-10 13:10:55 sun hongkai

1/1

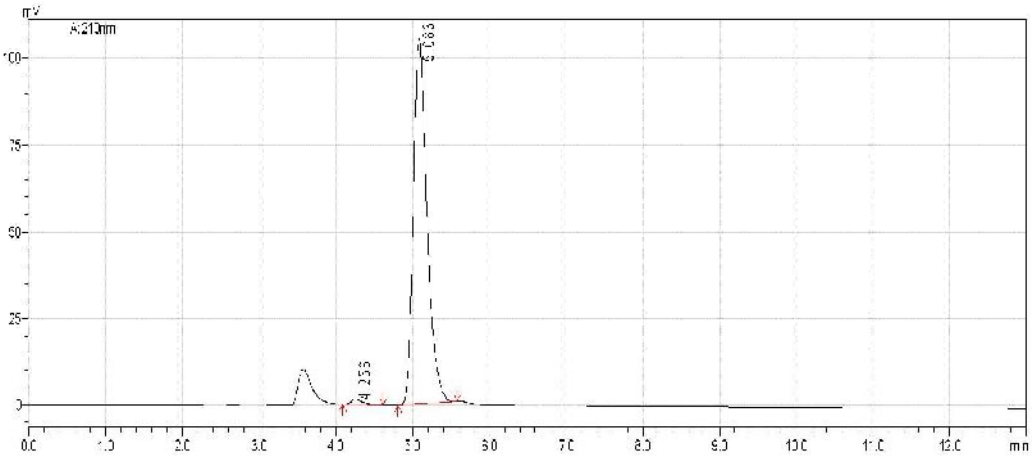
C-5 HPLC Chromatogram for RM95 Batch 20131016

Data File: E:\DATA\2013\1018-1\001-0101.D

Operator	: sun hongkai	Line	: 1
Instrument	: Instrument 3	Location	: 1
Injection Date	: 2013-10-18 10:31:42	Vial	: 1
		Inj Volume	: 20.0 µl

Acq.Method : D:\CHEMSTA\1\METHODS\SUGAR-D_LC.M
Last Changed : 2013-10-18 10:58:45 sun hongkai
(modified after loading)
Analysis Method : D:\CHEMSTA\1\METHODS\C18_LC.M
Last Changed : 2013-10-18 11:43:21 sun hongkai
(modified after loading)

Sample Info : GLG-RM95-20131016



External Standard Report

Sorted By	:	Signal
Multiplier	:	1.0000
Dilution	:	1.0000

Use Multiplier and Dilution Factor with ISTDs

Signal: VWD1 A, Sig=210,0 nm

Peak #	Ret. Time [min]	Type	Peak Width [min]	Area [mAU*s]	Peak Height [mAU]	Amt/Area %	Name
1	4.256	VV	0.1373	2.69722	1.05042e-1	1.20	Reb D
2	4.083	BB	0.2421	1295.9276	53.93421	98.80	Reb M
Total:				1298.6049	53.98471		

*** End of report ***

Instrument 3 2013-10-18 17:01:23 sun hongkai

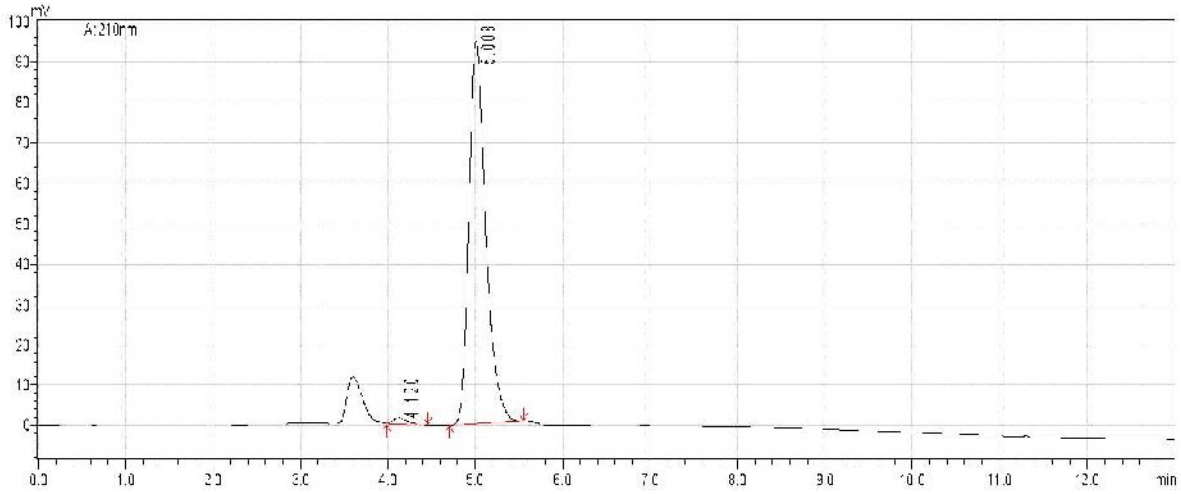
C-6 HPLC Chromatogram for RM95 Batch 20131020

Data File: D:\DATA\2013\1020-1\002-0601.D

Operator	: sun hongkai	Line :	6
Instrument	: Instrument 3	Location :	2
Injection Date	: 2013-10-20 13:36:50	Vial :	1
		Inj Volume :	20.0 µl

Acq.Method : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed : 2013-10-20 14:12:13 sun hongkai
(modified after loading)
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed : 2013-10-20 15:24:56 sun hongkai
(modified after loading)

Sample Info : GLG-RM95-20131020



External Standard Report

Sorted By	:	Signal
Multiplier	:	1.0000
Dilution	:	1.0000

Use Multiplier and Dilution Factor with ISTDs

Signal 1: VWD1 A, Sig=210 nm

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Area %	Name
1	4.120	VV	0.1294	2.57821	1.123430e-1	1.11	Reb D
2	5.008	BB	0.3698	1299.6875	57.88765	98.89	Reb M
Total :				1302.2658	59.0211		

*** End of report ***

Instrument 3 2013-10-20 17:46:38 sun hongkai

1/1

APPENDIX D

Certificates of Analysis for Multiple Production Batches of Rebpure™ RM95

D-1 Certificate of Analysis for Rebpure RM95 Batch 20131002

D-2 Certificate of Analysis for Rebpure RM95 Batch 20131008

D-3 Certificate of Analysis for Rebpure RM95 Batch 20131012

D-4 Certificate of Analysis for Rebpure RM95 Batch 20131016

D-5 Certificate of Analysis for Rebpure RM95 Batch 20131020

D-1 Certificate of Analysis for Rebpure RM95 Batch 20131002

Certificate of Analysis

GLG
Research and Development
GLG Life Tech Corporation
www.glglifetech.com
GLG-QA-COA-062

Product: Rebpure™ RM95
Lot Number: GLG-RM95-20131002

Manufacturing Date: Oct. 5th, 2013
Country of Origin: China
Shelf Life: 2 years

Product Description: Rebpure™ RM95 is a highly purified extract containing rebaudioside M (MW: 1291.3) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages. Rebpure™ RM95 meets JECFA requirements completely.

Distributed By: GLG Life Tech Corporation
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Fax:1.604.662.8858
Email: sales@glglifetech.com
Web: www.glglifetech.com

Manufacturing By: Qingdao Runde Biotechnology Co., Ltd
Lingshanwei Town, Jiaonan County
Qingdao, Shandong, China 266427
Phone: +86.532.83181169
Fax:+86.532.83181838

Qingdao Runde Biotechnology Company, Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Date of Analysis: Oct. 10th, 2013

CHARACTERISTIC	SPECIFICATION	RESULT	METHOD
Rebaudioside M (wt/wt)	≥95.0% (on dry basis)	96.3%	JECFA HPLC
Total Steviol Glycosides (wt/wt)	≥97.0% (on dry basis)	97.4%	JECFA HPLC
Arsenic (as As)	≤1.0 ppm	0.05ppm	AAS
Lead (as Pb)	≤1.0 ppm	0.01ppm	AAS
Cadmium (as Cd)	≤1.0 ppm	0.01ppm	AAS
Mercury (as Hg)	≤1.0 ppm	0.02ppm	AAS
Residual solvents -Ethanol	≤5000 ppm	360ppm	GC
-Methanol	≤200 ppm	78ppm	GC
Loss on Drying	≤4.0 %	2.8%	105℃,2hr
PH	4.5-7.0	5.18	1 in 100 solution,wt/v
Residue on Ignition (wt/wt)	≤1.0%	0.08%	FCC 6
Total Plate Count	<1000 CFU/g	< 10 cfu/g	FDA-BAM current ed.
Yeast & Mold	<100 CFU/g	< 10cfu/g	FDA-BAM current ed.
E.coli	Negative	Negative	FDA-BAM current ed.
Staphylococcus aureus	Negative	Negative	FDA-BAM current ed.
Salmonella (/25g)	Negative	Negative	FDA-BAM current ed.

Conclusion		(b) (6)

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

Analyzed by: (b) (6) **Date:** 10/10/2013

Checked by: (b) (6) **Date:** 10/10/2013

Approved by: (b) (6) (Quality Manager) **Date:** 10/10/2013

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D-2 Certificate of Analysis for Rebpure RM95 Batch 20131008

Certificate of Analysis

GLG
Research and Development
GLG Life Tech Corporation
www.glglifetech.com
GLG-QA-COA-062

Product: Rebpure™ RM95
Lot Number: GLG-RM95-20131008

Manufacturing Date: Oct. 8th, 2013
Country of Origin: China
Shelf Life: 2 years

Product Description: Rebpure™ RM95 is a highly purified extract containing rebaudioside M (MW: 1291.3) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages. Rebpure™ RM95 meets JECFA requirements completely.

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Manufacturing By:
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Fax:+86.532.83181836

Qingdao Runde Biotechnology Company, Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Date of Analysis: Oct. 13th, 2013

CHARACTERISTIC	SPECIFICATION	RESULT	METHOD
Rebaudioside M (wt/wt)	≥95.0% (on dry basis)	96.4%	JECFA HPLC
Total Steviol Glycosides (wt/wt)	≥97.0% (on dry basis)	97.5%	JECFA HPLC
Arsenic (as As)	≤1.0 ppm	0.03ppm	AAS
Lead (as Pb)	≤1.0 ppm	0.02ppm	AAS
Cadmium (as Cd)	≤1.0 ppm	0.02ppm	AAS
Mercury (as Hg)	≤1.0 ppm	0.01ppm	AAS
Residual solvents -Ethanol	≤5000 ppm	289ppm	GC
-Methanol	≤200 ppm	56ppm	GC
Loss on Drying	≤4.0 %	3.2%	105℃,2hr
PH	4.5-7.0	5.11	1 in 100 solution,wt/v
Residue on Ignition (wt/wt)	≤1.0%	0.06%	FCC 6
Total Plate Count	<1000 CFU/g	< 10 cfu/g	FDA-BAM current ed.
Yeast & Mold	<100 CFU/g	< 10cfu/g	FDA-BAM current ed.
E.coli	Negative	Negative	FDA-BAM current ed.
Staphylococcus aureus	Negative	Negative	FDA-BAM current ed.
Salmonella (/25g)	Negative	Negative	FDA-BAM current ed.

Conclusion	(b) (6)

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

Analyzed by: (b) (6) **Date:** 13/10/2013

Checked by: (b) (6) **Date:** 13/10/2013

Approved by: (b) (6) (Quality Manager) **Date:** 13/10/2013

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D-3 Certificate of Analysis for Rebpure RM95 Batch 20131012

Certificate of Analysis

GLG
Research and Development
GLG Life Tech Corporation
www.glglifetech.com
GLG-QA-COA-062

Product: Rebpure™ RM95
Lot Number: GLG-RM95-20131012
Manufacturing Date: Oct.10th, 2013
Country of Origin: China
Shelf Life: 2 years

Product Description: Rebpure™ RM95 is a highly purified extract containing rebaudioside M (MW: 1291.3) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages. Rebpure™ RM95 meets JECFA requirements completely.

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Email: sales@glglifetech.com
Web: www.glglifetech.com

Manufacturing By: Qingdao Runde Biotechnology Co., Ltd
Lingshanwei Town, Jiaonan County
Qingdao, Shandong, China 266427
Phone: +86.532.83181169
Fax:+86.532.83181836

Qingdao Runde Biotechnology Company, Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Date of Analysis: Oct. 15th, 2013

CHARACTERISTIC	SPECIFICATION	RESULT	METHOD
Rebaudioside M (wt/wt)	≥95.0% (on dry basis)	98.2%	JECFA HPLC
Total Steviol Glycosides (wt/wt)	≥97.0% (on dry basis)	97.3%	JECFA HPLC
Arsenic (as As)	≤1.0 ppm	0.01ppm	AAS
Lead (as Pb)	≤1.0 ppm	0.02ppm	AAS
Cadmium (as Cd)	≤1.0 ppm	0.01ppm	AAS
Mercury (as Hg)	≤1.0 ppm	0.01ppm	AAS
Residual solvents -Ethanol	≤5000 ppm	465ppm	GC
-Methanol	≤200 ppm	82ppm	GC
Loss on Drying	≤4.0 %	3.3%	105℃,2hr
PH	4.5-7.0	5.14	1 in 100 solution,wt/v
Residue on Ignition (wt/wt)	≤1.0%	0.09%	FCC 6
Total Plate Count	<1000 CFU/g	< 10 cfu/g	FDA-BAM current ed.
Yeast & Mold	<100 CFU/g	< 10cfu/g	FDA-BAM current ed.
E.coli	Negative	Negative	FDA-BAM current ed.
Staphylococcus aureus	Negative	Negative	FDA-BAM current ed.
Salmonella (/25g)	Negative	Negative	FDA-BAM current ed.

Conclusion	(b) (6)

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

Analyzed by: (b) (6) **Date:** 15/10/2013

Checked by: (b) (6) **Date:** 15/10/2013

Approved by: (b) (6) (Quality Manager) **Date:** 15/10/2013

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D-4 Certificate of Analysis for Rebpure RM95 Batch 20131016

Certificate of Analysis

GLG
Research and Development
GLG Life Tech Corporation
www.glglifetech.com
GLG-QA-COA-062

Product: Rebpure™ RM95
Lot Number: GLG-RM95-20131016

Manufacturing Date: Oct.18th, 2013
Country of Origin: China
Shelf Life: 2 years

Product Description: Rebpure™ RM95 is a highly purified extract containing rebaudioside M (MW: 1291.3) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages. Rebpure™ RM95 meets JECFA requirements completely.

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Manufacturing By: Qingdao Runde Biotechnology Co., Ltd
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Fax:+86.532.83181836

Qingdao Runde Biotechnology Company, Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Date of Analysis: Oct. 23rd, 2013

CHARACTERISTIC	SPECIFICATION	RESULT	METHOD
Rebaudioside M (wt/wt)	≥95.0% (on dry basis)	96.4%	JECFA HPLC
Total Steviol Glycosides (wt/wt)	≥97.0% (on dry basis)	97.4%	JECFA HPLC
Arsenic (as As)	≤1.0 ppm	0.03ppm	AAS
Lead (as Pb)	≤1.0 ppm	0.01ppm	AAS
Cadmium (as Cd)	≤1.0 ppm	0.02ppm	AAS
Mercury (as Hg)	≤1.0 ppm	0.03ppm	AAS
Residual solvents -Ethanol	≤5000 ppm	392ppm	GC
-Methanol	≤200 ppm	59ppm	GC
Loss on Drying	≤4.0 %	2.9%	105℃,2hr
PH	4.5-7.0	5.10	1 in 100 solution,wt/v
Residue on Ignition (wt/wt)	≤1.0%	0.06%	FCC 6
Total Plate Count	<1000 CFU/g	< 10 cfu/g	FDA-BAM current ed.
Yeast & Mold	<100 CFU/g	< 10cfu/g	FDA-BAM current ed.
E.coli	Negative	Negative	FDA-BAM current ed.
Staphylococcus aureus	Negative	Negative	FDA-BAM current ed.
Salmonella (/25g)	Negative	Negative	FDA-BAM current ed.

Conclusion	(b) (6)

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

Analyzed by: (b) (6) **Date:** 23/10/2013

Checked by: (b) (6) **Date:** 23/10/2013

Approved by: (b) (6) (Quality Manager) **Date:** 23/10/2013

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Suite 2168-1050 west Pender Street * Vancouver, B.C.* Canada * V6E 3S7
Phone: 1.604.669.2602 * Fax: 1.604.662.8858 * Email: sales@glglifetech.com * Web: glglifetech.com

D-5 Certificate of Analysis for Rebpure RM95 Batch 20131020

Certificate of Analysis

GLG
Research and Development
GLG Life Tech Corporation
www.glglifetech.com
GLG-QA-COA-062

Product: Rebpure™ RM95
Lot Number: GLG-RM95-20131020
Manufacturing Date: Oct.20th, 2013
Country of Origin: China
Shelf Life: 2 years

Product Description: Rebpure™ RM95 is a highly purified extract containing rebaudioside M (MW: 1291.3) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages. Rebpure™ RM95 meets JECFA requirements completely.

Distributed By: GLG Life Tech Corporation
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Email: sales@glglifetech.com
Web: www.glglifetech.com

Manufacturing By: Qingdao Runde Biotechnology Co., Ltd
Lingshanwei Town, Jiaonan County
Qingdao, Shandong, China 266427
Phone: +86.532.83181169
Fax:+86.532.83181836

Qingdao Runde Biotechnology Company, Ltd. is a wholly owned foreign subsidiary of GLG Life Tech Corporation.

Date of Analysis: Oct. 25th, 2013

CHARACTERISTIC	SPECIFICATION	RESULT	METHOD
Rebaudioside M (wt/wt)	≥95.0% (on dry basis)	96.3%	JECFA HPLC
Total Steviol Glycosides (wt/wt)	≥97.0% (on dry basis)	97.4%	JECFA HPLC
Arsenic (as As)	≤1.0 ppm	0.01ppm	AAS
Lead (as Pb)	≤1.0 ppm	0.01ppm	AAS
Cadmium (as Cd)	≤1.0 ppm	0.02ppm	AAS
Mercury (as Hg)	≤1.0 ppm	0.01ppm	AAS
Residual solvents -Ethanol	≤5000 ppm	421ppm	GC
-Methanol	≤200 ppm	71ppm	GC
Loss on Drying	≤4.0 %	2.8%	105℃,2hr
PH	4.5-7.0	5.15	1 in 100 solution,wt/v
Residue on Ignition (wt/wt)	≤1.0%	0.08%	FCC 6
Total Plate Count	<1000 CFU/g	< 10 cfu/g	FDA-BAM current ed.
Yeast & Mold	<100 CFU/g	< 10cfu/g	FDA-BAM current ed.
E.coli	Negative	Negative	FDA-BAM current ed.
Staphylococcus aureus	Negative	Negative	FDA-BAM current ed.
Salmonella (/25g)	Negative	Negative	FDA-BAM current ed.

Conclusion	(b) (6)

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

Analyzed by: (b) (6) **Date:** 25/10/2013

Checked by: (b) (6) **Date:** 25/10/2013

Approved by: (b) (6) (Quality Manager) **Date:** 25/10/2013

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APPENDIX E

Pesticide Analytical Report for Rebpure™ RM95 from Intertek

Test Report for Pesticides for Lot GLG-RM95-20131008

Intertek

No.: **SHF141B0548**

TEST REPORT
检测报告

Name of Sample: 样品名称：	甜菊糖苷/Steviol Glycosides Rebaudioside M 95
Applicant: 委托单位：	Qingdao Runde Biotechnology Co.,Ltd. 青岛润德生物科技有限公司 Lingshanwei Town,Jiaonan City Qingdao, Shangdong, China 266427 青岛胶南市灵山卫镇驻地
Test Purpose: 检验类别：	**ENTRUST TEST** 委托检验

Intertek Testing Services Ltd., Shanghai
6/F., No. 2 Building, Shanghai Comalong Industrial Park,
No. 889 Yishan Road, Shanghai, 200233, China.
上海天祥质量技术服务有限公司
中国 上海市宜山路 889 号齐来工业城 2 号楼 6 层西侧 邮政编码: 200233
Tel: 0086-21-61206565，Fax: 0086-21-64954500

Intertek

TEST REPORT (检测报告) **REPORT No.: SHF141B0548**

NAME OF SAMPLE 产品名称	甜菊糖苷/Steviol Glycosides Rebaudioside M 95	TEST PURPOSE 检验类别	ENTRUST TEST 委托检测
SPECIFICATION 型号、规格、等级	RM95	TRADEMARK 商标	/
SAMPLE DESCRIPTION 样品描述	Solid in bulk 散装固体		
CLIENT 委托单位	Qingdao Runde Biotechnology Co.,Ltd. 青岛润德生物科技有限公司		
MANUFACTURER 生产单位	Qingdao Runde Biotechnology Co.,Ltd. 青岛润德生物科技有限公司		
DATE SAMPLE RECEIVED 委托样品收到日期	Feb. 17, 2014 2014 年 02 月 17 日	SAMPLE QUANTITY 样品数量	100g
PRODUCTION DATE 生产日期	2014.2.11	CODE / NUMBER 批号或编号	Lot No: GLG-RM95-20131008
TEST STANDARD 检验依据或判定标准	实验室方法		
TEST PERIOD 检验日期	Feb. 17, 2014 To Feb. 21, 2014 2014 年 02 月 17 日至 2014 年 02 月 21 日		
TEST CONTENT 检验内容	CIRCUMSTANCES ARE IN THE REPORT ATTACHMENT.详见检验报告附页。		
CONCLUSION 检验结论	SUPPLY REAL MEASURING DATA 提供实测数据 CIRCUMSTANCES ARE IN THE REPORT ATTACHMENT 详见检验报告附页 签批日期 2014 年 02 月 21 日		
REMARK 备注	THE TESTING RESULTS ARE ONLY VALID FOR THE SAMPLE TESTED. 检测结果仅对来样负责。 WITHOUT CONSENT OF THE TESTING ORGANIZATION, THE CLIENTS SHALL NOT BE UNAUTHORIZED USE OF TEST RESULTS FOR IMPROPER PROPAGANDA. 未经检测机构同意，委托人不得擅自使用检验结果进行不当宣传。 THE TESTING RESULTS ARE ONLY FOR THE REFERENCE OF TESTING RESEARCH, CAN NOT BE USED AS SOCIAL JUSTICE DATA. 该检测数据仅供测试研究用参考，不做为社会公正性数据。		

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PREPARED AND CHECKED BY:
INTERTEK TESTING SERVICES
LTD., SHANGHAI
(b) (6)
STELLA LUO
SUPERVISOR

AUTHORIZED BY:
INTERTEK TESTING SERVICES
LTD., SHANGHAI
(b) (6)
SAMMY SHEN
ASSISTANT MANAGER

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Intertek

TEST REPORT (检测报告) REPORT No.: SHF141B0548

GC/MS Results Table – GC/MS 结果表
Pesticides Found and Concentration – 发现的农药及其浓度

No 编号	Compound Name 化合物名称		Rpt Limit, mg/kg 检出限	MRLs,mg/kg 限量标准	Result,mg/kg 检测结果	Item Judgement 单项判定
1	2-phenylphenol	2-苯基苯酚	0.01	——	ND 未检出	——
2	2，4'-DDE	o,p'-滴滴伊	0.01	——	ND 未检出	——
3	2，4'-DDD	o,p'-滴滴滴	0.01	——	ND 未检出	——
4	2，4'-DDT	o,p'-滴滴涕	0.01	——	ND 未检出	——
5	4，4'-DDD	p-p'-滴滴滴	0.01	——	ND 未检出	——
6	4，4'-DDE	p,p'-滴滴伊	0.01	——	ND 未检出	——
7	4，4'-DDT	p,p'-滴滴涕	0.01	——	ND 未检出	——
8	Acephate	乙酰甲胺磷	0.02	——	ND 未检出	——
9	BHC	六六六	0.01	——	ND 未检出	——
10	Acetochlor	乙草胺	0.01	——	ND 未检出	——
11	Ametryn	莠灭净	0.01	——	ND 未检出	——
12	Aldrin	艾氏剂	0.005	——	ND 未检出	——
13	Dieldrin	狄氏剂	0.005	——	ND 未检出	——
14	Atrazine	莠去津	0.01	——	ND 未检出	——
15	Benalaxyl	苯霜灵	0.01	——	ND 未检出	——
16	Benfluralin	乙丁氟灵	0.01	——	ND 未检出	——
17	Benoxacor	解草酮	0.01	——	ND 未检出	——
18	Bifenthrin	联苯菊酯	0.01	——	ND 未检出	——
19	Bromopropylate	溴螨酯	0.01	——	ND 未检出	——
20	Bupirimate	乙嘧酚磺酸酯	0.01	——	ND 未检出	——
21	Buprofezin	噻嗪酮	0.01	——	ND 未检出	——
22	Butachlor	丁草胺	0.01	——	ND 未检出	——
23	Cadusafos	硫线磷	0.01	——	ND 未检出	——
24	Captan	克菌丹	0.01	——	ND 未检出	——
25	Chlordane	克氯丹	0.01	——	ND 未检出	——
26	Chlorfenapyr	溴虫腈	0.05	——	ND 未检出	——
27	Chlorfenvinphos	毒虫畏	0.01	——	ND 未检出	——
28	Chlorfluazuron	氟啶脲	0.01	——	ND 未检出	——
29	Chlorpropham	氯苯胺灵	0.01	——	ND 未检出	——
30	Chlorpyrifos	毒死蜱	0.01	——	ND 未检出	——

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TEST REPORT (检测报告) REPORT No.: SHF141B0548

GC/MS Results Table – GC/MS 结果表
Pesticides Found and Concentration – 发现的农药及其浓度

No 编号	Compound Name 化合物名称		Rpt Limit, mg/kg 检出限	MRLs,mg/kg 限量标准	Result,mg/kg 检测结果	Item Judgement 单项判定
31	Chlorpyrifos-methyl	甲基毒死蜱	0.01	——	ND 未检出	——
32	Clethodim	烯草酮	0.01	——	ND 未检出	——
33	Coumaphos	蝇毒磷	0.01	——	ND 未检出	——
34	Cyanazine	草净津	0.05	——	ND 未检出	——
35	Cyflufenamid	环氟菌胺	0.02	——	ND 未检出	——
36	Cyfluthrin	氟氯氰菊酯	0.01	——	ND 未检出	——
37	Cypermethrin	氯氰菊酯	0.01	——	ND 未检出	——
38	Cyprodinil	嘧菌环胺	0.05	——	ND 未检出	——
39	Deltamethrin	溴氰菊酯	0.01	——	ND 未检出	——
40	Diazinon	二嗪磷	0.01	——	ND 未检出	——
41	Dichlofluanid	苯氟磺胺	0.01	——	ND 未检出	——
42	Dichlorvos	敌敌畏	0.01	——	ND 未检出	——
43	Dicloran	氯硝胺	0.01	——	ND 未检出	——
44	Dicofol	三氯杀螨醇	0.01	——	ND 未检出	——
45	Diethofencarb	乙霉威	0.01	——	ND 未检出	——
46	Difenoconazole	苯醚甲环唑	0.05	——	ND 未检出	——
47	Diphenylamine	二苯胺	0.01	——	ND 未检出	——
48	Edifenphos	克瘟散	0.01	——	ND 未检出	——
49	Endosulfan-1	硫丹-1	0.01	——	ND 未检出	——
50	Endosulfan-2	硫丹-2	0.01	——	ND 未检出	——
51	Endosulfan-sulfate	硫丹硫酸酯	0.01	——	ND 未检出	——
52	Endrin	异狄氏剂	0.01	——	ND 未检出	——
53	EPN	苯硫磷	0.01	——	ND 未检出	——
54	Ethion	乙硫磷	0.01	——	ND 未检出	——
55	Ethoprophos	灭线磷	0.01	——	ND 未检出	——
56	Etofenprox	醚菊酯	0.01	——	ND 未检出	——
57	Etrimfos	乙嘧硫磷	0.01	——	ND 未检出	——
58	Fenamiphos	苯线磷	0.01	——	ND 未检出	——
59	Fenarimol	氯苯嘧啶醇	0.01	——	ND 未检出	——
60	Fenitrothion	杀螟硫磷	0.01	——	ND 未检出	——

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TEST REPORT (检测报告) REPORT No.: SHF141B0548

GC/MS Results Table – GC/MS 结果表
Pesticides Found and Concentration – 发现的农药及其浓度

No 编号	Compound Name 化合物名称		Rpt Limit, mg/kg 检出限	MRLs,mg/kg 限量标准	Result,mg/kg 检测结果	Item Judgement 单项判定
61	Fenoxycarb	苯醚威	0.01	——	ND 未检出	——
62	Fenpropathrin	甲氰菊酯	0.05	——	ND 未检出	——
63	Fenpropimorph	丁苯吗啉	0.05	——	ND 未检出	——
64	Fenobucarb	仲丁威	0.01	——	ND 未检出	——
65	Fenthion	倍硫磷	0.01	——	ND 未检出	——
66	Fenvalerate	氰戊菊酯	0.01	——	ND 未检出	——
67	Fipronil	氟虫腈	0.005	——	ND 未检出	——
68	Fluazifop-P-butyl	吡氟禾草灵	0.01	——	ND 未检出	——
69	Flucythrinate	氟氰戊菊酯	0.01	——	ND 未检出	——
70	Flufenoxuron	氟虫脲	0.01	——	ND 未检出	——
71	Flumioxazin	氟恶嗪酮	0.05	——	ND 未检出	——
72	Flusilazole	氟硅唑	0.01	——	ND 未检出	——
73	Fluvalinate	氟胺氰菊酯	0.05	——	ND 未检出	——
74	Gamma-HCH	林丹	0.01	——	ND 未检出	——
75	Halfenprox	卤醚菊酯	0.01	——	ND 未检出	——
76	Heptachlor	七氯	0.01	——	ND 未检出	——
77	Hexachlorobenzene	六氯苯	0.01	——	ND 未检出	——
78	Hexythiazox	噻螨酮	0.05	——	ND 未检出	——
79	Imazalil	稀菌灵	0.01	——	ND 未检出	——
80	Iprobenfos	异稻瘟净	0.01	——	ND 未检出	——
81	Iprodione	异菌脲	0.01	——	ND 未检出	——
82	Isocarbophos	水胺硫磷	0.01	——	ND 未检出	——
83	Isofenphos	异柳磷	0.01	——	ND 未检出	——
84	Isoprothiolane	稻瘟灵	0.01	——	ND 未检出	——
85	Isoprocarb	异丙威	0.01	——	ND 未检出	——
86	Kresoxim-methyl	肟菌酯	0.01	——	ND 未检出	——
87	Lambda-cyhalothrin	高效氯氟氰菊酯	0.01	——	ND 未检出	——
88	Malathion	马拉硫磷	0.01	——	ND 未检出	——
89	Mefenoxam	精甲霜灵	0.01	——	ND 未检出	——
90	Metalaxyl	甲霜灵	0.01	——	ND 未检出	——

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TEST REPORT (检测报告) REPORT No.: SHF141B0548

GC/MS Results Table – GC/MS 结果表
Pesticides Found and Concentration – 发现的农药及其浓度

No 编号	Compound Name 化合物名称		Rpt Limit, mg/kg 检出限	MRLs,mg/kg 限量标准	Result,mg/kg 检测结果	Item Judgement 单项判定
91	Metamitron	灭它通	0.05	——	ND 未检出	——
92	Methamidophos	甲胺磷	0.01	——	ND 未检出	——
93	Methidathion	杀扑磷	0.01	——	ND 未检出	——
94	Metolachlor	异丙甲草胺	0.01	——	ND 未检出	——
95	Mevinphos	速灭磷	0.01	——	ND 未检出	——
96	Myclobutanil	腈菌唑	0.01	——	ND 未检出	——
97	Napropamide	敌草胺	0.01	——	ND 未检出	——
98	Nitrothal-isopropyl	酞菌酯	0.01	——	ND 未检出	——
99	Oxadixyl	恶霜灵	0.01	——	ND 未检出	——
100	Oxadiazon	恶草灵	0.01	——	ND 未检出	——
101	Oxyfluorfen	乙氧氟草醚	0.01	——	ND 未检出	——
102	Paclobutrazol	多效唑	0.01	——	ND 未检出	——
103	Paraoxon-ethyl	对氧磷	0.01	——	ND 未检出	——
104	Parathion	对硫磷	0.01	——	ND 未检出	——
105	Parathion-methyl	甲基对硫磷	0.01	——	ND 未检出	——
106	Propham	苯胺灵	0.01	——	ND 未检出	——
107	Penconazole	戊菌唑	0.01	——	ND 未检出	——
108	Pendimethalin	二甲戊灵	0.01	——	ND 未检出	——
109	Permethrin	氯菊酯	0.01	——	ND 未检出	——
110	Phenthoate	稻丰散	0.01	——	ND 未检出	——
111	Phorate	甲拌磷	0.01	——	ND 未检出	——
112	Phosalone	伏杀硫磷	0.01	——	ND 未检出	——
113	Phosmet	亚胺硫磷	0.01	——	ND 未检出	——
114	Pirimicarb	抗蚜威	0.01	——	ND 未检出	——
115	Pirimiphos-ethyl	嘧啶磷	0.01	——	ND 未检出	——
116	Pirimiphos-methyl	甲基嘧啶磷	0.01	——	ND 未检出	——
117	Prochloraz	咪鲜胺	0.05	——	ND 未检出	——
118	Propetamphos	胺丙畏	0.01	——	ND 未检出	——
119	Phosphamidon	磷胺	0.01	——	ND 未检出	——
120	Procymidone	腐霉利	0.01	——	ND 未检出	——

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TO BE CONTINUED(接下页)

Intertek

TEST REPORT (检测报告) REPORT No.: SHF141B0548

GC/MS Results Table – GC/MS 结果表
Pesticides Found and Concentration – 发现的农药及其浓度

No 编号	Compound Name 化合物名称		Rpt Limit, mg/kg 检出限	MRLs,mg/kg 限量标准	Result,mg/kg 检测结果	Item Judgement 单项判定
121	Profenofos	丙溴磷	0.01	——	ND 未检出	——
122	Prometryn	扑草净	0.01	——	ND 未检出	——
123	Propargite	炔螨特	0.01	——	ND 未检出	——
124	Propiconazole	丙环唑	0.01	——	ND 未检出	——
125	Propoxur	残杀威	0.01	——	ND 未检出	——
126	Propyzamide	炔苯酰草胺	0.01	——	ND 未检出	——
127	Pymetrozine	抑嘧酮	0.02	——	ND 未检出	——
128	Pyrazophos	吡菌磷	0.01	——	ND 未检出	——
129	Pyridaben	哒螨灵	0.01	——	ND 未检出	——
130	Pyridaphenthion	哒嗪硫磷	0.01	——	ND 未检出	——
131	Pyrimethanil	甲基嘧菌胺	0.01	——	ND 未检出	——
132	Quinalphos	喹硫磷	0.05	——	ND 未检出	——
133	Quintozene	五氯硝基苯	0.01	——	ND 未检出	——
134	Simazine	西玛津	0.05	——	ND 未检出	——
135	Spiroxamine	谷物和葡萄杀菌剂	0.01	——	ND 未检出	——
136	S-421	S-421	0.01	——	ND 未检出	——
137	TBHQ	特丁基对苯二酚	0.01	——	ND 未检出	——
138	Tebuconazole	戊唑醇	0.05	——	ND 未检出	——
139	Tecnazene	四氯硝基苯	0.01	——	ND 未检出	——
140	Tetrachlorvinphos	杀虫畏	0.01	——	ND 未检出	——
141	Tetradifon	三氯杀螨砜	0.01	——	ND 未检出	——
142	Thiamethoxam	噻虫嗪	0.05	——	ND 未检出	——
143	Tolclofos-methyl	甲基立枯磷	0.01	——	ND 未检出	——
144	Tolyfluanid	甲苯氟磺胺	0.05	——	ND 未检出	——
145	Triadimefon	三唑酮	0.01	——	ND 未检出	——
146	Triadimenol	三唑醇	0.01	——	ND 未检出	——
147	Triazophos	三唑磷	0.01	——	ND 未检出	——
148	Trifloxystrobin	肟菌酯	0.01	——	ND 未检出	——
149	Trifluralin	氟乐灵	0.01	——	ND 未检出	——
150	Vinclozolin	乙烯菌核利	0.01	——	ND 未检出	——

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Intertek

TEST REPORT (检测报告) **REPORT No.: SHF141B0548**

LC/MS/MS Results Table – LC/MS/MS 结果表
Pesticides Found and Concentration – 发现的农药及其浓度

No 编号	Compound Name 化合物名称		Rpt Limit, mg/kg 检出限	MRLs,mg/kg 限量标准	Result,mg/kg 检测结果	Item Judgement 单项判定
1	3-hydroxycarbofuran	3-羟基呋喃丹	0.01	——	ND 未检出	——
2	Abamectin	阿维菌素	0.01	——	ND 未检出	——
3	Acetamiprid	啶虫脒	0.01	——	ND 未检出	——
4	Aldicarb	涕灭威	0.01	——	ND 未检出	——
5	Aldicarb sulfone	涕灭威砜	0.01	——	ND 未检出	——
6	Aldicarb sulfoxide	涕灭威亚砜	0.01	——	ND 未检出	——
7	Aldoxycarb	铁灭威	0.01	——	ND 未检出	——
8	Allethrin	丙烯菊酯	0.01	——	ND 未检出	——
9	Azinphos-methyl	保棉磷	0.01	——	ND 未检出	——
10	Azoxystrobin	嘧菌酯	0.005	——	ND 未检出	——
11	Bendiocarb	恶虫威	0.005	——	ND 未检出	——
12	Benfuracarb	丙硫克百威	0.005	——	ND 未检出	——
13	Bensulfuron-methyl	苄嘧磺隆	0.005	——	ND 未检出	——
14	Benzoyl Peroxide	过氧化苯甲酰	0.005	——	ND 未检出	——
15	Boscalid	啶酰菌胺	0.005	——	ND 未检出	——
16	Butocarboxim	丁硫威	0.01	——	ND 未检出	——
17	Captafol	敌菌丹	0.01	——	ND 未检出	——
18	Carbaryl	甲萘威	0.005	——	ND 未检出	——
19	Carbendazim	多菌灵	0.005	——	ND 未检出	——
20	Carbofuran	虫螨威	0.01	——	ND 未检出	——
21	Chlorbenzuron	灭幼脲	0.01	——	ND 未检出	——
22	Chlorothalonil	百菌清	0.01	——	ND 未检出	——
23	Cyclamic Acid	甜蜜素	0.005	——	ND 未检出	——
24	Cymoxanil	霜脲氰	0.01	——	ND 未检出	——
25	Cyromazine	灭蝇胺	0.005	——	ND 未检出	——
26	Daminozide	丁酰肼	0.01	——	ND 未检出	——
27	Dimethoate	乐果	0.005	——	ND 未检出	——
28	Omethoate	氧乐果	0.002	——	ND 未检出	——
29	Dimethomorph	烯酰吗啉	0.005	——	ND 未检出	——
30	Dithiocarbamates	二硫代氨基甲酸酯	0.01	——	ND 未检出	——

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LC/MS/MS Results Table – LC/MS/MS 结果表
Pesticides Found and Concentration – 发现的农药及其浓度

No 编号	Compound Name 化合物名称		Rpt Limit, mg/kg 检出限	MRLs,mg/kg 限量标准	Result,mg/kg 检测结果	Item Judgement 单项判定
31	Emamectin benzoate	甲胺基阿维菌素苯甲酸盐	0.01	——	ND 未检出	——
32	Ethiofencarb	乙硫甲威	0.005	——	ND 未检出	——
33	Ethoxyquin	乙氧喹啉	0.01	——	ND 未检出	——
34	Fenhexamid	环乙酰胺类化合物	0.01	——	ND 未检出	——
35	Fthalide	四氯苯酞	0.01	——	ND 未检出	——
36	Furathiocarb	呋线威	0.005	——	ND 未检出	——
37	Glyphosate	草甘磷	0.01	——	ND 未检出	——
38	Heptenophos	庚烯磷	0.005	——	ND 未检出	——
39	Imidacloprid	吡虫啉	0.01	——	ND 未检出	——
40	Indoxacarb	茚虫威	0.005	——	ND 未检出	——
41	Iprovalicarb	丙森锌	0.005	——	ND 未检出	——
42	Isofenphos-methyl	甲基异硫磷	0.005	——	ND 未检出	——
43	Isoproturon	异丙隆	0.002	——	ND 未检出	——
44	Linuron	利谷隆	0.002	——	ND 未检出	——
45	Methiocarb	灭虫威	0.005	——	ND 未检出	——
46	Methomyl	灭多威	0.01	——	ND 未检出	——
47	Thiodicarb	硫双威	0.01	——	ND 未检出	——
48	Methoxyfenozide	甲氧虫酰肼	0.005	——	ND 未检出	——
49	Monocrotophos	久效磷	0.005	——	ND 未检出	——
50	Naled	二溴磷	0.01	——	ND 未检出	——
51	Nicosulfuron	烟嘧磺隆	0.005	——	ND 未检出	——
52	Oxydemeton-methyl	砜吸磷	0.01	——	ND 未检出	——
53	Phoxim	辛硫磷	0.005	——	ND 未检出	——
54	Promecarb	猛杀威	0.01	——	ND 未检出	——
55	Propamocarb	霜霉威	0.005	——	ND 未检出	——
56	Quizalofop-ethyl	禾草克	0.005	——	ND 未检出	——
57	Rimsulfuron	砜嘧磺隆	0.01	——	ND 未检出	——
58	Spinosad	多杀菌素	0.01	——	ND 未检出	——
59	Tebufenozide	虫酰肼	0.01	——	ND 未检出	——
60	Terbufos	丁硫磷	0.01	——	ND 未检出	——

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TEST REPORT (检测报告) REPORT No.: SHF141B0548

LC/MS/MS Results Table – LC/MS/MS 结果表
Pesticides Found and Concentration – 发现的农药及其浓度

No 编号	Compound Name 化合物名称		Rpt Limit, mg/kg 检出限	MRLs,mg/kg 限量标准	Result,mg/kg 检测结果	Item Judgement 单项判定
61	Thiabendazole	噻菌灵	0.01	——	ND 未检出	——
62	Thiacloprid	噻虫啉	0.005	——	ND 未检出	——
63	Thifensulfuron-methyl	阔叶散	0.01	——	ND 未检出	——
64	Thiofanox-sulfone	久效威砜	0.005	——	ND 未检出	——
65	Thiofanox-sulfoxid	久效威亚砜	0.01	——	ND 未检出	——
66	Thiophanate-methyl	甲基托布津	0.01	——	ND 未检出	——
67	Tralomethrin	四溴菊酯	0.01	——	ND 未检出	——
68	Triasulfuron	醚苯磺隆	0.01	——	ND 未检出	——
69	Trichlorfon	敌百虫	0.005	——	ND 未检出	——
70	Triflumizole	氟菌唑	0.005	——	ND 未检出	——
71	Triflusulfuron-methyl	氟胺磺隆	0.01	——	ND 未检出	——
72	Vamidothion	完灭硫磷	0.01	——	ND 未检出	——

Remark: ND=less than the limitation of detection
备注:未检出表示低于检出限

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END OF REPORT (结束)

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APPENDIX F

Sweetness Intensity Test Report for Rebpure™ RM95

GLG LIFE TECH CORPORATION	Issue Date:20/12/2013
Sweetness Intensity Data of Steviol Glycosides RM95	File No:GLG-QA-SD-109

Sweetness Data for Steviol Glycosides

Prepared by: Zhang Lei (QA/QC Manager)

Date: 20/12/2013

Approved by: Kevin Li (VP of Technology)

Date: 20/12/2013

GLG LIFE TECH CORPORATION	Issue Date:20/12/2013
Sweetness Intensity Data of Steviol Glycosides RM95	File No:GLG-QA-SD-109

Objective

To determine sweetness intensity of *Steviol Glycosides RM95* produced by *GLG Life Tech Corporation.*

Samples

Samples representing commercial lot of *Steviol Glycosides* labeled as " GLG-RM95-20131002 "

Solvents and Reagents

- Sucrose
- Purified water,
- Unsalted crackers

Apparatus

1. Analytical balance, XS205, (Mettler Toledo, USA);
2. Volumetric (class ***A***) and Laboratory glassware.

ASSAY and Procedures

The sweetness intensity tests are following with "ISO 8587:2006 Sensory Analysis-Methodology-Ranking" testing method.
28 panelists have been previously qualified for taste acuity and trained for the sweetness intensity test. The panelists were presented with 2 samples (5.0% of sucrose water solution and RM95 water solutions).

Test Results

Test results, see Table 1.

Table 1, Sweetness Potency of Steviol Glycoside RM95

Sample	Steviol glycosides concentration % (Sweetness equivalent to 5.0% of sucrose at 20 ° C)	Sweetness Intensity
RM95	0.013	About 380 times sweeter than sucrose

APPENDIX G

Stability Testing Report for Rebpure™ RM95

GLG LIFE TECH CORPORATION	Issue Date:10/12/2013
GLG Storage Stability Data of Steviol Glycosides	File No: GLG-QA-SSD-RM95

GLG Storage Stability Data for Steviol Glycosides

Prepared by: Zhang Lei (QA/QC Manager, GLG Life Tech Corporation)

Date: 10/12/2013

Approved by: Kevin Li (VP of Technology, GLG Life Tech Corporation)

Date: 10/12/2013

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GLG LIFE TECH CORPORATION	Issue Date:10/12/2013
GLG Storage Stability Data of Steviol Glycosides	File No: GLG-QA-SSD-RM95

Objective

To determine storage stability of Steviol Glycosides produced by GLG.

Samples

One sample representing commercial lot of Steviol Glycosides labeled as "GLG-RM95-20131002".

Standards

1. Rebaudioside M Standard; (Chromadex Inc. Irvine, CA，USA);
2. Rebaudioside D Standard; (Chromadex Inc. Irvine, CA，USA);
3.Stevioside Standard; (Chromadex Inc. Irvine, CA，USA);
4, Rebaudioside A Standard; (Chromadex Inc. Irvine, CA USA)

Solvents and Reagents

- Acetonitrile, HPLC grade (Merck, Germany);
- Water, HPLC grade (Millipore, Germany);
- Ammonium acetate, reagent grade (Merck, Germany);
- Acetic acid, reagent grade (Merck, Germany).

Apparatus

1. Agilent1200 HPLC system equipped with binary pump, auto sampler, thermostatted column compartment and UV detector, (Agilent Technologies, USA);
2. Analytical column, Luna 5μ C18 (2) 100A (Phenomenex, USA)
3. Analytical balance, XS205, (Mettler Toledo, USA);
4. Karl Fischer coulometer, Mettler Toledo DL-39, (Mettler Toledo, USA);
6. Volumetric (class ***A)*** and Laboratory glassware.

Sample Storage

Sample was stored in original packaging at 25°C± 5°C and 60% ± 5% relative humidity.

Solution Preparation

Sample solutions were prepared at approx. 5,000 ppm concentration in Diluent

Determination of rebaudioside M and related steviol glycosides

Analysis and calculations were performed as per *FCC* 6 monograph method for Rebaudioside M and Related Steviol Glycosides .The assay results are summarized in Table 1.

GLG LIFE TECH CORPORATION	Issue Date:10/12/2013
GLG Storage Stability Data of Steviol Glycosides	File No: GLG-QA-SSD-RM95

Table 1

Lot# GLG-RM95-20131002, Steviol Glycosides, % dry basis							
Duration	Reb M	Reb D	TSG	Total plate count	Salmonella	E. Coli	Staphylococcus
t=0	96.3	1.04	97.4	<10cfu/g	Negative	Negative	Negative
2 weeks	96.2	1.05	97.4	<10cfu/g	Negative	Negative	Negative
4 weeks	96.4	1.0	97.4	<10cfu/g	Negative	Negative	Negative
6 weeks	96.2	1.03	97.2	<10cfu/g	Negative	Negative	Negative
8 weeks	95.9	0.98	96.9	<10cfu/g	Negative	Negative	Negative

References

1 Stability testing of new drug substances and products, *ICH QIA (R) stability testing guidelines,* The European Agency for the Evaluation of Medicinal Products, 2000.
2. Rebaudioside A monograph, *Food Chemicals Codex (FCC) 6, Second Supplement,* 2009, p. 1438-42.

ASSAY PROCEDURE

Acetate buffer: Dissolve 0.125 g ammonium acetate in 900 mL of water, adjust pH to 2.6 with glacial acetic acid solution and dilute to 1 L. [NOTE: It may be necessary to adjust the ratio of ammonium acetate to acetic acid. Changing the pH adjusts the retention time of rebaudioside M and related glycosides. Decreasing the pH of the buffer will decrease the retention time of rebaudioside M.]
Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L ammonium acetate buffer (pH 2.6). After that, flush the column with Mobile phase until the retention time of rebaudioside M is less than 8 min (may take up to one day of flushing).]
RebaudiosideM standard solutions: 4500, 4750, 5250, and 5500 mg/L Rebaudioside A RS in Diluent
Stevioside standard stock solution: 250 mg/L Stevioside RS in Diluent
Stevioside standard solutions: 2.5, 5.0, 10, 25, and 50 mg/L USP Stevioside RS in Diluent: from
Sample solution: 5000 mg/L in Diluent
Chromatographic system
Mode: High-performance liquid chromatography
Detector: UV (210 nm)

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Column: Capcell pak C_{18} 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)

GLG LIFE TECH CORPORATION	Issue Date:10/12/2013
GLG Storage Stability Data of Steviol Glycosides	File No: GLG-QA-SSD-RM95

Column temperature: 40°C
Flow **rate:** 1.0 mL/min
Injection size: 20 µ L
System suitability
Samples: 5250 mg/L Rebaudioside M standard solution, 2.5 mg/L Stevioside standard solution.

Suitability Requirements
Detector response: Peak to noise ratio (peak height /baseline noise) is NLT 3 for the stevioside peak from the 2.5 mg/L Stevioside standard solution, where peak height is expressed in mAU, and baseline noise is the maximum deflection of the baseline (mAU) in a blank at the retention time of stevioside over the same baseline peak width in min.
Relative standard deviation: NMT 2.0% for rebaudioside M peak area and retention time from the 5250 mg/L Rebaudioside M standard solution.
Column efficiency: NLT 5000 theoretical plates count, *N*, using the rebaudioside B peak from the Sample solution.
Retention time: The retention time for the rebaudioside M peak from the 5250 mg/L Rebaudioside M standard solution is less than 5.5 min.
Tailing factor: NMT 2.0 for the rebaudioside B peak from the Sample solution, Stevioside standard solutions, and Sample solution into the chromatograph.

Analysis: Separately inject equal volumes of the Rebaudioside M standard solutions, Stevioside standard solutions, Rebaudioside A Standard solution, and Sample solution into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [NOTE: The approximate retention times for rebaudioside M and its related steviol glycosides are listed below in *Chromotographic Profile Table*1. If the retention time for rebaudioside M is below 5.5 min, adjust the ratio of ammonium acetate to acetic acid.]

*Chromotographic Profile Table*1

Name	ABBR.	Molecular	R_1	R_2	CAS NO	MW	RT(min)
Rebaudioside D	RD	$C_{50}H_{80}O_{28}$	Glcβ(1-2)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	63279-13-0	1129.15	**4.3**
Rebaudioside M (X)	RM/RX	$C_{56}H_{90}O_{33}$	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	1220616-44-3	1291.29	5.1
Rebaudioside A	RA	$C_{44}H_{70}O_{23}$	Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	58543-16-1	967.01	**10.3**

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Prepare a standard curve for rebaudioside M by plotting rebaudioside M peak areas versus concentrations in mg/L, corrected for purity, based on the USP Reference Standard label claim. [NOTE: Peak responses for all other steviol glycosides besides rebaudioside M are used below in the Related Steviol Glycosides impurities test procedure]. From the standard curve, calculate the concentration *(CU)* of rebaudioside *M* in the Sample solution in mg/L. Calculate the percentage of rebaudioside M in the portion of the sample taken by the formula:

CU/CSMPX 100%

Cu = Concentration of rebaudioside M in the *Sample solution* determined from the standard curve (mg/L)

CsMp = Concentration of the sample in the Sample solution (mg/L)

RELATED STEVIOLG LYCOSIDES

Acetate buffer, Mobile phase, Diluent, Rebaudioside M standard solutions, Stevioside standard stock solution, Stevioside standard solutions, Sample solution, Chromatographic system, and System suitability: Prepare as directed in the *Assay* (above).

Analysis: Proceed as directed above in the *Assay,* but with the following modifications for the standard curve and calculations.

Using the peak area responses from the Stevioside standard solutions, prepare a standard curve for stevioside by plotting stevioside peak areas versus concentrations, in mg/L, corrected for purity, based on the USP Reference Standard label claim. From this standard curve, determine the concentration (mg/L) of stevioside in the Sample solution.

Calculate the percent of stevioside in the sample taken using the following formula:

Cu/Csmp x 100%

Cu = Concentration of stevioside in the Sample solution determined from the standard curve (mg/L)

Csmp = Concentration of the sample in the Sample solution (mg/L)

For the seven other steviol glycoside impurities (rubusoside, dulcoside A, rebaudioside C rebaudioside F rebaudioside D, steviolbioside, and rebaudioside B) use the stevioside standard curve prepared above to calculate the mg/L stevioside equivalents for each. Separately calculate the percents of each analyte (rubusoside, dulcoside A, rebaudioside C, rebaudioside F, rebaudioside D, steviolbioside, and rebaudioside B) in the sample taken using the following formula which takes into account the differences in molecular weights between the analytes and stevioside:

(Cu X M_{r} /Me) / CsMp X 100%

Cu = concentration of stevioside equivalents in the *Sample solution* determined from the standard curve (mg/L)

M_{r} = Molecular weight of the analyte (see *Chromotographic Profile Table* 1, above under Assay)

M_{r2}= Molecular weight of stevioside 804.88

$CsMp$ = Concentration of the sample in the Sample solution (mg/L).

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APPENDIX H

Estimated Daily Intake Levels of Steviol Glycosides

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

As part of its safety deliberations, JECFA reviewed various estimates of possible daily intake of steviol glycosides (WHO, 2006). These estimates are presented in Table H-1. Merisant also listed intended use levels of rebaudioside A for various food applications in their GRAS Notification (Table H-2). 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 precentile daily consumption levels of rebaudioside A were estimated as 2.0 and 4.7 mg/kg bw/day, respectively. In its notification, Cargill (2008) utilized a different approach in estimating dietary intake figures for rebaudioside A when incorporated as a general sweetener in a broad cross-section of processed foods. Cargill considered that, with a few minor exceptions, rebaudioside A uses and use levels would be comparable to those of aspartame uses in the US. Using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008), Cargill performed a side-by-side consumption analysis for rebaudioside A versus aspartame. Findings from the above-described different sources along with FSANZ estimates and the intake estimates are presented in Table H-3.

D. Estimated Daily Intake

The very conservative consumer intake estimates provided by JECFA as shown in Table H-1 were utilized to gauge the potential human exposures of rebaudioside A and steviol glycosides and in foods as reported in the US and in other countries. As rebaudioside A is about twice as sweet as the mixed glycosides, these levels can be adjusted accordingly.

Table H-1. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents

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

[a] Reproduced from WHO, 2006.
[b] Calculated by Expert Panel assuming twice the sweetness intensity for rebaudioside A and three-fold difference in molecular weight between rebaudioside A and steviol.

Table H-2. 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."

Further consideration was given to anticipated human exposures as projected independently and with different approaches by JECFA (WHO, 2006), Merisant (2008), and Cargill (2008). As described below, the multiple approaches tended to converge to yield estimated daily intakes (EDIs) in the range of 1.3 – 4.7 mg/kg bw/day that, when compared to the acceptable daily intake (ADI), constitutes supporting information in the subject GRAS evaluation.

JECFA evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that were prepared for the European Commission by the Scientific Committee on Food. JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1. The intakes ranged from 1.3 mg/kg bw/day with the African diet to 3.5 mg/kg bw/day with the European diet. Additionally, JECFA also estimated the per capita exposure derived from disappearance (poundage) data supplied by Japan and China. The Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US. The exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee are summarized in Table H-4.

JECFA concluded that the replacement estimates were highly conservative---that is, the calculated dietary exposure overestimates likely consumption---and that true dietary intakes of steviol glycosides (as steviol) would probably be 20 – 30% of these values or 1.0 - 1.5 mg/kg bw/day on a steviol basis or 3.0 – 4.5 mg/kg bw/day for rebaudioside A based on the molecular weight adjustment. 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, or 0.5 – 1.5 mg/kg bw/day for rebaudioside A when making both the molecular weight and sweetness equivalency calculations. FSANZ examined consumption in other age groups and concluded that there were no safety concerns for 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 Reb A (Renwick, 2008).

Table H-3. Summary of Estimated Daily Intake Assessments for Rebaudioside A & Calculation of Rebaudioside A Values from JECFA & FSANZ Estimates of EDI

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

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

Table H-4. 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.

In October 2009, Cargill applied to FSANZ to increase the maximum usage levels of high purity steviol glycosides in the high volume food categories of ice cream and various beverages. Cargill supported its application with increased usage levels by presenting market share analyses which overestimate actual intake while remaining well below the generally accepted ADI. In December 2010, FSANZ recommended accepting the increased usage levels as requested since no public health and safety issues were identified (FSANZ, 2010). Subsequently, FSANZ approved the Cargill application to increase the allowed maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages and flavored soy beverages up to 200 mg/kg and in plain soy beverages up to 100 mg/kg (FSANZ, 2011).

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

There have been many scholarly estimates of potential dietary intake of replacement sweeteners---including steviol glycosides---that have been published (FSANZ, 2008; Renwick, 2008; WHO, 2003) or submitted to FDA (Merisant, 2008). In GRN 301, a simplified estimate was proposed to and accepted by FDA based on the estimates of exposure in “sucrose equivalents” (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90th percentile consumer of a sweetener which is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg/kg bw/day for any population subgroup.

APPENDIX I

Summary of Published Safety Reviews

1. Summary of JECFA Reviews

At an early review during 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.*
>
> In view of the absence of information for the elaboration of specifications for stevioside and since the evaluation of the available toxicological data revealed several limitations, the Committee was unable to relate the results of the toxicological investigations to the commercial product and could not allocate an ADI to stevioside.
>
> Before reviewing stevioside again, the Committee considered that it would be necessary to develop specifications to ensure that the material tested was representative of the commercial product. Further information on the nature of the substance that was tested, data on the metabolism of stevioside in humans and the results of suitable *in vivo* genotoxicity studies with steviol would also be necessary.

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 new toxicology studies on test materials that 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 *in vivo* conditions, 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), 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. FSANZ agreed with JECFA in setting an ADI of 4 mg steviol equivalents/kg bw/day, which was derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day established by a 2-year rat study (Toyoda et al., 1997). The FSANZ review discussed the adequacy of the existing database and several new studies, including the clinical studies reviewed by JECFA in the summer of 2007, most notably the work of Barriocanal et al. (2008), which was later published in 2008.

In their draft document, FSANZ also indicated that the new data in humans provides a basis for revising the uncertainty factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore, FSANZ established an ADI of 4 mg/kg bw/day for steviol glycosides as steviol equivalents, derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study (FSANZ, 2008). In December 2010, FSANZ recommended accepting the increased usage levels since no public health and safety issues were identified (FSANZ, 2010). Subsequently, FSANZ approved an increase in the maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages and flavored soy beverages up to 200 mg/kg and in plain soy beverages up to 100 mg/kg (FSANZ, 2011).

3. Summary of EFSA Review of Steviol Glycosides

On March 10, 2010, EFSA adopted a scientific opinion on the safety of steviol glycosides (mixtures that comprise not less than 95% of stevioside and/or rebaudioside A) as a food additive. Earlier---in 1984, 1989 and 1999---the Scientific Committee for Food (SCF) evaluated stevioside as a sweetener. At the time, the SCF concluded that the use of stevioside was “toxicologically not acceptable” due to insufficient available data to assess its safety. However, in light of JECFA’s 2008 findings, and in response to a June 2008 request by the European Commission, EFSA reevaluated the safety of steviol glycosides as a sweetener. As both rebaudioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for both glycosides, the EFSA Panel agreed that the results of toxicology studies on either stevioside or rebaudioside A are applicable for the safety assessment of steviol glycosides. Considering the available safety data (*in vitro* and *in vivo* animal studies and some human tolerance studies), the EFSA Panel concluded that steviol glycosides, complying with JECFA specifications, are not carcinogenic, genotoxic, or associated with any reproductive/developmental toxicity. The EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on the application of a 100-fold uncertainty factor to the

NOAEL in the 2-year carcinogenicity study in the rat when administering 2.5% stevioside in the diet. This is equal to 967 mg stevioside/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day). Conservative estimates of steviol glycosides exposures both in adults and in children suggest that the ADI could possibly be exceeded by European consumers of certain ages and geographies at the maximum proposed use levels.

Recently, EFSA (2011a) revised its exposure assessment of steviol glycosides from its uses as a food additive for children and adults, and published the reduced usage levels in 16 foods by a factor of 1.5 to 3, with no changes for 12 food groups. Additionally, 15 other foods were removed, mainly within the category of desserts and other products, while 3 new food uses were added. The mean estimated exposure to steviol glycosides (equivalents) in European children (aged 1-14 years) ranged from 0.4 to 6.4 mg/kg bw/day and from 1.7 to 16.3 mg/kg bw/day at the 95th percentile. A correction was considered to be necessary for the consumption of non-alcoholic flavored drinks (soft drinks) by children, and the corrected exposure estimate at the 95th percentile for children ranged from 1.0 to 12.7 mg/kg bw/day. For adults, the mean and 97.5th percentile intakes were estimated to range from 1.9 to 2.3 and 5.6 to 6.8 mg/kg bw/day, respectively. Non-alcoholic flavored drinks (soft drinks) are the main contributors to the total anticipated exposure to steviol glycosides for both consumer categories. For high consumers, EFSA noted that revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg/kg bw (steviol equivalent).

In addition, EFSA (2011b) recently accepted rebaudioside A as a flavoring agent in a variety of foods. EFSA reviewed the available safety data on rebaudioside A and agreed that the ADI of 4mg/kg bw/day established for steviol glycosides applied also to rebaudioside A in a purified form. The dietary intake for use as a flavoring agent was calculated by two different methods, and EFSA determined that the worst-case exposure would be 10,888 microgram/person/day, which is equivalent to 181 microgram rebaudioside A/kg bw/day, for a person weighing 60 kg. This corresponds to a daily intake of 60 microgram steviol/kg bw/day, using a conversion factor of 0.33 for converting the amount of rebaudioside A into steviol equivalents.

4. Other Published Reviews

Stevia and steviol glycosides have been extensively investigated for their biological, toxicological, and clinical effects (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002). Four additional reviews have appeared on the toxicology and biological activity of stevia extracts and steviol glycosides (Yadav and Guleria, 2012; Brown and Rother, 2012; Brahmachari et al., 2011; Chatsudthipong and Muanprasat, 2009). In reviewing these studies, caution is warranted since these reviews do not differentiate well between studies on crude stevia extract and purified steivol glycosides. In addition, many of the reviewed studies on biological activity used routes of administration other than oral, and they may have used doses that are much higher than expected dietary exposures of steviol glycosides as a sweetener. In a letter to the editor of the Journal of Pharmacology and Therapeutics, Roberts and Munro (2009) criticized the Chatsudthipong and Muanprasat (2009) review with some important points that are applicable in general to these four reviews. Important excerpts from this letter are as follows:

> "It is well established that some stevia extracts are crude mixtures that contain multiple components of the stevia leaf, including those components that do not provide a sweet taste. These mixtures also vary considerably in quality, purity, and composition. Therefore, it is not surprising that sometimes these crude and uncharacterized materials may contain substances that possess some degree of pharmacologic activity but any such effects cannot be attributed specifically to the steviol

glycosides. In contrast to studies conducted with less pure steviol glycoside preparations, studies conducted with purified preparations do not indicate any evidence of pharmacological effects."

"The authors consistently cite pharmacological, toxicological, and biochemical effects from in vitro studies or from studies in whichanimals were dosed intravenously (e.g., Melis, 1992a,b,c). Steviol glycosides are hydrolyzed completely by the gut microflora to steviolprior to absorption, with no systemic absorption of the glycone form following oral exposure. Therefore, the results of in vitro and intravenous, intraperitoneal, or subcutaneous dosing studies of the glycone form are not relevant to the safety of steviol glycosides consumed orally."

"Collectively, the report of Chatsudthipong and Muanprasat (2009) is incomplete and lacking discussion of key studies of the safety of stevioside and rebaudioside A. It focuses on alleged effects of stevia and steviol glycosides of low or unknown purity, fails to consider the route of exposure in relation to metabolism and safety assessment and does not include recent opinions expressed by world wide regulatory authorities affirming the safety of purified forms of stevioside and rebaudioside A as a food ingredient."

APPENDIX J

Studies on Principal Metabolite: Steviol

Studies on 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 below.

Acute Toxicity Studies

The oral LD_{50} 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 LD_{50} values in hamsters given steviol orally were 5.2 g/kg bw in males and 6.1 g/kg bw in females. Histopathological examination of the kidneys revealed severe degeneration of the proximal tubular cells, and these structural alterations were correlated with increased serum blood urea nitrogen and creatinine. The authors concluded that the cause of death was acute renal failure (Toskulkao et al., 1997).

Developmental Toxicity Studies

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1,000 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).

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 J-1.

Table J-1. Mutagenicity & Genotoxicity Studies on Steviol

	In Vivo/In Vitro	System	Test Sample Purity	Author Conclusion	Results and Remarks
Sekihashi et al., 2002[a]	*In Vivo/In Vitro*	Comet Assay	Not reported	Negative	In *in vitro* study, steviol at 62.5, 125, 250 and 500 µg/ml did not damage DNA of TK6 and WTK1 cells in presence or absence of S9 mix. In *in vivo* study, mice sacrificed 3 or 24 hours after one-time oral administration of 250, 500, 1000 or 2000 mg/kg of steviol. Stomach, colon, kidneys, testis and liver DNA not damaged. An identical *in vivo* 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 *S.typhimurium* TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene. Magnitude of increase of these mutations over the control not reported.
Klongpanich-pak et al., 1997[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Steviol and stevioside inactive in TA strains of *S. typhimurium, e. coli WP2, uvrA/PKM101* and rec assay using *B. subtilis* 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 *E. coli*. The chromosomal DNA of TM677 and steviol-induced TM677 mutants digested by restriction enzymes and probed. No significant differences found in fragment length between wild-type and mutant DNA.
Matsui et al., 1996[a]	*In Vitro*	Bacterial Mutagenicity	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.

	In Vivo/In Vitro	System	Test Sample Purity	Author Conclusion	Results and Remarks
Compadre et al., 1988[a]	*In Vitro*	BacterialMutagenicity, 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]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Using *S. typhimurium* 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]	*In Vivo*	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]	*In Vivo*	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]	*In Vivo*	Micronucleus (mouse)	Not Reported	Negative	Steviol did not increase number of micronuclei observed in this study.
Temacharoen et al., 2000[c]	*In Vivo*	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 WHO, 2006.
[c] As reviewed by Geuns, 2003.
[d] Full article.

APPENDIX K

Studies on Steviol Glycosides Preparations that are Primarily Mixtures of Stevioside & Rebaudioside A

This appendix summarizes studies on stevioside or stevia extracts that were identified compositionally as predominantly stevioside. In some of the published literature, the terms stevia, stevioside, and stevia glycoside are used interchangeably. However, wherever possible, an attempt has been made to identify the specific substance studied.

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).

Koyama et al. (2003b) published an *in vitro* study in which α-glucosylated steviol glycosides were degraded by fecal microflora to steviol glycosides. These are subsequently hydrolyzed to the aglycone, steviol, demonstrating that the metabolic fate of α-glucosylated steviol glycosides follows that of non-modified steviol glycosides. Due to the similarities in metabolic fate, the safety of α-glucosylated steviol glycosides can be established based on studies conducted with non-modified steviol glycosides. Furthermore, as individual steviol glycosides show similar pharmacokinetics in the rat and humans, the results of toxicology studies on individual steviol glycosides are applicable to the safety of steviol glycosides in general.

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%) three 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 glycosides. 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. Given the similarity in the microbial metabolism of stevioside and rebaudioside A, with the formation of steviol as the single hydrolysis product that is absorbed from the intestinal tract, these investigators concluded that the toxicological data on stevioside are relevant to the risk assessment of rebaudioside M.

Table K-1. 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	*In vivo* rat	Reb A		Up to 2000 mg/kg bw	No increase in unscheduled DNA synthesis	Williams and Burdock (2009)
DNA damage (comet assay)	*Male BDF1 mouse stomach, colon, liver*	Stevia extract	Stevio-side, 52%; Reb A, 22%	250 - 2000 mg/kg bw	Negative[a]	Sekihashi et al. (2002)

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCENTRATION / DOSE	RESULT	REFERENCE
Chromosomal aberration	*CHL/IU Chinese hamster lung fibroblasts*	Reb A	NS	1.2 - 55 mg/mL	Negative[b]	Nakajima (2000a)
Micronucleus formation	*BDF1 mouse bone marrow*	Reb A	NS	500-2000 mg/kg bw per day for 2 days	Negative[c]	Nakajima (2000b)
Forward mutation	*S. typhimurium* TM677	Reb A	NS	10 mg/plate	Negative[b]	Pezzuto et al. (1985)

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

2. Acute Toxicity Studies

The oral LD_{50} studies of stevioside (purity, 96%) following administration of a single dose to rodents are summarized in Table K-2. No lethality was noted within 14 days after the administration, and no clinical signs of toxicity, or morphological or histopathological changes were found, indicating that stevioside is relatively harmless.

Table K-2. 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 five 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 F_0, F_1, and F_2 generations. These studies are summarized in Table J-3. 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, 1,300, and 2,500 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).

In a recently published exploratory subchronic toxicity study, Awney et al. (2011) investigated the effects of 97% pure stevioside on body weight, organ relative weight, hematological and biochemical parameters, and enzyme activities in Sprague Dawley rats. In this 12-week toxicity study, groups of male rats (8/group) were given drinking water containing stevioside. The groups were assigned to drink distilled water (control), low-dose stevioside solution (15 mg/kg/day), high-dose stevioside solution (1500 mg/kg/day), or low-dose stevioside (15 mg/kg/day) plus inulin solution for 12 weeks as the sole source of liquid. Fluid intake was recorded daily, and levels of test articles were adjusted weekly to receive the appropriate target concentration. Low-dose stevioside (15 mg/kg bw/day) administration, with or without inulin, for 12 weeks did not reveal any adverse effects on body weight, organs relative weight, hematological and biochemical parameters, or enzyme activities. However, treatment with high-dose stevioside was reported to cause significant changes in several investigated toxicological parameters. Among the hematological parameters, significant changes were noted in all except WBCs, RBCs, and PCV%, and in all clinical chemistry parameters except proteins, total lipids, serum ATL and AST. These data support the NOEL of 15 mg/kg/day. However, critical review of the publication reveals that the study was poorly designed and implemented. Design deficiencies include: insufficient numbers of animals; group-housing with the potential for stress-related changes; unreliable access to steviol *via* drinking water, resulting in suspect dosing calculations in group-housed cages; no indication of fasting prior to blood collection, which affects many chemistry and hematological values; no urine collection; and no histopathological evaluations for confirmation of findings beyond the controls. In addition to these study design deficiencies, the report fails to adequately present mean or individual organ weight data and, in general, there appears to be inadequate comparison of study findings against laboratory historical control data. Any one of these oversights could have adversely affected the results and/or interpretation of the hematological and chemistry data.

In addition to the above described parameters, tartrate-resistant alkaline phosphatase (TRAP) levels were measured and found to be significantly decreased (Awney et al., 2011). TRAP is an enzyme that is expressed by bone-resorbing osteoclasts, inflammatory macrophages, and dendritic cells. This enzyme was not measured in any previous steviol glycosides studies nor has it been adequately vetted for application in toxicological studies. These investigators did not identify the specific TRAP isomer measured, the methodology employed, the handling of the samples, or any historical data on TRAP levels. The significance and relevance of this poorly documented toxicological endpoint, which lacks histopathological confirmation, does not appear to have a distinct role in determining the toxicological profile of a material in a test animal. The data presented by Awney et al. (2011) are probably not representative of changes due to the subchronic dietary administration of steviol glycosides because of overall inadequate study design and reliance on the findings of the untested enzyme TRAP. The preponderance of the data from several well designed studies on steviol glycosides suggest that differences noted in hematological and chemistry data are probably random, nonspecific, and not toxicologically significant.

Critical reviews of the publication by Carakostas (2012) and Waddell (2011) revealed a poor study design that included: insufficient numbers of animals; group-housing with the potential for stress-related changes; unreliable access to steviol *via* drinking water resulting in suspect dosing calculations in group-housed cages; no indication of fasting prior to blood collection, which affects many chemistry and hematological values; no urine collection; and no histopathological evaluations for confirmation of findings beyond the controls. Additionally, the report did not

adequately describe mean or individual organ weight data and lacked comparison of study findings against laboratory historical control data.

Table K-3. Summary of Subchronic Studies on Stevioside

Study	Animal Model/ Group size	Test Material/ Sample Purity	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results and Remarks
Aze et al., 1991[a]	F344 rat/ 10 females & 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 & histopathological investigation indicated increased incidence of single-cell liver necrosis in all male treated groups, but not in clear dose-response relationship. Investigators did not consider these changes to be treatment related due to small magnitude & low severity of changes, the lack of clear dose relationship & limitation to males only. Organ weights, urine chemistry & gross necropsy not discussed. Authors concluded that 5% stevioside in diet is tolerable dose for 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 & F_2 generations in reproductive study dosed for 90 days. Histological examination showed no effect at any dose. Weights of organs, blood analysis, urine chemistry & gross necropsy not discussed. The F_1 & 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 & 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 & histopathology not discussed.
Awney et al., 2011	Sprague Dawley rats	Stevioside 97%	Drinking water (15, 1500 mg/kg bw /day)	15	Treatment with high dose stevioside caused significant changes in several investigated toxicological parameters Among hematological parameters, significant changes noted in all except WBCs, RBCs& PCV% & in all clinical chemistry parameters except proteins, total lipids, ATL AST.

[a] Abstract only. [b] As reported by Geuns, 2003.

4. Chronic Toxicity Studies

Chronic effects of stevioside have been studied in three separate studies (Table K-4). 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 (WHO, 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.

Table K-4. 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	*Ad libitum* 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)	Significant decrease in survival rates in males receiving 5%. General condition, body weight, food intake, mortality, hematological, histopathological& organ weights observed. Body weight gains dose-dependently decreased in both sexes. Kidney weights significantly lower in 5% males& ovary, kidney, & brain weights significantly increased in 5% females. Tumors& non-neoplastic lesions found in all groups& not correlated to treatment. Conclusion–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 & 24 months 5 rats from each group 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 unrelated tolevel of stevioside in 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 &12 months, 10 males & 10 females sacrificed for analysis. General behavior, growth & mortality were same among groups throughout experiment. At 6 months, protein urea significantly increased in females, & blood glucose increased in both sexes, although urinary glucose not detected. Weights of liver, kidney, heart, prostate & testes increased in males at 6 months, &weight of ovaries 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 not found at 12 months. Authors concluded that there were no significant changes after 2 years.

[a] Only abstract available.

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; Schvartzman 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. No effect on fertility or reproductive parameters was seen in a three-generation study in hamsters at doses up to 2,500 mg/kg/day (Yodyingyuad and Bunyawong, 1991). There was an absence of statistically significant effects at doses up to 3% (equivalent to 3,000 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).

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1,000 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).

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 K-5.

Table K-5. 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 & Remarks
Kumar and Oommen, 2008	Swiss albino mice/ 4 groups of 5 females	Not reported	500 & 800 mg/kg bw/15 days	800	Stevioside & stevia extract (purity & 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/day on days 6-15 of gestation & were sacrificed on day 20 of gestation. Fetuses examined for malformations in addition to maternal & fetal body weight, number of live fetuses, sex distribution& numbers of resorptions or dead fetuses. No treatment-related effects observed. Authors concluded that orally administered stevioside 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 mated to females from respective dose group. Each female allowed to bear 3 litters during course of experiment. Stevioside had no effect on pregnancies of females at any dose. The F_1 & F_2 hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents); showed normal growth & 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, & pancreas; water content of testes & prostate; body-weight gain; & final weights of testes, prostate, seminal vesicle, submandibular salivary gland& 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 & during mating with females who received same diet (as mated male) 14 days before mating & 7 days during gestation. No effect due to treatment on fertility or mating performance& 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 & 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 last 6 days. Fertility reduced to 21% of fertility in control rats & remained reduced in a 50-60 day recovery. Histological examination, weights of organs, blood analysis, urine chemistry and & necropsy not discussed.

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

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 K-6. 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 by Geuns (2007), Williams, (2007), and Brusick (2008), and responded to by the authors (Nunes et al., 2007b, c). Recently, the genotoxicity data on steviol glycosides were reviewed and considered to be adequate to support the safety of its use as a sweetener in foods (Urban et al., 2013).

Table K-6. Mutagenicity & Genotoxicity Studies on Stevia Extracts & Stevioside

End-Point	Test System	Material	Purity (%)	Concen-tration / Dose	Result	Reference
In Vitro						
Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Stevioside	83	5 mg/plate[a] 1 mg/plate[b]	Negative	Matsui et al. (1996)
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	99	50 mg/plate	Negative[c]	Suttajit et al. (1993)
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	NS	50 mg/plate	Negative	Klongpanichpak et al (1997)
Forward mutation	*S. typhimurium* TM677	Stevioside	83	10 mg/plate	Negative[c]	Matsui et al. (1996)
Forward mutation	*S .typhimurium* TM677	Stevioside	NS	10 mg/plate	Negative[c]	Pezzuto et al. (1985)
Forward mutation	*S. typhimurium* 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)	*S. typhimurium* TA1535/pSK1002	Stevioside	83	5 mg/plate	Negative[c]	Matsui et al. (1996)
Gene mutation	*B. subtilis* 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)
In Vivo						
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	*D. melanogaster* 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.

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 2 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 1,500 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 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 regimen, 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).

In a paired cross-over study, 12 patients with type 2 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).

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, alanine transaminase (ALT), glutamic-pyruvate transaminase (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 68th 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.

In a follow-up study, Barriocanal et al. (2008) evaluated the effects of steviol glycosides on blood glucose and blood pressure (BP) for three months in subjects with type 1 diabetes, subjects with type 2 diabetes, and subjects without diabetes and with normal/low-normal BP levels. Patients in each group received either 250 mg t.d.s. (total dissolved solids) steviol glycoside, stevioside, or placebo treatment. The purity of the steviol glycosides was ≥ 92%. Three months of follow up revealed no changes in systolic BP, diastolic BP, glucose, or glycated hemoglobin from baseline. In placebo type 1 diabetics, there was a significant difference in systolic BP and glucose. There were no adverse effects observed in either treatment group, and the authors concluded that oral steviol glycosides are well-tolerated and have no pharmacological effect.

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.

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 alanine transaminase (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 68th meeting that the product used in this study 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, 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).

APPENDIX L

Studies on Steviol Glycosides Preparations that are Primarily Rebaudioside A

Safety Data on Rebaudioside A[16]

Since 2008, several well-designed toxicology studies that followed the current regulatory and scientific 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 recent 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. These studies confirm that rebaudioside A is metabolized similarly to other steviol glycosides, and they exhibited an absence of toxicological effects in the key studies reviewed by JECFA. It should be noted that rebaudioside A, as the steviol glycoside with high sweetness intensity and relatively high prevalence in the stevia leaves, remains an active topic of scientific research. For example, a study found in a recent literature search examined the anti-hyperglycemic activity of rebaudioside A in diabetic rats (Saravanan et al., 2012). These investigators found that the effects of streptozotocin-induced diabetes on glucose and insulin levels were at least partially reversed in a dose-dependent manner with oral administration of rebaudioside A at doses in the range of 50-200 mg/kg bw. The doses used are 10-40 times higher than expected from the use of rebaudioside A as a sweetener. The known anti-hyperglycemic activity of steviol glycosides led JECFA to require clinical studies at reasonably high doses to show that—at levels used in food—there would be no effect on glucose homeostasis or blood pressure in human consumers. The clinical studies described below on rebaudioside A (Maki et al., 2008a,b) demonstrate the lack of these pharmacological effects of rebaudioside A at expected levels of consumption.

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

Studies investigating the ADME of extracts from stevia are available on stevioside, Reb A, and other steviol glycosides. Data evaluating the absorption and fate of these extracts from various animal species and humans indicate that one can extrapolate these results from rats to humans. Stevioside is metabolized to steviol *via* intestinal microflora, and the absorption of stevioside after oral administration has been shown to be very low (Koyama et al., 2003a; Geuns et al., 2003).

Studies investigating the hydrolysis of steviol glycosides by intestinal microflora have demonstrated that both stevioside and Reb A are hydrolyzed to steviol following *in vitro* incubation with various cecal microflora (Wingard et al., 1980; Hutapea et al., 1997; Gardana et al., 2003; Geuns et al., 2003). In addition, the *in vitro* hydrolysis of Reb A to steviol was found to be slower than that of stevioside (Koyama et al., 2003a), which is thought to be partly due to the presence of one additional glucose moiety and to differences in structural complexities. Koyama et al. (2003a)

[16] Questions about the safety of rebaudioside A were previously raised by Huxtable (2002), and Kobylewski and Eckhert (2008). Their respective concerns, as well as opposing views supporting the safety of designated food uses of rebaudioside A expressed by Expert Panels, have been outlined in other GRAS notifications that were submitted to FDA. A more detailed account can be found in GRAS notifications 278, 287, 303, and 304. This matter is discussed by the Expert Panel in Section VI.C.

suggest that the major pathway for Reb A is conversion to stevioside with a minor pathway of conversion to Reb B prior to being ultimately converted to steviol. Stevioside is further converted to steviolbioside, steviolmonosides, and finally steviol, with glucose being released with each subsequent hydrolysis.

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

For comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, rebaudioside A, toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol were examined in rats (Roberts and Renwick, 2008). Orally administered single doses of the radiolabeled compounds were extensively and rapidly absorbed with plasma concentration-time profiles following similar patterns for stevioside and rebaudioside A.

Roberts and Renwick (2008) identified free steviol (82 to 86%), steviol, glucuronide (10 to 12%), and two unidentified metabolites (5-6%) in rat plasma following treatment with either stevioside or Reb A eight hours post-oral administration. A comparable pharmacokinetic profile was noted following oral treatment of rats with radiolabeled Reb A or stevioside, with the time of maximum plasma concentration (T_{max}) for radioactivity ranging between 2 and 8 hours. In comparison, steviol T_{max} for plasma was noted within 30 minutes of oral administration. 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 unidentified metabolites. It is believed that this delay between the occurrence of radioactivity in the plasma and time of administration of steviol glycosides is due to the fact that the Reb A and stevioside are first cleaved to steviol before absorption.

Within 72 hours of administration, elimination of radioactivity from plasma was essentially complete. Following elimination in the bile, steviol is available to be released again from its conjugated form by microflora activity and may enter enterohepatic circulation. Consequently, free and conjugated steviol are secreted in the feces along with any unhydrolyzed fraction of the administered glycosides. Following Reb A treatment, significant amounts of unchanged rebaudioside A (29% in males and 19% in females) and stevioside (3% in males and 4% in females) were excreted in the feces. Following oral stevioside administration, unchanged stevioside was excreted in rat feces. Other unidentified metabolites are also present in fecal samples of rats treated with either glycoside. Rebaudioside A, stevioside, and steviol were metabolized and excreted rapidly, with ~60% 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 (Roberts and Renwick, 2008).

In summary, Roberts and Renwick (2008) found that steviol was the predominant component found in plasma samples after oral administration of Reb A, stevioside, and steviol in rats. Lower amounts of steviol glucuronide(s) and one or two unidentified metabolites were also found. The majority of all samples were found to be excreted rapidly---primarily in the feces---within 48 hours. This is in agreement with the previous *in vitro* hydrolysis data that indicated that both Reb A and stevioside are metabolized to steviol by intestinal microflora. The predominant compound detected

in the bile was steviol glucuronide, while the prominent material in the intestine was steviol, which the authors suggest indicates that deconjugation occurs 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 each compound. Administration of rebaudioside A resulted in a significantly (~22%) lower steviol glucuronide geometric mean C_{max} value (1,472 ng/mL) than administration of stevioside (1,886 ng/mL). The geometric mean AUC_{0-t} value for steviol glucuronide after administration of rebaudioside A (30,788 ng*hr/mL) was approximately 10% lower than after administration of stevioside (34,090 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 2,000 mg/kg bw/day (Sloter, 2008a). Extremely low levels of rebaudioside A and total steviol were detected in peripheral blood of rats during daily administration of 2,000 mg/kg bw/day of rebaudioside A, with mean plasma concentrations of approximately 0.6 and 12 μg/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 estimated dose recovery of approximately 84%.

2. Subchronic Toxicity Studies

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 (9,938 and 11,728 mg/kg bw/day for males and females, respectively) for 4 weeks, or 50,000 ppm (4,161 and 4,645 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 4,161 and 4,645 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, 1,000, and 2,000 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 2,000 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 ≥ 2,000 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, 1,000, or 2,000 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 2,000 mg/kg bw/day and the assigned NOAEL was ≥ 2,000 mg/kg bw/day.

3. Mutagenicity Studies

In a set of *in vitro* and *in vivo* genotoxicity assays covering mutation, chromosome damage, and DNA strand breakage, rebaudioside A consistently and uniformly revealed negative results (Pezzuto et al., 1985; Nakajima, 2000a; Nakajima, 2000b; Sekihashi et al., 2002). These studies were 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. Furthermore, 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 L-1.

4. 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 F_0 or F_1 generations. The survival and general condition of the F_1 and F_2 offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by rebaudioside A treatment. The NOAEL for reproductive effects was 25,000 ppm, and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm, or 2,048 to 2273 mg/kg body weight/day (the highest dose tested).

Table L-1. Mutagenicity & Genotoxicity Studies on Rebaudioside A

End-Point	Test System	Material	Purity (%)	Concentration / Dose	Result	Reference
Bacterial Mutagenicity	5 Salmonella strains with & without exogenous metabolic activation system	Reb A	99.5	1.5, 5.0, 15, 50, 150, 500, 1500 & 5000 µg per plate	No mutagenic response	Wagner and Van Dyke (2006)
Bacterial Mutagenicity	4 Salmonella strains & 1 *E. coli* strain with & without exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg per plate	No mutagenic response	Williams and Burdock (2009)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence & presence of exogenous metabolic activation system	Reb A	99.5	Cloning conc. of 500, 1000, 2000, 3000, 4000 & 5000 µg/mL	No mutagenic or clastogenic response	Clarke (2006)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence & presence of exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Chromosom e Aberration	Human lymphocytes in absence & presence of exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Mouse Micronucleus	Micronucleus study in groups of 5 male & 5 female ICR mice	Reb A	99.5	500, 1000 & 2000 mg/kg bw	No increase in micronuclei formation	Krsmanovic and Huston (2006)
Mouse Micronucleus	Micronucleus study in groups of 5 male & 5 female NMRI mice	Reb A	95.6	Up to 750 mg/kg bw	No increase in micronuclei formation	Williams and Burdock (2009)
Unscheduled DNA Synthesis	Unscheduled DNA synthesis in one group of 4 Wistar rats	Reb A	95.6	Up to 2000 mg/kg bw	No increase in unscheduled DNA synthesis	Williams and Burdock (2009)

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCENTRATION / DOSE	RESULT	REFERENCE
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevio-side, 52%; Reb A, 22%	250 - 2000 mg/kg bw	Negative[a]	Sekihashi et al. (2002)
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Reb A	NS	1.2 - 55 mg/mL	Negative[b]	Nakajima (2000a)
Micronucleus formation	BDF1 mouse bone marrow	Reb A	NS	500-2000 mg/kg bw/ day for 2 days	Negative[c]	Nakajima (2000b)
Forward mutation	*S. typhimurium* TM677	Reb A	NS	10 mg/plate	Negative[b]	Pezzuto et al. (1985)

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

The results from two unpublished studies with rebaudioside A (Sloter 2008a, b) further support the above described findings from published studies. In a two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were fed either basal diet or the diet containing rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter 2008a). For the F_0 and F_1 generations, rebaudioside A doses were 0, 500, 1,000, and 2,000 mg/kg/day. At initiation of study, F_0 animals were approximately 7 weeks of age. The test diet was offered to the offspring selected to become the F_1 generation following weaning [beginning on postnatal day (PND) 21]. The F_0 and F_1 males continued to receive rebaudioside A throughout mating, continuing through the day of euthanasia. The F_0 and F_1 females continued to receive rebaudioside A throughout mating, gestation and lactation until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females 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 ≥ 2,000 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 were investigated. 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 ≥ 2,000 mg/kg bw/day (highest dose administered) was considered to be the NOAEL for maternal and embryo/fetal developmental toxicity.

5. Clinical Studies on Rebaudioside A

In a four week randomized, double-blind, placebo controlled trial, hemodynamic effects of rebaudioside A, at a dose of 1,000 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 1,000 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 1,000 mg/person/day 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 ± 0.06%, mean ± standard error) and placebo (0.09 ± 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 μU/mL and 3.3 ± 1.5 μU/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 investigators suggested that chronic use of 1,000 mg/person/day rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

6. Safety of Rebaudioside A

There have been a significant number of studies regarding the safety and toxicity of rebaudioside A, including many that have been published since the two initial GRAS notifications were submitted to FDA by Cargill (GRN 253) and Merisant (GRN 252). These, and some other unpublished studies, formed the basis of the two initial GRAS notifications to FDA by Cargill (GRN 253) and Merisant (GRN 252). Prior to this, a limited number of toxicology studies specifically on rebaudioside A were conducted. Even before these new studies were completed, and as noted in the previous section, JECFA concluded that 7 (which was later expanded to 9) common steviol glycosides are safe for use as sweetener preparations when present in any combination, as long as a combined purity of 95% or more was established.

Since a majority of the previous pharmacokinetic research was conducted with steviol glycosides, the presumed strategy adopted for the more recent research on rebaudioside A was to conduct a limited number of well-designed and executed toxicology studies on rebaudioside A itself, and to demonstrate that rebaudioside A is handled pharmacokinetically similarly to stevioside in rats and humans. This approach appears to have been undertaken to justify the JECFA-generated ADI without having to conduct a chronic study in rats with rebaudioside A. Additionally, the Merisant group conducted three mutagenicity assays on rebaudioside A that FDA generally considers to be most predictive for carcinogenicity potential. The Cargill group conducted two clinical studies to assure that rebaudioside A does not have potentially problematic pharmacological effects on blood glucose and blood pressure.

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

- Steviol glycosides, rebaudioside A, and stevioside are not genotoxic *in vitro*.
- In well-conducted *in vivo* assays, steviol glycosides, rebaudioside A, and stevioside have not been found to be genotoxic.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes, et al., 2007a) and was improperly interpreted as a positive response.

- Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.
- The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- While studies with Reb A indicated slight GI absorption of the glycoside *per se*, the predominant metabolic pathway is comparable to that of stevioside and the use of the ADI established by JECFA, which was determined on studies employing stevioside as the main component, can be used as the ADI for rebaudioside A.
- The dietary levels expected from consumption of rebaudioside A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.

The Expert Panel concurs that the consumption estimates described by JECFA, Renwick (2008), and the GRN 252 and GRN 253 Expert Panels that very conservatively represent a potential high user of rebaudioside A if this non-nutritive sweetener becomes widely available in food.

Regarding the available aggregate safety information, the Panel has concluded that JECFA has critically and extensively evaluated the use of steviol glycosides in foods and agrees that, at the present time, the ADI for steviol glycosides of adequate purity, as defined by JECFA specifications, has been properly determined to be 4 mg/kg bw/person as steviol equivalents, which corresponds to 12 mg/kg bw/day for rebaudioside A, on a dry weight basis. The Panel agrees that unwanted pharmacological effects are not likely to occur at this level and, moreover, that high consumers of rebaudioside A are not likely to exceed this level. Therefore, the Panel adopts the JECFA-derived ADI as a safe exposure for rebaudioside A and that food uses meeting the specifications within the limits determined by this esteemed international body of food safety experts can be considered to be generally recognized as safe (GRAS).

The Panel recognizes that JECFA---which is composed of dozens of scientists that are internationally known experts on food ingredient safety---has established ADIs for food ingredients over the last 40 years. Both Merisant and Cargill took rather rigorous scientific approaches to demonstrate the safety of rebaudioside A. The studies were equally well conducted. The safety profiles compiled by Merisant and Cargill differ somewhat, yet the results are complementary and are mutually reinforcing of rebaudioside A safety.

The studies conducted by Cargill provided significant insight into the pharmacokinetics of rebaudioside A, while demonstrating clinical safety of rebaudioside A regarding lack of effects on blood pressure and glucose metabolism that could result from doses expected from use in food. The Merisant notification augmented genotoxicity data in three systems recognized by FDA as good predictors of carcinogenic potential. Two of these assays were conducted in mouse systems. Additional mutagenicity and genotoxicity studies have been published on rebaudioside A (Williams and Burdock, 2009). Merisant added a subchronic study in dogs and a teratology study in rats. Both Cargill and Merisant relied on the JECFA ADI for steviol glycosides as determined largely by published chronic studies in rats. Both groups justified the use of the ADI on pharmacokinetic arguments showing the similarity of stevioside and rebaudioside A metabolism and excretion.

SUBMISSION END