

GRAS Notice (GRN) No. 493

<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>

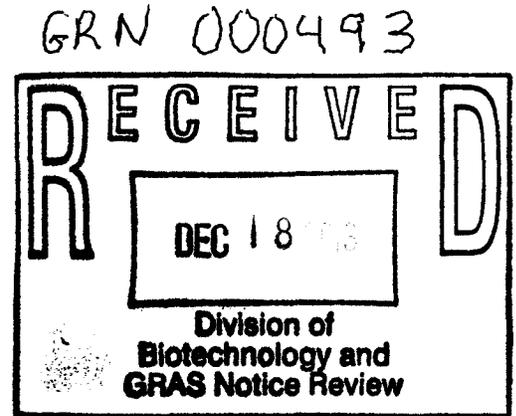
**ORIGINAL SUBMISSION**



20482 Jacklight Lane  
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541-678-5522  
mcquate@gras-associates.com

December 9, 2013

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 Steviol Glycosides ( $\geq 95\%$ )

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 Steviol Glycosides ( $\geq 95\%$ ). The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

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  
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[www.gras-associates.com](http://www.gras-associates.com)

Enclosure: GRAS Notification for GLG Life Tech Corporation – High Purity Steviol Glycosides ( $\geq 95\%$ )

**FDA USE ONLY**

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

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

**PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION**

1. Type of Submission (*Check one*)

New       Amendment to GRN No. \_\_\_\_\_       Supplement to GRN No. \_\_\_\_\_

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

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

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

**PART II – INFORMATION ABOUT THE NOTIFIER**

<b>1a. Notifier</b>	Name of Contact Person Brian R. Meadows		Position President & CFO	
	Company ( <i>if applicable</i> ) GLG Life Tech Corporation			
	Mailing Address ( <i>number and street</i> ) 1050 West Pender Street, Suite 2168			

City Vancouver		State or Province British Columbia	Zip Code/Postal Code V6E 3S7	Country Canada
Telephone Number 604-669-2602 (ext 105)		Fax Number 604-662-8858	E-Mail Address brian.meadows@glglifetech.com	

<b>1b. Agent or Attorney (if applicable)</b>	Name of Contact Person Robert S McQuate		Position CEO	
	Company ( <i>if applicable</i> ) GRAS Associates, LLC			
	Mailing Address ( <i>number and street</i> ) 20482 Jacklight Lane			

City Bend		State or Province Oregon	Zip Code/Postal Code 97702-3074	Country United States of America
Telephone Number 541-678-5522		Fax Number 541-678-5522 call first	E-Mail Address mcquate@gras-associates.com	

**PART III – GENERAL ADMINISTRATIVE INFORMATION**

1. Name of Substance

High Purity Steviol Glycosides (minimum purity 95%)

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

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

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**

	<b>Name of Substance<sup>1</sup></b>	<b>Registry Used (CAS, EC)</b>	<b>Registry No.<sup>2</sup></b>	<b>Biological Source (if applicable)</b>
1	High purity steviol glycosides - predominantly Rebaudioside A and Stevioside			
2	Rebaudioside A	CAS	58543-16-1	Stevia rebaudiana Bertoni
3	Stevioside	CAS	57817-89-7	Stevia rebaudiana Bertoni

<sup>1</sup> 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)

<sup>2</sup> 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.

Subject blend of steviol glycosides extracted from the leaves of Stevia rebaudiana Bertoni and subsequently purified to meet the detailed specifications provided on page 19 within Table 3 in volume 1.

Chemical structures provided on page 14 in Figures 1 and 2 in volume 1.

Molecular weights: Rebaudioside A - 967.03 daltons and Stevioside - 804.88 daltons. See Table 2 on page 13 of volume 1.

Chemical formulas: Rebaudioside A - C<sub>44</sub>H<sub>70</sub>O<sub>23</sub> and Stevioside - C<sub>38</sub>H<sub>60</sub>O<sub>18</sub> as found in Table 2 on page 13 of volume 1.

**3. Synonyms**

Provide as available or relevant:

1	Rebaudioside A --> 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D- glucopyranosyl) oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester - from page 13 of Table 2 in volume 1
2	Stevioside -->13-[2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester - from page 13 of Table 2 in volume 1
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 Steviol Glycosides (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**

(b) (6)

**Printed Name and Title**

Robert S McQuate

**Date (mm/dd/yyyy)**

11/25/2013

**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)
	Volume 2 contains multiple appendices---Appendices A through O---with supporting safety information as listed in the attached 2 pages.	

**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.

APPENDICES -- GLG NOTIFICATION ON HIGH PURITY STEVIOL GLYCOSIDES  
 FORM 3667 Continuation Pages 6 & 7

APPENDIX A	Specifications & Certificates of Analysis for Production Processing Aids.....	4
APPENDIX A-1	GLG Specifications for Ethanol.....	5
APPENDIX A-2	GLG Specifications for Methanol.....	6
APPENDIX A-3	Certificate of Analysis for GLG Active Carbon.....	7
APPENDIX B	Analytical Method.....	8
APPENDIX C	HPLC Chromatograms for Anysweet RA50.....	16
APPENDIX C-1	HPLC Chromatogram for Batch 20130801.....	17
APPENDIX C-2	HPLC Chromatogram for Batch 20130804.....	19
APPENDIX C-3	HPLC Chromatogram for Batch 20130807.....	21
APPENDIX C-4	HPLC Chromatogram for Batch 20130810.....	23
APPENDIX C-5	HPLC Chromatogram for Batch 20130814.....	25
APPENDIX D	HPLC Chromatograms for Anysweet RA60.....	27
APPENDIX D-1	HPLC Chromatogram for Batch 20130801.....	28
APPENDIX D-2	HPLC Chromatogram for Batch 20130803.....	30
APPENDIX D-3	HPLC Chromatogram for Batch 20130806.....	32
APPENDIX D-4	HPLC Chromatogram for Batch 20130809.....	34
APPENDIX D-5	HPLC Chromatogram for Batch 20130811.....	36
APPENDIX E	HPLC Chromatograms for Rebsweet RA80.....	38
APPENDIX E-1	HPLC Chromatogram for Batch 20130801.....	39
APPENDIX E-2	HPLC Chromatogram for Batch 20130805.....	41
APPENDIX E-3	HPLC Chromatogram for Batch 20130807.....	43
APPENDIX E-4	HPLC Chromatogram for Batch 20130810.....	45
APPENDIX E-5	HPLC Chromatogram for Batch 20130815.....	47
APPENDIX F	HPLC Chromatograms for Rebsweet RA85.....	49
APPENDIX F-1	HPLC Chromatogram for Batch 20130802.....	50
APPENDIX F-2	HPLC Chromatogram for Batch 20130806.....	52
APPENDIX F-3	HPLC Chromatogram for Batch 20130808.....	54
APPENDIX F-4	HPLC Chromatogram for Batch 20130812.....	56
APPENDIX F-5	HPLC Chromatogram for Batch 20130816.....	58
APPENDIX G	Certificates of Analysis for Multiple Production Batches of Anysweet RA50 .....	60
APPENDIX G-1	Certificate of Analysis for Batch 20130801.....	61
APPENDIX G-2	Certificate of Analysis for Batch 20130804.....	62
APPENDIX G-3	Certificate of Analysis for Batch 20130807.....	63
APPENDIX G-4	Certificate of Analysis for Batch 20130810.....	64
APPENDIX G-5	Certificate of Analysis for Batch 20130814.....	65
APPENDIX H	Certificates of Analysis for Multiple Production Batches of Anysweet RA60.....	66
APPENDIX H-1	Certificate of Analysis for Batch 20130801.....	67
APPENDIX H-2	Certificate of Analysis for Batch 20130803.....	68
APPENDIX H-3	Certificate of Analysis for Batch 20130806.....	69
APPENDIX H-4	Certificate of Analysis for Batch 20130809.....	70
APPENDIX H-5	Certificate of Analysis for Batch 20130811.....	71
APPENDIX I	Certificates of Analysis for Multiple Production Batches of Rebsweet RA80.....	72
APPENDIX I-1	Certificate of Analysis for Batch 20130801.....	73
APPENDIX I-2	Certificate of Analysis for Batch 20130805.....	74
APPENDIX I-3	Certificate of Analysis for Batch 20130807.....	75
APPENDIX I-4	Certificate of Analysis for Batch 20130810.....	76
APPENDIX I-5	Certificate of Analysis for Batch 20130815.....	77

APPENDICES -- GLG NOTIFICATION ON HIGH PURITY STEVIOL GLYCOSIDES  
FORM 3667 Continuation Pages 6 & 7

APPENDIX J	Certificates of Analysis for Multiple Production Batches of Rebsweet RA85.....	78
APPENDIX J-1	Certificate of Analysis for Batch 20130802.....	79
APPENDIX J-2	Certificate of Analysis for Batch 20130806.....	80
APPENDIX J-3	Certificate of Analysis for Batch 20130808.....	81
APPENDIX J-4	Certificate of Analysis for Batch 20130812.....	82
APPENDIX J-5	Certificate of Analysis for Batch 20130816.....	83
APPENDIX K	Pesticide Analytical Reports from SGS-CSTC Standards Technical Services, Co. Ltd....	84
APPENDIX K-1	Test Report for Pesticides GLG RA60 Lot 20130501.....	85
APPENDIX K-2	Test Report for Pesticides GLG RA80 Lot 20130401.....	95
APPENDIX L	Summary of Regulatory & Expert Body Safety Reviews.....	105
APPENDIX M	Studies on Principal Metabolite: Steviol.....	109
APPENDIX N	Studies on Steviol Glycosides Preparations That Are Primarily Stevioside.....	112
APPENDIX O	Studies on Rebaudioside A.....	123



## **GRAS ASSESSMENT**

of

**HIGH PURITY STEVIOL GLYCOSIDES ( $\geq 95\%$ )**

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

**VOLUME 1 of 2**

for

**GLG Life Tech Corporation**

Suite 2168-1050 West Pender St.

Vancouver, B.C. 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)

November 25, 2013



## TABLE OF CONTENTS

<b>I. GRAS EXEMPTION CLAIM.....</b>	<b>5</b>
A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1).....	5
B. Name & Address of Notifier.....	5
C. Common Name & Identity of Notified Substance.....	6
D. Conditions of Intended Use in Food.....	6
E. Basis for GRAS Determination.....	6
F. Availability of Information.....	6
<b>II. INTRODUCTION.....</b>	<b>6</b>
A. Objective.....	6
B. Foreword.....	7
C. Summary of Regulatory History of Stevia & Stevia-Derived Sweeteners.....	7
D. FDA Regulatory Framework.....	11
<b>III. CHEMISTRY &amp; MANUFACTURE OF GLG-SG.....</b>	<b>12</b>
A. Common or Usual Name.....	12
B. Description.....	12
C. Chemistry of Steviol Glycosides.....	12
D. Accepted Identity Specifications for Food Grade Steviol Glycosides.....	16
E. Manufacturing Processes.....	16
1. Scientific & Patent Literature.....	16
2. GLG's Manufacturing Process for Purified GLG-SG.....	16
F. Product Specifications & Supporting Methods.....	17
1. JECFA Specifications for Steviol Glycosides.....	17
2. Specifications for GLG-SG Preparations & Supporting Methods.....	17
G. Stability Data.....	20
1. Stability Data on Steviol Glycosides .....	20
2. Stability Data for GLG-SG Preparations.....	21
<b>IV. INTENDED FOOD USES &amp; ESTIMATED DIETARY INTAKE .....</b>	<b>22</b>
A. Intended Uses.....	22
B. Food Uses as Addressed by JECFA, Merisant & Cargill.....	22
C. Estimated Daily Intake.....	23
D. Other Information on Human Exposure to Stevia: Use as Food Ingredient & Other Uses.....	28
<b>V. SAFETY INVESTIGATIONS FOR STEVIOL GLYCOSIDES.....</b>	<b>28</b>
A. Safety Data on Steviol Glycosides: Recent Reports & Reviews by Expert Bodies & Other Scientists....	28
B. Safety Data on Rebaudioside A.....	29
<b>VI. GRAS CRITERIA &amp; PANEL SAFETY FINDINGS.....</b>	<b>31</b>
A. GRAS Criteria.....	31
B. Panel Findings on Safety Studies of High Purity Steviol Glycosides.....	32
C. Safety of Rebaudioside A.....	34
D. Common Knowledge Elements for GRAS Determinations.....	36

TABLE OF CONTENTS continued

VII. CONCLUSIONS..... 38

VIII. REFERENCES..... 39

TABLES

Table 1. FDA's GRAS Notice Inventory on Rebaudioside & Steviol Glycosides..... 8

Table 2. Chemical Identity of Rebaudioside A & Stevioside..... 13

Table 3. Specifications for GLG-SG Preparations..... 19

Table 4. GLG-SG Storage Stability Data of Steviol Glycosides..... 21

Table 5. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents..... 23

Table 6. Proposed Uses & Levels of Rebaudioside A by Merisant..... 23

Table 7. Summary of Estimated Daily Intake Assessments for Rebaudioside A & Calculation of Rebaudioside A Values from JECFA & FSANZ Estimates of EDI..... 24

Table 8. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)..... 25

Table 9. Sweetness Intensity of GLG-SG Preparations Relative to Sucrose..... 26

Table 10. Daily Intake of Sweeteners (In Sucrose Equivalents) & Estimated Daily Intakes of High Purity GLG-SG Preparations..... 27

FIGURES

Figure 1. Chemical Structure of Rebaudioside A..... 14

Figure 2. Chemical Structure of Stevioside..... 14

Figure 3. Chemical Structures of Various Steviol Glycosides..... 15

Figure 4. GLG Production Process for High Purity Steviol Glycosides (GLG-SG)..... 18

VOLUME 2 -- APPENDICES

APPENDIX A Specifications & Certificates of Analysis for Production Processing Aids..... 4

APPENDIX A-1 GLG Specifications for Ethanol..... 5

APPENDIX A-2 GLG Specifications for Methanol..... 6

APPENDIX A-3 Certificate of Analysis for GLG Active Carbon..... 7

APPENDIX B Analytical Method..... 8

APPENDIX C HPLC Chromatograms for Anysweet RA50..... 16

APPENDIX C-1 HPLC Chromatogram for Batch 20130801..... 17

APPENDIX C-2 HPLC Chromatogram for Batch 20130804..... 19

APPENDIX C-3 HPLC Chromatogram for Batch 20130807..... 21

APPENDIX C-4 HPLC Chromatogram for Batch 20130810..... 23

APPENDIX C-5 HPLC Chromatogram for Batch 20130814..... 25

APPENDIX D HPLC Chromatograms for Anysweet RA60..... 27

APPENDIX D-1 HPLC Chromatogram for Batch 20130801..... 28

APPENDIX D-2 HPLC Chromatogram for Batch 20130803..... 30

APPENDIX D-3 HPLC Chromatogram for Batch 20130806..... 32

APPENDIX D-4 HPLC Chromatogram for Batch 20130809..... 34

APPENDIX D-5 HPLC Chromatogram for Batch 20130811..... 36

TABLE OF CONTENTS continued

APPENDIX E	HPLC Chromatograms for Rebsweet RA80.....	38
APPENDIX E-1	HPLC Chromatogram for Batch 20130801.....	39
APPENDIX E-2	HPLC Chromatogram for Batch 20130805.....	41
APPENDIX E-3	HPLC Chromatogram for Batch 20130807.....	43
APPENDIX E-4	HPLC Chromatogram for Batch 20130810.....	45
APPENDIX E-5	HPLC Chromatogram for Batch 20130815.....	47
APPENDIX F	HPLC Chromatograms for Rebsweet RA85.....	49
APPENDIX F-1	HPLC Chromatogram for Batch 20130802.....	50
APPENDIX F-2	HPLC Chromatogram for Batch 20130806.....	52
APPENDIX F-3	HPLC Chromatogram for Batch 20130808.....	54
APPENDIX F-4	HPLC Chromatogram for Batch 20130812.....	56
APPENDIX F-5	HPLC Chromatogram for Batch 20130816.....	58
APPENDIX G	Certificates of Analysis for Multiple Production Batches of Anysweet RA50 .....	60
APPENDIX G-1	Certificate of Analysis for Batch 20130801.....	61
APPENDIX G-2	Certificate of Analysis for Batch 20130804.....	62
APPENDIX G-3	Certificate of Analysis for Batch 20130807.....	63
APPENDIX G-4	Certificate of Analysis for Batch 20130810.....	64
APPENDIX G-5	Certificate of Analysis for Batch 20130814.....	65
APPENDIX H	Certificates of Analysis for Multiple Production Batches of Anysweet RA60.....	66
APPENDIX H-1	Certificate of Analysis for Batch 20130801.....	67
APPENDIX H-2	Certificate of Analysis for Batch 20130803.....	68
APPENDIX H-3	Certificate of Analysis for Batch 20130806.....	69
APPENDIX H-4	Certificate of Analysis for Batch 20130809.....	70
APPENDIX H-5	Certificate of Analysis for Batch 20130811.....	71
APPENDIX I	Certificates of Analysis for Multiple Production Batches of Rebsweet RA80.....	72
APPENDIX I-1	Certificate of Analysis for Batch 20130801.....	73
APPENDIX I-2	Certificate of Analysis for Batch 20130805.....	74
APPENDIX I-3	Certificate of Analysis for Batch 20130807.....	75
APPENDIX I-4	Certificate of Analysis for Batch 20130810.....	76
APPENDIX I-5	Certificate of Analysis for Batch 20130815.....	77
APPENDIX J	Certificates of Analysis for Multiple Production Batches of Rebsweet RA85.....	78
APPENDIX J-1	Certificate of Analysis for Batch 20130802.....	79
APPENDIX J-2	Certificate of Analysis for Batch 20130806.....	80
APPENDIX J-3	Certificate of Analysis for Batch 20130808.....	81
APPENDIX J-4	Certificate of Analysis for Batch 20130812.....	82
APPENDIX J-5	Certificate of Analysis for Batch 20130816.....	83
APPENDIX K	Pesticide Analytical Reports from SGS-CSTC Standards Technical Services, Co. Ltd....	84
APPENDIX K-1	Test Report for Pesticides GLG RA60 Lot 20130501.....	85
APPENDIX K-2	Test Report for Pesticides GLG RA80 Lot 20130401.....	95
APPENDIX L	Summary of Regulatory & Expert Body Safety Reviews.....	105
APPENDIX M	Studies on Principal Metabolite: Steviol.....	109
APPENDIX N	Studies on Steviol Glycosides Preparations That Are Primarily Stevioside.....	112
APPENDIX O	Studies on Rebaudioside A.....	123

## I. GRAS EXEMPTION CLAIM

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

GLG Life Tech Corporation (“GLG”) has determined that its high purity steviol glycosides (≥ 95%) preparations with rebaudioside A and stevioside as the principal components, referred to as individual products---Anysweet RA50 Plus; Anysweet RA60 Plus; Rebsweet RA80; and Rebsweet RA85---and which meet the specifications described below, are 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 sweeteners.

Signed:

(b) (6)



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

Date: November 25, 2013

### 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 (“GLG”) accepts responsibility for the GRAS determination that has been made for its high purity steviol glycosides preparations,<sup>2</sup> primarily containing rebaudioside A and stevioside, as described in the subject notification; consequently, these high purity steviol glycosides preparations meeting the conditions described herein are exempt from premarket approval requirements for food ingredients.

---

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

<sup>2</sup> GLG refers to its high purity steviol glycoside preparations from the leaves of *Stevia rebaudiana* Bertone with the following trade names: Anysweet RA50 Plus, Anysweet RA60 Plus, Rebsweet RA80, and Rebsweet RA85.

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

High purity steviol glycosides is the common name for the notified substances; also see Section III.A.

### **D. Conditions of Intended Use in Food**

The high purity steviol glycosides preparations, primarily containing rebaudioside A and stevioside, are intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into foods in general, other than 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 steviol glycosides ( $\geq 95\%$ ) preparations, with rebaudioside A and stevioside as the principal components extracted from the leaves of *Stevia rebaudiana* Bertoni have been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

### **F. Availability of Information**

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

## **II. INTRODUCTION**

### **A. Objective**

At the request of GLG, GRAS Associates, LLC ("GA") has undertaken an independent safety evaluation of GLG's steviol glycosides preparations ("GLG-SG"): Anysweet RA50 Plus; Anysweet RA60 Plus; Rebsweet RA80; and Rebsweet RA85. The GLG-SG preparations are extracted from the leaves of *Stevia rebaudiana* Bertoni and purified to yield 50%, 60%, 80%, or 85% rebaudioside A and stevioside with a total steviol glycosides content  $\geq 95\%$ . The purpose of the evaluation is to ascertain whether the intended food uses of steviol glycosides 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; the history of use of stevia in food; and compositional details, specifications, and method of preparation of the notified substance. GLG was asked to provide adverse reports, as well as those that supported conclusions of safety. Safety/toxicity studies performed with animals were noted to have value, along with available human testing. GLG was also asked to supply past and present human food use information. Knowing how much steviol glycosides have been safely consumed, i.e., the use levels, is critical in extrapolating to safe exposures for highly purified steviol glycosides when consumed as a food ingredient. The composite safety/toxicity studies, in concert with exposure information, ultimately provide the specific scientific foundation for the GRAS determination.

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 October 29, 2013. 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 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).

Based on available information from FDA's GRAS Notice Inventory<sup>3</sup> website as of November 25, 2013, the agency has issued 27 "no questions" letters on GRAS notices on rebaudioside A 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) has reviewed steviol glycosides at its 51<sup>st</sup>, 63<sup>rd</sup>, 68<sup>th</sup> and 73<sup>rd</sup> meetings. In 2000, JECFA published the original review on steviol glycosides (WHO, 2000). JECFA established a temporary ADI (acceptable daily intake) of 0-2 mg/kg (on a steviol basis) at its 63<sup>rd</sup> meeting (WHO, 2006). Additionally, JECFA finalized food grade specifications (FAO, 2007a), although they were subsequently updated in 2008 (FAO, 2008) and 2010 (FAO, 2010) (see below). At the 69<sup>th</sup> meeting, the temporary status of the ADI was removed, and the ADI was raised to 0-4 mg/kg bw/day (on a steviol basis) as a result of the JECFA review of recently completed clinical studies with steviol glycosides (WHO, 2008). In 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009).

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

**Table 1. FDA’s GRAS Notice Inventory on Rebaudioside & Steviol Glycosides Preparations<sup>a, b</sup>**

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	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	GRN 348	High-Purity Stevioside ≥95%	General-purpose & table top sweetener, excluding meat, poultry products & infant formulas
14. GLG Life Tech Ltd	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
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 Freeman 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

COMPANY	FDA GRAS IDENTIFIER	MATERIAL IDENTITY	INTENDED FOOD USES
20. GLG Life Tech Ltd	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. Daepyeong Co., Ltd.	GRN 393	Purified Reb A	General purpose & table top sweetener, excluding meat & poultry products
24. Daepyeong 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. Daepyeong Co., Ltd.	GRN 448	Enzyme Modified Steviol Glycosides	General-purpose sweetener, excluding meat, poultry products & infant formulas.
27. Almendra, Ltd.	GRN 461	High-Purity Reb A ≥97%	General-purpose sweetener, excluding meat, poultry products & infant formulas.

<sup>a</sup> This table was derived, in part, from McQuate (2011). <sup>b</sup> GRN 452, submitted by Daepyeong Co., Ltd regarding glucosylated steviol glycosides, was filed by FDA on January 14, 2013 and is presently under review; GRN 456, submitted by PureCircle USA, Inc. regarding rebaudioside D, was filed by FDA on January 23, 2013 and is presently under review; GRN 467, submitted by Qufu Xiangzhou Stevia Products Co., Ltd. Regarding rebaudioside A, was filed by FDA on April 30, 2013 and is presently under review; GRN 473, submitted by PureCircle, Ltd. Regarding rebaudioside X, was filed by FDA on May 23, 2013 and is presently under review.

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.<sup>4</sup> 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, 2011b).

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 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

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<sup>4</sup> From a historical perspective, it is noted that the UK's Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food on September 24, 1998 rejected an application for use of steviol glycosides as a sweetener in herbal teas because "the applicant had not provided all of the information necessary to enable an assessment to be made." See <http://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 also reiterated "its earlier opinion that stevioside is not acceptable as a sweetener on the presently available data" (European Commission, 1999b).

‘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 December 10, 2012, multiple food registrations have been granted by FDA Philippines to stand-alone steviol glycosides sweeteners or foods containing steviol glycosides as ingredients: FR-104390, Steviten Light Brand Steviol Glycosides 95% Sweetener Powder; FR-109427, Del Monte Pineapple Chunks in Extra Light Syrup Reduced Calorie with Steviol Glycosides from Stevia; FR-101120, Diebetamil Zero Calorie Sweetener with Stevia (stick pack); and FR-102127, Sawayaka Stevia Sweetener (1 g sticks) (Republic of the Philippines, Food and Drug Administration, 2013).

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 accessed on their website on 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.<sup>5</sup>

In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process. At that time, the petitioning process was replaced with a notification procedure.<sup>6</sup> 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.

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<sup>5</sup> See 21 CFR 170.3(i)(3).

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

### III. CHEMISTRY & MANUFACTURE OF GLG-SG

#### A. Common or Usual Name

High purity steviol glycosides is the common or usual name of the non-nutritive sweetener derived from *Stevia rebaudiana* Bertoni that is the subject of the GRAS evaluation. The compositional features of the subject high purity steviol glycosides ( $\geq 95\%$ ), primarily containing rebaudioside A and stevioside, are described in more detail in this section. GLG-SG is the general term used by GLG to encompass the high purity steviol glycosides preparations in referring to the notified substance. GLC-SG, therefore, refers to the following commercial names of the high purity steviol glycosides preparations: Anysweet RA50 Plus; Anysweet RA60 Plus; Rebsweet RA80; and Rebsweet RA85.

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

#### B. Description

In 2010, Food Chemicals Codex (FCC) prepared a monograph with a description and specifications for rebaudioside A. In this monograph, rebaudioside A is described as a white to off-white, hygroscopic fine crystal, granule, or powder having a sweet taste (FCC, 2010). It is freely soluble in ethanol:water 50/50 (v/v), and is sparingly soluble in water and in ethanol. Rebaudioside A is obtained from the leaves of the *Stevia rebaudiana* Bertoni plant in a multistep separation and purification process. The principal steps of manufacturing include extraction of steviol glycosides from the leaves using an aqueous or aqueous alcoholic (ethanol or methanol) solvent, and purification of rebaudioside A from the resulting mixture of steviol glycosides by resin absorption followed by recrystallization from an aqueous or aqueous alcoholic (ethanol or methanol) solvent. It is primarily composed of rebaudioside A, a glycoside of the *ent*-kaurenoid diterpenoid aglycone known as steviol (FCC, 2010).

#### C. Chemistry of Steviol Glycosides

At its 51<sup>st</sup> meeting, JECFA reviewed the safety related information on steviol glycosides, including the identity and chemistry of these compounds. The following 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).

Of the nine different steviol glycosides, the two principal sweetener components of stevia extracts have been identified as rebaudioside A and stevioside. The chemical identities and key chemical identifiers for the two major components are presented in Table 2.

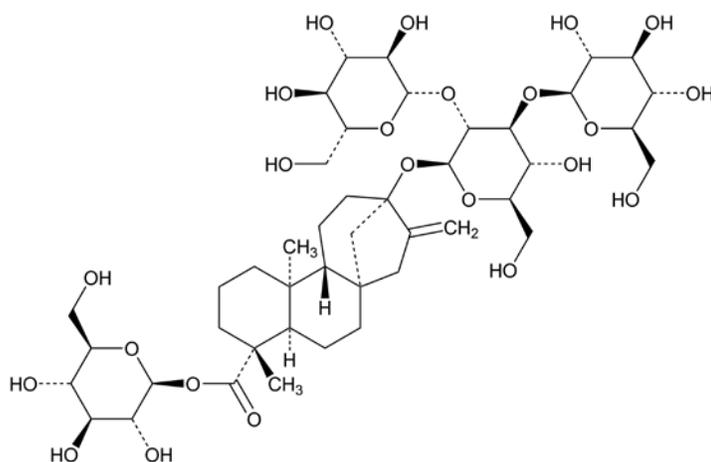
**Table 2. Chemical Identity of Rebaudioside A & Stevioside**

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

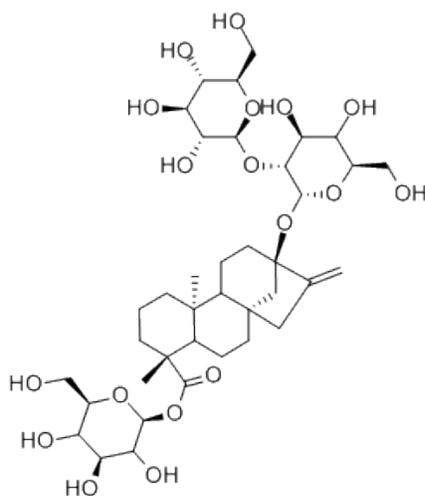
The chemical structure of rebaudioside A is presented in Figure 1, and the chemical structure of stevioside is presented in Figure 2.

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 3.

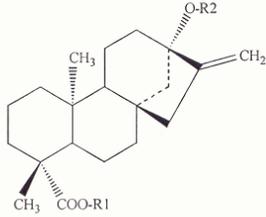
**Figure 1. Chemical Structure of Rebaudioside A**



**Figure 2. Chemical Structure of Stevioside**



**Figure 3. Chemical Structures of Various Steviol Glycosides<sup>a, b</sup>**



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

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

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

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.

## D. Accepted Identity Specifications for Food Grade Steviol Glycosides

In addition to the manufacturing process, the composition of *Stevia rebaudiana* Bertoni extract depends upon the composition of the harvested leaves, which, in turn, is influenced by soil, climate, etc. (FAO, 2007b). As discussed in Section III.F.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.

## E. 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 GLC-SG, which is also specifically discussed in Section III.E.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<sub>2</sub>, and extract clarification with zeolite have been employed.

At the 68<sup>th</sup> JECFA meeting, steviol glycosides were defined as the products obtained from the leaves of *Stevia rebaudiana* Bertoni. As described by JECFA, the typical 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 GLG-SG

For the manufacturing of steviol glycosides, GLG employs a fairly typical process that is used in the industry for the production of stevia-derived sweeteners that are prepared in accordance with current Good Manufacturing Practices regulations (cGMP). The manufacturing process is summarized in a flow chart provided in Figure 4. The source of GLG's high purity steviol glycoside (>95%) preparations is the leaves of the *Stevia rebaudiana* (Bertoni) plant. GLG extracts the steviol glycosides from the stevia leaves with water. The aqueous extract is then re-extracted with

ethanol and/or methanol, and the resulting steviol glycosides are crystallized. The steviol glycosides are concentrated via filtration to obtain the appropriate Reb A concentration (either 50%, 60%, 80% or 85% Reb A). The material is then dissolved in purified water at a temperature of 70-80°C. The steviol glycoside solution undergoes additional filtration, decolorization, and concentration steps prior to sterilization and spray drying. 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 for each of the GLG-SG preparations are detailed in Appendix B, Appendices C-F, and Appendices G-J, respectively. Results from pesticide analysis of representative GLG-SG samples are provided in Appendix K. The content of steviol glycosides in the final GLG-SG product in all cases is  $\geq 95\%$ .

The ethanol and methanol, and activated charcoal used in the purification process comply with FCC 5th Edition specifications, and the filters used in the manufacturing comply with 21 CFR 173.25. Specifications and certificates of analysis are provided in Appendix A.

## **F. Product Specifications & Supporting Methods**

### **1. JECFA Specifications for Steviol Glycosides**

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

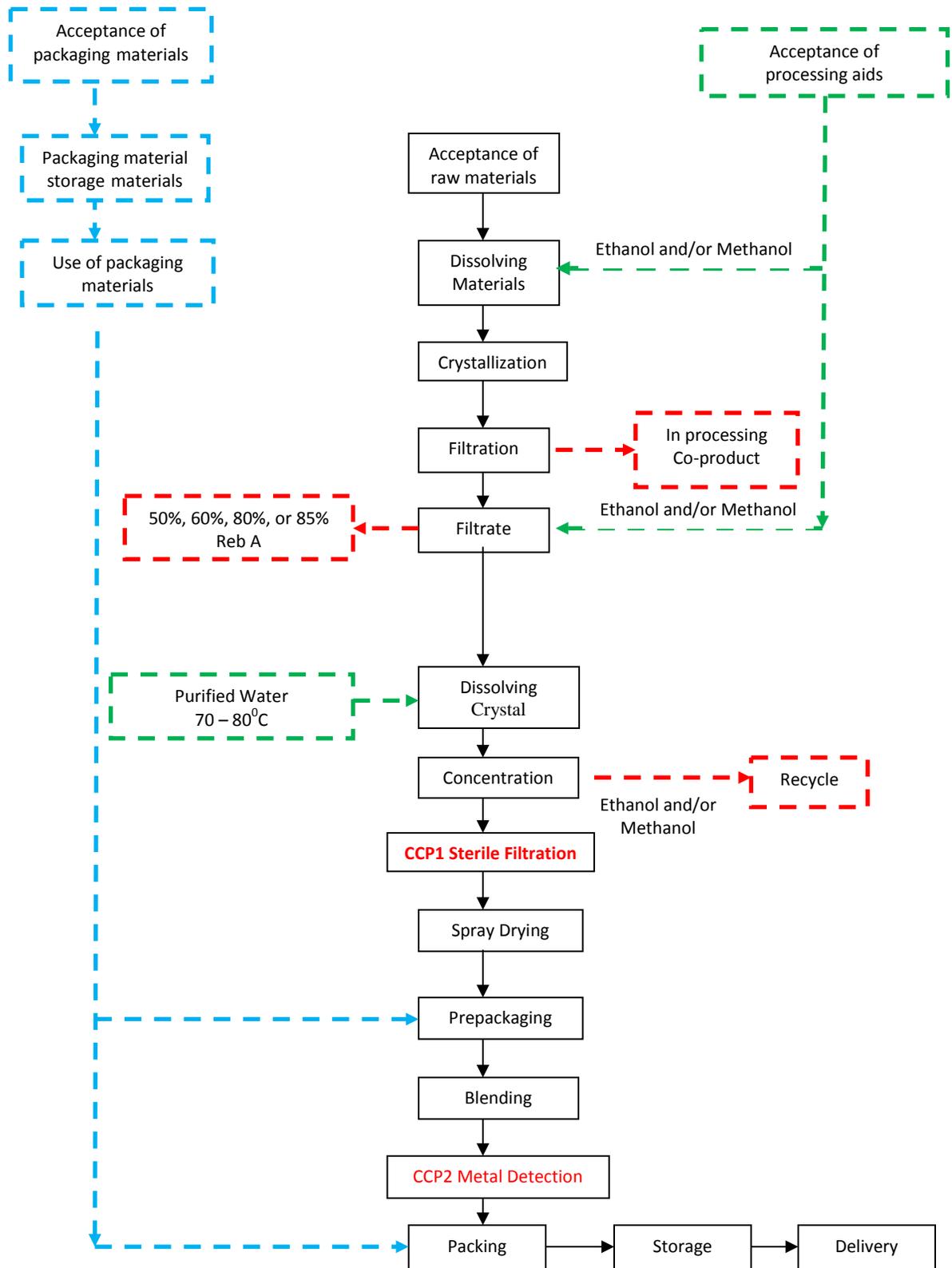
In 2007, JECFA recommended that the method of assay should include a minimum requirement of 95% of the total of 7 specific steviol glycosides on a dried weight basis, and JECFA finalized food grade specifications at the 68<sup>th</sup> JECFA meeting with publication in the FAO JECFA Monograph 4 (FAO, 2007a). Stevioside and rebaudioside A are the major component glycosides of interest because of their sweetening property. The five other associated glycosides found in preparations of steviol glycosides accepted by the JECFA specifications with the 95% requirement are rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B. These, however, are typically found at much lower levels than stevioside or rebaudioside A. JECFA updated the specifications for steviol glycosides in 2008 (FAO, 2008), and then again in 2010, when the specifications were expanded to include the original seven specific steviol glycosides plus Reb D and Reb F (FAO, 2010).

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 - 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.

### **2. Specifications for GLG-SG Preparations & Supporting Methods**

GLG has adopted product specifications for its purified steviol glycosides products that meet or exceed JECFA recommendations, while also complying with Food Chemicals Codex (FCC, 2010)

**Figure 4. GLG Production Process for High Purity Steviol Glycosides (GLG-SG)**



specifications for rebaudioside A as a consumable human food substance. The compositions of five product batches from each of the GLG-SG preparations are compared to the JECFA and FCC specifications, and are presented, in full, in Appendices G-J. Representative lots of each of the four GLG-SG preparations compared to the JECFA and FCC specifications are presented in Table 3.

**Table 3. Specifications for GLG-SG Preparations**

PARAMETER	JECFA <sup>a</sup> SPECIFICATIONS STEVIOL GLYCOSIDES	FCC <sup>b</sup> SPECIFICATIONS REBAUDIOSIDE A	GLG SPECIFICATIONS			
			ANYSWEET RA50 PLUS	ANYSWEET RA60 PLUS	REBSWEET RA80	REBSWEET RA85
Appearance	White to light yellow powder	White to off-white, hygroscopic fine crystal, granule, or powder	White/off-white hygroscopic powder	White/off-white hygroscopic powder	White/off-white hygroscopic powder	White/off-white hygroscopic powder
Sweetness	200-300 times sweeter than sucrose	NS	NS	NS	NS	NS
Solubility	Freely soluble in water	Freely soluble in water:ethanol (50:50)	Freely Soluble	Freely Soluble	NS	Freely soluble
Rebaudioside A	NS	NLT 95%	NLT 50%	NLT 60.0%	NLT 80%	NLT 85.0%
Stevioside	NS	NS	NLT 25%	NLT 15.0%	NS	NS
Total Steviol Glycosides	NLT 95%	NA	NLT 95%	NLT 95.0%	NLT 95%	NLT 95.0%
Residue on Ignition	NS	NS	NMT 1.0%	NMT 1.0%	NMT 1.0%	NMT 1.0%
Moisture (loss on drying)	NMT 6%	NMT 6%	NMT 4.0%	NMT 4.0%	NMT 4.0%	NMT 4.0%
pH (1% solution)	4.5 - 7.0	4.5 - 7.0	4.5-7.0	4.5-7.0	4.5-7.0	4.5-7.0
Specific Rotation	NS	NS	-30°to -38°	-30°to -38°	NS	NS
<b>RESIDUAL SOLVENT LEVELS</b>						
Total Solvents	NS	NS	NMT 5200 ppm	NMT 5200 ppm	NMT 5200 ppm	NMT 5200 ppm
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	NMT 200 ppm	NMT 200 ppm	NMT 200 ppm	NMT 200 ppm
Residual Ethanol	NMT 5000 mg/kg	NMT 0.5%	NMT 5000 ppm	NMT 5000 ppm	NMT 5000 ppm	NMT 5000 ppm
<b>HEAVY METALS</b>						
Total Metals	NS	NS	NMT 10 ppm	NMT 10 ppm	NMT 10 ppm	NMT 10.0 ppm
Lead	NMT 1 mg/kg	NMT 1 mg/kg	NMT 1.0 ppm	NMT 1.0 ppm	NMT 1.0 ppm	NMT 1.0 ppm
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	NMT 1.0 ppm	NMT 1.0 ppm	NMT 1.0 ppm	NMT 1.0 ppm
<b>MICROBIOLOGICAL</b>						
Total Plate Count (cfu/g)	NA	NA	<1000	<1000	<1000	<1000
Yeast & Mold Plate Count (cfu/g)	NA	NA	<100	<100	<100	<100
<i>Salmonella</i>	NA	NA	Negative	Negative	Negative	Negative
<i>Escherichia coli</i>	NA	NA	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	NA	NA	Negative	Negative	Negative	Negative

<sup>a</sup> Prepared at 73rd JECFA (2010).

<sup>b</sup> FCC, 2010. Rebaudioside A monograph. Food Chemicals Codex (7th Ed.)

NS = not specified; NA = not applicable; NLT = not less than; NMT = not more than

Details of the analytical methodology employed to determine steviol glycosides is provided in Appendix B, the chromatograms for representative GLG-SG preparations are provided in Appendices C-F, and certificates of analysis for five representative lots of each of the GLG-SG preparations are provided in Appendices G-J. Pesticide residue screening is periodically conducted on various product lots. Test reports for analysis of pesticide residues in representative lots of RA60 and RA80 are located in Appendix K. The collection of these reports demonstrates that the substance is well characterized and meets the established purity criteria.

## G. Stability Data

### 1. Stability Data on 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, it rapidly decomposes (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 resulted in approximately 20% loss of rebaudioside A. Heating at 60°C for 6 days resulted in 0-6% loss of rebaudioside A.

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

Cargill (2008) also conducted extensive stability testing on rebaudioside A as a powder under various storage conditions and under a range of 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.

## 2. Stability Data for GLG-SG Preparations

Due to the favorable stability profile for products of similar composition as noted in Section III.G.1., GLG conducted a shelf-stability test study on its RA60 and RA80 preparations as representative for the 4 subject products. Over the course of 24 months, samples of one lot of each preparation were stored at 25°C ± 5°C at a relative humidity of 60% ± 5% for 0, 3, 6, 12, 18 and 24 months. The stability samples were then tested for steviol glycosides, including rebaudioside A and stevioside, and microbial parameters. A summary of the shelf-stability results is presented in Table 4.

**Table 4. GLG-SG Storage Stability Data of Steviol Glycosides**

Lot# GLG-RA60 Plus-20130801, Steviol Glycosides, % dry basis							
Duration	Reb A	Stevioside	Total Steviol Glycosides	Total plate count	<i>Salmonella</i>	<i>E. coli</i>	<i>Staphylococcus</i>
t=0	61.63	22.43	96.34	<10cfu/g	Negative	Negative	Negative
3 months	61.58	22.36	96.16	<10cfu/g	Negative	Negative	Negative
6 months	61.42	22.28	95.89	<10cfu/g	Negative	Negative	Negative
12 months	61.36	22.16	95.68	<10cfu/g	Negative	Negative	Negative
18 months	61.12	22.09	95.42	10cfu/g	Negative	Negative	Negative
24 months	60.86	21.98	95.11	10cfu/g	Negative	Negative	Negative
Lot# GLG-RA80-20130815, Steviol Glycosides, % dry basis							
Duration	Reb A	Stevioside	Total Steviol Glycosides	Total plate count	<i>Salmonella</i>	<i>E. coli</i>	<i>Staphylococcus</i>
t=0	81.02	5.32	95.93	<10cfu/g	Negative	Negative	Negative
3 months	81.02	5.31	95.92	<10cfu/g	Negative	Negative	Negative
6 months	80.98	5.28	95.81	<10cfu/g	Negative	Negative	Negative
12 months	80.62	5.26	95.58	<10cfu/g	Negative	Negative	Negative
18 months	80.56	5.25	95.40	10cfu/g	Negative	Negative	Negative
24 months	80.32	5.25	95.15	30cfu/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, along with GLG's stability testing results, support the position that GLG's high purity steviol glycosides preparations are well-suited for the intended food uses.

#### **IV. INTENDED FOOD USES & ESTIMATED DIETARY INTAKE**

##### **A. Intended Uses**

The subject GLG-SG preparations with steviol glycosides ( $\geq 95\%$ ), containing rebaudioside A and stevioside as the principal components, are intended to be used as a table top sweetener and general purpose non-nutritive sweetener in various foods other than infant formulas and meat and poultry products. The intended use will be as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19).<sup>7</sup> The intended use levels will vary by actual food category, but the actual levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts of GLG-SG preparations to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation.<sup>8</sup>

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

As part of its safety deliberations, JECFA reviewed various estimates of possible daily intake of steviol glycosides (WHO, 2006). These estimates are presented in Table 5. Merisant also listed intended use levels of rebaudioside A for various food applications in their GRAS Notification (Table 6). Merisant utilized food consumption survey data from 2003-2004 NHANES to determine the estimated daily intake from the proposed uses of rebaudioside A. On a per user basis, the mean and 90<sup>th</sup> percentile daily consumption of rebaudioside A was estimated as 2.0 and 4.7 mg/kg bw/day, respectively. In its notification, Cargill (2008) utilized a different approach in estimating dietary intake figures for rebaudioside A when incorporated as a general sweetener in a broad cross-section of processed foods. Cargill considered that, with a few minor exceptions, rebaudioside A uses and use levels would be comparable to those of aspartame uses in the US. Using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008), Cargill performed a side-by-side consumption analysis for rebaudioside A versus aspartame. Findings from the above-described different sources along with FSANZ estimates are further discussed in Section IV.C, and the intake estimates are presented in Table 7.

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<sup>7</sup> Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.

<sup>8</sup> See 21 CFR 182.1(b)(1).

### C. Estimated Daily Intake

The very conservative consumer intake estimates provided by JECFA as shown in Table 5 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. Since rebaudioside A is about twice as sweet as the mixed glycosides, these levels can be adjusted accordingly. GLG intends to use GLG-SG preparations in a number of food categories at levels that comply with GMP uses. The application of GLG-SG to the same foods and at the same levels as those described in earlier FDA notices by Merisant and Cargill is unlikely to affect the dietary intake of rebaudioside A from introduction into the market by another supplier who will have to compete in essentially the same markets and foods. This also negates the need for a cumulative intake analysis.

**Table 5. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents**

FOOD TYPE	MAXIMUM USE LEVEL REPORTED <sup>a</sup> (MG STEVIOL GLYCOSIDES /KG OF FOOD)	MAXIMUM USE LEVEL CALCULATED FOR REBAUDIOSIDE A <sup>b</sup> MG REBAUDIOSIDE A /KG OF FOOD	MAXIMUM USE LEVEL CALCULATED FOR REBAUDIOSIDE A <sup>b</sup> MG STEVIOL EQUIVALENTS /KG OF FOOD
Desserts	500	250	83
Cold confectionery	500	250	83
Pickles	1000	500	167
Sweet corn	200	100	33
Biscuits	300	150	50
Beverages	500	250	83
Yogurt	500	250	83
Sauces	1000	500	167
Delicacies	1000	500	167
Bread	160	80	27

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

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

**Table 6. Proposed Uses & Levels of Rebaudioside A by Merisant<sup>a</sup>**

FOOD GROUP	REBAUDIOSIDE A (PPM)
Table top sweeteners	30,000 <sup>b</sup>
Sweetened ready-to-drink teas	90-450
Fruit juice drinks	150-500
Diet soft drinks	150-500
Energy drinks	150
Flavored water	150
Cereals (oatmeal, cold cereal, cereal bars)	150

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

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

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

JECFA evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that were prepared for the European Commission by the Scientific Committee on Food. JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars, at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1. The intakes ranged from 1.3 mg/kg bw/day with the African diet to 3.5 mg/kg bw/day with the European diet. Additionally, JECFA also estimated the per capita exposure derived from disappearance (poundage) data supplied by Japan and China. The Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US. The exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee are summarized in Table 8.

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

**Table 7. Summary of Estimated Daily Intake Assessments for Rebaudioside A & Calculation of Rebaudioside A Values from JECFA & FSANZ Estimates of the EDI**

SCENARIOS	EDI		
	AS STEVIOL <sup>a</sup> (MG/KG BW/DAY)	AS REBAUDIOSIDE A <sup>b</sup> (MG/KG BW/DAY)	TOTAL DAILY INTAKE <sup>c</sup> (MG/DAY)
<b>JECFA</b>			
100% Reb A replacement of sugars	5.0	7.5	450
20-30% Reb A replacement of sugars	1.0 - 1.5	1.5 - 2.3	90 - 140
<b>FSANZ</b>			
100% Reb A replacement of sugars	0.3 - 1.0	0.5 - 1.5	30 - 90
<b>MERISANT</b>			
		2.0 - 4.7 <sup>d</sup>	120 - 282
<b>CARGILL</b>			

		1.3 - 3.4 <sup>d</sup>	78 - 204
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- <sup>a</sup> Published values for mixed steviol glycosides consumption listed in this column were used for the calculation of Reb A consumption values appearing in next two columns.
- <sup>b</sup> Estimates for Reb A consumption were calculated from JECFA and FSANZ estimates as steviol by multiplying by 3 to correct for the molecular weight of Reb A compared to steviol and by subsequently dividing by 2 because of the increased inherent sweetness of Reb A compared to the mixed steviol glycosides.
- <sup>c</sup> Total daily intake figures were calculated for a 60 kg adult.
- <sup>d</sup> Published values are shown for comparison purposes.

**Table 8. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)**

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

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

Similar to JECFA, FSANZ (2008) also estimated steviol glycosides dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario which resulted in estimated exposures of 0.3 - 1.0 mg/kg bw/day on a steviol basis, or 0.5 – 1.5 mg/kg bw/day for rebaudioside A, when making both the molecular weight and sweetness equivalency calculations. Merisant also calculated a dietary estimate for rebaudioside A of 2.0 mg/kg bw/day for the average consumer of the foods listed in Table 6, and 4.7 mg/kg bw/day for a 90<sup>th</sup> percentile consumer. In another review conducted on behalf of Cargill, and included in their GRAS notification, the intake of rebaudioside A when used as a complete sugar replacement was estimated at 1.3 – 3.4 mg/kg bw/day when calculated as rebaudioside A (Renwick, 2008). The estimated daily intake assessments have been compiled in Table 8. These different assessments suggest that total daily consumption of rebaudioside A for specified food categories and as a general purpose sweetener is unlikely to exceed 5 mg/kg bw/day, for a total daily dietary exposure of up to 300 mg rebaudioside A for an adult weighing 60 kg. EFSA also calculated the daily intake of steviol glycosides (EFSA, 2010) following the JEFCA guidelines. EFSA (2010) considers that the results of toxicology studies on either stevioside or rebaudioside A are applicable for 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 each.

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 that 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 European 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, 2011a). It should be noted that this estimate is based on European consumption patterns. All other estimates of consumption do not suggest that there is a concern of exceeding the ADI.

There have been many scholarly estimates of potential dietary intake of replacement sweeteners---including steviol glycosides---that have been published (FSANZ, 2008; Renwick, 2008; WHO, 2003) or submitted to FDA (Merisant, 2008). In GRAS notification 301, a simplified estimate was proposed to and accepted by FDA based on the estimates of exposure in “sucrose equivalents” (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90<sup>th</sup> percentile consumer of a sweetener that 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. Using the WHO GEMS/Food database assumption that steviol glycosides, in general, exhibit a minimum relative sweetness intensity of 200:1 when compared to sucrose, one can assume that high purity rebaudioside A also shows a minimal 200-fold sweetness to that of sucrose (WHO, 2006).

GLG’s steviol glycoside preparations have varying sweetness intensities when compared to sucrose; specific sweetness intensities are provided in Table 9.

**Table 9. Sweetness Intensity of GLG-SG Preparations Relative to Sucrose**

PRODUCT	SWEETNESS INTENSITY
Anysweet RA50 Plus	280
Anysweet RA60 Plus	320
Rebsweet RA80	360
Rebsweet RA85	380

Therefore, the highest 90<sup>th</sup> percentile consumption by any population subgroup of GLG’s high purity steviol glycosides preparations would be approximately 3.54 mg/kg bw/day (for Anysweet RA50). A weighted sum estimate was used to determine the steviol equivalency factor, on a worst-case scenario basis. For example, GLG’s Anysweet RA50 steviol equivalence factor was calculated from the molecular weight ratios of steviol to rebaudioside A, stevioside, and remaining steviol glycosides (as steviolbioside), on a percent composition basis, as follows:

$$SteviolEquivalenceFactor = \left( \frac{MW_{Steviol}}{MW_{RebA}} \bullet 0.50 \right) + \left( \frac{MW_{Steviol}}{MW_{Stevioside}} \bullet 0.25 \right) + \left( \frac{MW_{Steviol}}{MW_{Steviolbioside}} \bullet 0.25 \right)$$

Based on a weighted sum estimate for steviol equivalents,<sup>9</sup> the consumption would be less than 1.37 mg/kg bw/day on a steviol equivalents basis for any population group, on a worst-case scenario basis. These calculations are summarized in Table 10.

**Table 10. Daily Intake of Sweeteners (In Sucrose Equivalents) & Estimated Daily Intakes of High Purity GLG-SG Preparations**

Population Group	Intakes of Sweeteners (g sucrose/kg bw/day) <sup>a</sup>		Intake of RA50 (mg/kg bw/day) <sup>b</sup>		Intake of RA50 as Steviol Equivalents <sup>c</sup>	
	Low	High	Low	High	Low	High
Healthy Population	255	675	0.91	2.41	0.35	0.93
Diabetic Adults	280	897	1.00	3.20	0.39	1.24
Healthy Children	425	990	1.52	3.54	0.59	1.37
Diabetic Children	672	908	2.40	3.24	0.93	1.25
Population Group	Intakes of Sweeteners (g sucrose/kg bw/day) <sup>a</sup>		Intake of RA60 (mg/kg bw/day) <sup>b</sup>		Intake of RA60 as Steviol Equivalents <sup>c</sup>	
	Low	High	Low	High	Low	High
Healthy Population	255	675	0.80	2.11	0.30	0.80
Diabetic Adults	280	897	0.88	2.80	0.33	1.07
Healthy Children	425	990	1.33	3.09	0.51	1.18
Diabetic Children	672	908	2.10	2.84	0.80	1.08
Population Group	Intakes of Sweeteners (g sucrose/kg bw/day) <sup>a</sup>		Intake of RA80 (mg/kg bw/day) <sup>b</sup>		Intake of RA80 as Steviol Equivalents <sup>c</sup>	
	Low	High	Low	High	Low	High
Healthy Population	255	675	0.71	1.88	0.26	0.68
Diabetic Adults	280	897	0.78	2.49	0.28	0.90
Healthy Children	425	990	1.18	2.75	0.43	1.00
Diabetic Children	672	908	1.87	2.52	0.68	0.91
Population Group	Intakes of Sweeteners (g sucrose/kg bw/day) <sup>a</sup>		Intake of RA85 (mg/kg bw/day) <sup>b</sup>		Intake of RA85 as Steviol Equivalents <sup>c</sup>	
	Low	High	Low	High	Low	High
Healthy Population	255	675	0.67	1.78	0.24	0.63
Diabetic Adults	280	897	0.74	2.36	0.26	0.83
Healthy Children	425	990	1.12	2.61	0.40	0.92
Diabetic Children	672	908	1.77	2.39	0.63	0.85

<sup>a</sup> Source: Renwick (2008).

<sup>b</sup> Calculated by dividing the sucrose intake by the average relative sweetness values from Table 9.

<sup>c</sup> Calculated based on the ratio of molecular weights of rebaudioside A, steviol, and steviol glycosides, as described above.

<sup>9</sup> Calculated by the Expert Panel as a percent of molecular weight of steviol to the molecular weight of rebaudioside A, stevioside, and steviol glycosides (as steviolbioside), on a percent composition basis. The total steviol glycosides content was assumed to be 100%. All steviol glycosides, with the exception of Reb A and stevioside, were treated as steviolbioside for calculation purposes.

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

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

For about 20 years, consumers in Japan and Brazil, where stevia has long been approved as a food additive, have been using stevia extracts as non-caloric sweeteners.<sup>10</sup> 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 glycoside 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).

### **V. SAFETY INVESTIGATIONS FOR STEVIOL GLYCOSIDES**

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

GLG's high purity steviol glycosides preparations contain rebaudioside A and stevioside as the major components, with not less than 95% total steviol glycosides. Given the structural similarity among rebaudioside A, stevioside, and other steviol glycosides, along with metabolic considerations, the scientific data on stevia and its other components, particularly the available extensive data on rebaudioside A, are relevant to the present safety assessment. This is further supported by the fact that EFSA (2010) considers that the results of toxicology studies on either stevioside or rebaudioside A are applicable to the safety assessment of steviol glycosides, as both

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<sup>10</sup> See Raintree Nutrition Tropical Plant Database <http://www.rain-tree.com/stevia.htm>.

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 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, 27 of the submitted notifications have had "no questions" letters of response from FDA (see Table 1).

More detailed reviews on safety of steviol glycosides by expert bodies such as JECFA, FSANZ and EFSA are summarized in Appendix L. A more detailed review on steviol, the principal metabolite of steviol glycosides, can be found in Appendix M. A more detailed review of steviol glycosides appears in Appendix N.

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

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, two studies found in a recent literature search examined the anti-hyperglycemic activity of rebaudioside A in diabetic rats (Saravanan et al., 2012; Saravanan and Ramachandran, 2013). 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. In the second study, the administration of 200 mg/kg bw Reb A to diabetic rats normalized levels of plasma glucose, insulin, lipid peroxidation products, enzymatic, non-enzymatic antioxidants and lipids (Saravanan and Ramachandran, 2013). The doses used are 10-40 times higher than expected from the use of rebaudioside A as a sweetener (Saravanan et al., 2012).

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.

Much of the supporting evidence with respect to the safety of rebaudioside A is derived from safety studies on purified steviol glycosides, which were largely composed of mixtures that were predominately stevioside and Reb A. These studies include a complete battery of toxicology and clinical studies on steviol glycosides, and evidence of poor GI absorption (Gardana et al., 2003; Geuns and Pietta, 2004; Koyama et al., 2003a) of steviol glycosides in the upper GI tract in concert with the conversion of the steviol glycosides to steviol by normal flora of the lower GI tract (Koyama et al., 2003b, Renwick and Tarka, 2008). Additional studies (Hutapea et al., 1997; Geuns et al., 2007) report that human digestive enzymes are not capable of hydrolyzing  $\beta$ -glycosidic bonds, and, thus, steviol glycosides are not digested in the upper gastrointestinal tract. Steviol is absorbed, but is rapidly converted to glucuronides that are subsequently excreted in the urine or eliminated by the enterohepatic circulation. Because of the structural similarity, it is reasonable to expect that Reb A is not absorbed in the GI tract but is similarly converted to steviol by the normal flora of the lower GI tract.

Based on the presumption that rebaudioside A is not appreciably absorbed from the GI tract and it is similarly converted to steviol by intestinal flora, the safety review of purified rebaudioside A can be further supported by the large body of published evidence supporting the safety of purified steviol glycosides extracts. Steviol is absorbed from the colon, subjected to glucuronidation in the liver, and excreted *via* bile primarily as steviol glucuronide in the feces of rats or the urine of humans. The differences in the route of elimination are due to the lower molecular weight thresholds for biliary excretion in rats (325 Da) compared to humans (500 to 600 Da). Although the primary routes of elimination of steviol glucuronide differ between rats and humans, the metabolisms of modified and non-modified steviol glycosides and pharmacokinetics are quite similar, which confirms that the rat is an acceptable model for risk assessment in humans (Roberts and Renwick, 2008; Wheeler, et al., 2008). There is an extensive database of literature on steviol glycosides extracts already in the published literature, along with in-depth reviews in numerous GRAS submissions. JECFA (WHO, 2008), after several years of review, established an ADI of 4 mg/kg bw expressed as steviol equivalents, based on studies on test samples of steviol glycosides with a minimum purity of 95% expressed on a dry weight basis. The critical studies leading to this decision included a chronic study in rats (Toyoda, et al. 1997), which indicated a lack of effects or carcinogenic activity at the highest doses, along with a series of clinical studies conducted at 11 mg/kg bw, which ruled out effects on blood glucose and blood pressure.

Given the chemical similarity of rebaudioside A and stevioside, the results of toxicology studies on stevioside and stevia extracts can be used to support the safety assessment of Reb A. A more detailed review on Rebaudioside A can be found in Appendix O.

## 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.”<sup>11</sup>

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

Furthermore, in discussing GRAS criteria, FDA notes that:

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

“General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information.”<sup>12</sup>

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called “common knowledge element,” in terms of the two following component elements:<sup>13</sup>

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and

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<sup>11</sup> See 21 CFR 170.3(i).

<sup>12</sup> See 21 CFR 170.30(a).

<sup>13</sup> See Footnote 1.

- 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).

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

## **B. Panel Findings on Safety Studies of High Purity Steviol Glycosides**

Because of their sweetness characteristics, steviol glycosides have viable uses as a non-nutritive sweetener in foods.<sup>14</sup> Periodic reviews by JECFA over the years indicate the progress of knowledge on the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple investigations with *in vivo* and *in vitro* models, which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years, as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic, and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably, the initially reported concerns related to the effects of stevia leaves or crude extracts on fertility were refuted by the well-designed reproductive studies with purified steviol glycosides. All other concerns failed to manifest themselves at the doses employed in the long-term rat studies.

As discussed in Section V, at its 51<sup>st</sup> 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 the use of high purity preparations of various steviol glycosides, are safe to use as a sugar substitute. Subsequently, the additional clinical data

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

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 Section IV.C and summarized in Table 7, the highest dietary estimate for use in foods for rebaudioside A is 4.7 mg/kg bw/day. The Panel agrees with the JECFA ADI of 4 mg/kg bw/day based on steviol equivalents, which corresponds to 12 mg/kg bw/day for rebaudioside A, and notes that the estimates of anticipated dietary intake, as contained in Table 7, 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). This study is summarized in Appendix N. 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 27 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 A, 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 steviol glycosides preparations to the composition of the test materials used in all the published studies. The Panel agrees that GLG's high purity steviol glycosides preparations are sufficiently similar to those used in all key studies reviewed by JECFA, and those on rebaudioside A subsequently reviewed by FDA, and there is no need for further studies to be conducted on the GLG-SG products. The Panel has also reviewed the expected levels of dietary intake and agrees that there is sufficient information to conclude that the subject GLG-SG products 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. Safety of Rebaudioside A

Since July 2008, over 10 papers describing the results of a comprehensive research program by two different groups on rebaudioside A have been published. These, and some other unpublished studies, formed the basis of the two initial GRAS notifications to FDA 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.

The Expert Panel discussed findings from a recently published exploratory subchronic toxicity study in rats by Awney et al. (2011), where a number of toxicological effects of stevioside treatment were reported. This study is summarized in Appendix N. 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. In contrast to the data presented by Awney et al. (2011), several well-designed and well-conducted subchronic toxicity studies did not reveal any adverse effects from rebaudioside A consumption.

#### **D. Common Knowledge Elements of GRAS Determinations**

The first common knowledge element for a GRAS determination is that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing published, peer-reviewed scientific journals. The majority of studies reviewed as part of this safety assessment have been published in the scientific literature as reported in Section V. The majority of the literature relied upon by JECFA has also been published---most importantly the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website. Thus, these studies become generally available to the scientific community. JECFA reviewed only a limited number of studies conducted specifically on rebaudioside A. The collection of supporting data on rebaudioside A has been enhanced by a series of studies published during 2008 and cited earlier. The clinical studies that address JECFA's concern on unwanted pharmacological effects with steviol glycosides (Barriocanal et al., 2008) and with rebaudioside A (Maki et al., 2008a, b) are also published in the peer-reviewed scientific literature.

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

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

The JECFA conclusion has been reviewed and validated by other respected regulatory agencies including FSANZ, the Switzerland Federal Office of Public Health, France's Agence Francais De Securite Sanitaire Des Alimenta, Health Canada, and others (FSANZ, 2008; Switzerland Federal Office of Public Health, 2008; AFSSA, 2009; Health Canada, 2012; Republic of South Africa Department of Health, 2012; AVA, 2013; FSSAI, 2012; Republic of the Philippines, 2013).

Furthermore, the favorable scientific opinion on the safety of steviol glycosides use as a sweetener in foods as issued by EFSA in 2010 reinforces the safety determinations of many other qualified organizations (EFSA, 2010). In addition, a number of individual well-respected scientists have indicated that steviol glycosides are safe for human consumption at doses in the range of the JECFA ADI (Xili et al., 1992; Toyoda et al., 1997; Geuns, 2003; Williams, 2007).

The common knowledge element has been embellished by the many respected scientists that participated in the Cargill-sponsored research conducted on rebaudioside A, most notably Brusick (2008) and Renwick (2008). An assertion of “general recognition of safety” was 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 two dozen GRAS notifications have been submitted to FDA for stevia-derived sweetener products, and FDA’s detailed reviews have yielded “no questions” letters in each case.

In summary, there are many diverse groups of scientists from all corners of the globe that together provide strong fulfillment of the consensus requirement. Of particular significance, from the perspective of establishing consensus for the safety of high purity steviol glycosides, are the 27 GRAS notifications with “no questions” determinations by FDA since 2008 (see Table 1). While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Expert Panel believes that a wide consensus does exist in the scientific community to support the GRAS conclusion on high purity rebaudioside A and high purity steviol glycosides, as described in this notification. The broader scientific community has concluded that past concerns expressed by others over the years (Huxtable, 2002), and earlier safety issues noted by FDA, have been resolved by newer data on more purified test materials and the rigid specifications for purity published by JECFA for steviol glycosides, including rebaudioside A. Indeed, scientists from FDA are members of JECFA and have not objected to the safety decision on steviol glycosides. There is also a wider consensus that the body of new research on rebaudioside A is sufficient, as opposed to the small group of scientists that argue that more studies need to be done before the sweetener is made available in the US.

## VII. CONCLUSIONS<sup>15</sup>

**GLG’s high purity steviol glycosides (≥ 95%), referred to as Anysweet RA50, Anysweet RA60, Rebsweet RA80, and/or Rebsweet RA85---primarily containing rebaudioside A and stevioside---when produced in accordance with FDA Good Manufacturing Practices requirements and when meeting at a minimum the JECFA purity specifications for steviol glycosides, are 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. In order to remain within the designated ADI, it is important to observe good manufacturing practices principles in that the quantity of a substance added to food should not exceed the amount reasonably required to accomplish its intended technical effect.**

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

(b) (6)

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

(b) (6)

(b) (6)

Robert S. McQuate, Ph.D.

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

Date: November 25, 2013

<sup>15</sup> 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](http://www.gras-associates.com). Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA’s GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. 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.

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**See Volume 2 of 2 for Appendices**



## **GRAS ASSESSMENT**

of

**HIGH PURITY STEVIOL GLYCOSIDES ( $\geq 95\%$ )**

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

**VOLUME 2 OF 2---APPENDICES**

for

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## APPENDICES

APPENDIX A	Specifications & Certificates of Analysis for Production Processing Aids.....	4
APPENDIX A-1	GLG Specifications for Ethanol.....	5
APPENDIX A-2	GLG Specifications for Methanol.....	6
APPENDIX A-3	Certificate of Analysis for GLG Active Carbon.....	7
APPENDIX B	Analytical Method.....	8
APPENDIX C	HPLC Chromatograms for Anysweet RA50.....	16
APPENDIX C-1	HPLC Chromatogram for Batch 20130801.....	17
APPENDIX C-2	HPLC Chromatogram for Batch 20130804.....	19
APPENDIX C-3	HPLC Chromatogram for Batch 20130807.....	21
APPENDIX C-4	HPLC Chromatogram for Batch 20130810.....	23
APPENDIX C-5	HPLC Chromatogram for Batch 20130814.....	25
APPENDIX D	HPLC Chromatograms for Anysweet RA60.....	27
APPENDIX D-1	HPLC Chromatogram for Batch 20130801.....	28
APPENDIX D-2	HPLC Chromatogram for Batch 20130803.....	30
APPENDIX D-3	HPLC Chromatogram for Batch 20130806.....	32
APPENDIX D-4	HPLC Chromatogram for Batch 20130809.....	34
APPENDIX D-5	HPLC Chromatogram for Batch 20130811.....	36
APPENDIX E	HPLC Chromatograms for Rebsweet RA80.....	38
APPENDIX E-1	HPLC Chromatogram for Batch 20130801.....	39
APPENDIX E-2	HPLC Chromatogram for Batch 20130805.....	41
APPENDIX E-3	HPLC Chromatogram for Batch 20130807.....	43
APPENDIX E-4	HPLC Chromatogram for Batch 20130810.....	45
APPENDIX E-5	HPLC Chromatogram for Batch 20130815.....	47
APPENDIX F	HPLC Chromatograms for Rebsweet RA85.....	49
APPENDIX F-1	HPLC Chromatogram for Batch 20130802.....	50
APPENDIX F-2	HPLC Chromatogram for Batch 20130806.....	52
APPENDIX F-3	HPLC Chromatogram for Batch 20130808.....	54
APPENDIX F-4	HPLC Chromatogram for Batch 20130812.....	56
APPENDIX F-5	HPLC Chromatogram for Batch 20130816.....	58
APPENDIX G	Certificates of Analysis for Multiple Production Batches of Anysweet RA50 .....	60
APPENDIX G-1	Certificate of Analysis for Batch 20130801.....	61
APPENDIX G-2	Certificate of Analysis for Batch 20130804.....	62
APPENDIX G-3	Certificate of Analysis for Batch 20130807.....	63
APPENDIX G-4	Certificate of Analysis for Batch 20130810.....	64
APPENDIX G-5	Certificate of Analysis for Batch 20130814.....	65
APPENDIX H	Certificates of Analysis for Multiple Production Batches of Anysweet RA60.....	66
APPENDIX H-1	Certificate of Analysis for Batch 20130801.....	67
APPENDIX H-2	Certificate of Analysis for Batch 20130803.....	68
APPENDIX H-3	Certificate of Analysis for Batch 20130806.....	69
APPENDIX H-4	Certificate of Analysis for Batch 20130809.....	70
APPENDIX H-5	Certificate of Analysis for Batch 20130811.....	71
APPENDIX I	Certificates of Analysis for Multiple Production Batches of Rebsweet RA80.....	72
APPENDIX I-1	Certificate of Analysis for Batch 20130801.....	73
APPENDIX I-2	Certificate of Analysis for Batch 20130805.....	74
APPENDIX I-3	Certificate of Analysis for Batch 20130807.....	75
APPENDIX I-4	Certificate of Analysis for Batch 20130810.....	76
APPENDIX I-5	Certificate of Analysis for Batch 20130815.....	77

APPENDIX J	Certificates of Analysis for Multiple Production Batches of Rebsweet RA85.....	78
APPENDIX J-1	Certificate of Analysis for Batch 20130802.....	79
APPENDIX J-2	Certificate of Analysis for Batch 20130806.....	80
APPENDIX J-3	Certificate of Analysis for Batch 20130808.....	81
APPENDIX J-4	Certificate of Analysis for Batch 20130812.....	82
APPENDIX J-5	Certificate of Analysis for Batch 20130816.....	83
APPENDIX K	Pesticide Analytical Reports from SGS-CSTC Standards Technical Services, Co. Ltd....	84
APPENDIX K-1	Test Report for Pesticides GLG RA60 Lot 20130501.....	85
APPENDIX K-2	Test Report for Pesticides GLG RA80 Lot 20130401.....	95
APPENDIX L	Summary of Regulatory & Expert Body Safety Reviews.....	105
APPENDIX M	Studies on Principal Metabolite: Steviol.....	109
APPENDIX N	Studies on Steviol Glycosides Preparations That Are Primarily Stevioside.....	112
APPENDIX O	Studies on Rebaudioside A.....	123

## **APPENDIX A**

### **Specifications and 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 (8<sup>th</sup>)**)

Characteristic	Specification
Assay ( $C_2H_6O$ , by volume)	$\geq 95.0\%$
Acidity (as acetic acid)	$\leq 0.003\%$
Alkalinity(as $NH_3$ )	$\leq 3\text{ppm}$
Fusel Oil	Passes test
Ketones, Isopropyl Alcohol	Passes test
Lead	$\leq 0.5\text{ppm}$
Methanol	Passes test
Nonvolatile Residue	$\leq 0.003\%$
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<sub>3</sub>OH 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 (8<sup>th</sup>))

Characteristic	Specification
Assay (CH <sub>3</sub> OH%)	>99.5
Acetone and Aldehydes	$\leq 0.003\%$
Acidity (as formic acid)	$\leq 0.0015\%$
Alkalinity (as NH <sub>3</sub> )	$\leq 3$ ppm
Distillation Range °C	64.5~65.5
Lead	$\leq 1$ ppm
Nonvolatile Residue	$\leq 10$ ppm
Readily Carbonizable Substance	Passes test
Solubility in water	Passes test
Substances Reducing Permanganate	Passes test
Water	$\leq 0.1\%$

### A-3 CERTIFICATE OF ANALYSIS FOR GLG ACTIVE CARBON



## Certificate of Analysis

**Product:** Active Carbon  
**Manufacturing Date:** May 10th, 2013  
**Analysis Date:** May 21st, 2013  
**Manufacture:** Ning Guo city Hengda Active Carbon Co.,Ltd  
**Country of Origin:** China  
**Accordinging:** GB/T13803.3-1999

**Lot No. 20130506**  
**Shelf Life: two years**

Inspection Item	Specification	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)      Checked by: (b) (6)      Approved by: (b) (6)  
21/05/2013

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

\*This product should be stored and sealed in a cool and dry place.

## **APPENDIX B**

### **Analytical Method**

 <b>GLG LIFE TECH CORPORATION</b>	Issued Date: 08/08/2010
<b>DETERMINATION OF REBAUDIOSIDE A AND RELATED STEVIOL GLYCOSIDES BY HPLC</b>	
File No: GLG-QA-STD-HPLC-03	PAGE 1 / 7

**PRINCIPLE:**

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

**SCOPE:**

The assay is capable of determining the content of rebaudioside A and related steviol glycosides in final product by LC analysis. The assay is applicable for rebaudioside A samples that have rebaudioside A concentrations in the range of 50%-102% and related steviol glycosides in the range of 5.0%- 0.05%.

**EQUIPMENT AND REAGENTS:**

1. Agilent1200 HPLC system equipped with binary pump, auto sampler), thermostatted column compartment and UV detector, (Agilent Technologies, USA);
2. LC Amine Column; Zorbax NH2, 4.6x250mm, 5um particle
3. Analytical balance capable of 0.00001g
4. Vacuum system
5. Sonicator
6. Volumetric flasks (10ml, 25ml, 50mL and 100ml)
7. Class A pipettes (1ml and 5 mL)
8. Acetonitrile, LC grade, suitable for analysis at 210nm
9. Ultra high purity water suitable for LC analysis (NanoPure, E-Pure or equivalent)
10. Ammonium acetate, reagent grade (VWR or equivalent)
11. Glacial acetic acid, reagent grade (VWR or equivalent)

**Standards**

1. Rebaudioside A Standard; (Chromadex Inc. Irvine, CA USA);
2. Stevioside Standard; (Chromadex Inc. Irvine, CA USA);
3. Rebaudioside B Standard; (Chromadex Inc. Irvine, CA USA);
4. Rebaudioside C Standard; (Chromadex Inc. Irvine, CA USA);
5. Rebaudioside D Standard; (Chromadex Inc. Irvine, CA USA);
6. Steviolbioside Standard; (Chromadex Inc. Irvine, CA USA);
7. Dulcoside A Standard; (Chromadex Inc. Irvine, CA USA);

**SAFETY NOTES:**

1. Always follow the Chemical Hygiene Plan and established safety procedures for handling materials, cleaning up spills and disposing of wastes.

 <b>GLG LIFE TECH CORPORATION</b>	Issued Date: 08/08/2010
	<b>DETERMINATION OF REBAUDIOSIDE A AND RELATED STEVIOL GLYCOSIDES BY PHLC</b> File No: GLG-QA-STD-HPLC-03   PAGE 2 / 7

2. Read and observe all precautionary measures and hazards noted in the Material Safety Data Sheets for all chemicals used in this procedure.
3. Steviol glycoside materials are typically powders that can become airborne if shaken, dropped or otherwise agitated. Once airborne they can be tasted and smelled by the analyst. Utilize caution to prevent material from becoming airborne.

**PROCEDURE:**

**A. Standard and Sample Equilibration to Moisture**

1. Rebaudioside A and the related steviol glycosides are hygroscopic compounds. Standards and samples require moisture equilibration before analysis. The standards and samples should be left out, in the same room as the analytical balance, for no less than 24 hours before weighing. Intermittent stirring of the dry powder will insure uniform sample absorption.
2. At time of weighing, a moisture value should be determined for all standards and samples.

**B. Mobile Phase Preparation**

1. Prepare the aqueous buffer solution (0.0125% acetic acid, 0.0125% ammonium acetate) by dissolving 0.125 g ammonium acetate (NH<sub>4</sub>OAc) and 125 µL glacial acetic acid (HOAc) in one liter of water. The aqueous buffer may be scaled up as needed. See Table 1 for appropriate amounts of ammonium acetate and glacial acetic acid.

Table 1: Amounts of NH<sub>4</sub>OAc, HOAc and water needed for buffer solution scale up

Volume of buffer Solution to be made (mL)	Amount of NH <sub>4</sub> OAc (mg)	Amount of HOAc (µL)	LC grade Water (mL)
1500	187.5	187.5	1500
2000	250.0	250.0	2000
2500	312.5	312.5	2500
3000	375.0	375.0	3000
4000	500.0	500.0	4000

 <b>GLG LIFE TECH CORPORATION</b>	Issued Date: 08/08/2010
	<b>DETERMINATION OF REBAUDIOSIDE A AND RELATED STEVIOL GLYCOSIDES BY HPLC</b> <b>File No: GLG-QA-STD-HPLC-03</b>   <b>PAGE 3 / 7</b>

2. Prepare the Mobile Phase (acetonitrile: buffer) at a ratio of 87:13 as to ensure sufficient separation of analytes.
  - a. Combine the appropriate volumes of acetonitrile and buffer.

Table 2: Volumes of acetonitrile and aqueous buffer for preparation of mobile phase

Acetonitrile:Aqueous Buffer	Volume of mobile phase to be made (mL)	Volume of ACN (mL)	Volume of buffer solution (mL)
87:13	4000	3480	520

- b. Combine the volumes, given above in Table 2, of acetonitrile and aqueous buffer, allow the solution to reach ambient temperature and degas the solution using a vacuum system and the sonicator.
3. Prepare the diluent solution (25% buffer in acetonitrile) by combining 750 mL of acetonitrile and 250 mL of aqueous buffer and mix thoroughly. Allow diluent to come to room temperature. The diluent solution may be scaled up as needed.

### C. Standard Preparation

1. Prepare the rebaudioside A standard curve
  - a. The rebaudioside A curve consists of 6 points varying in concentration from 2.5 mg/mL to 5.5 mg/mL.
  - b. Mass individual samples of rebaudioside A standard (equilibrated for moisture) at 62.5, 87.5, 100, 112.5, 125 and 137.5 mg (+/- 2 mg).
  - c. Dissolve in 25 ml volumetric flasks with diluent solution.
2. Prepare the stevioside standard curve
  - a. The stevioside calibration curve consists of seven points designed to span a concentration of 0.0005 to 0.25 mg/mL.
  - b. Weigh 125 mg of a stevioside standard (equilibrated for moisture) into a 50 ml volumetric flask, creating the 2.5 mg/mL stock A solution.
  - c. Prepare the stock B solution by diluting 1 mL of stock A in a 10 mL volumetric flask with diluent. The remaining dilutions can found in the Table 3.

 <b>GLG LIFE TECH CORPORATION</b>	Issued Date: 08/08/2010
	<b>DETERMINATION OF REBAUDIOSIDE A AND RELATED STEVIOL GLYCOSIDES BY HPLC</b>
File No: GLG-QA-STD-HPLC-03	PAGE 4 / 7

Table 3: Serial dilutions for the stevioside calibration curve

Standard	Stock Solution	Stock Std (mL)	Volumetric Flask (mL)	Std Conc. (mg/mL)
Stock A			50	2.5
Stock B	A	1	10	0.25
1	A	1	50	0.05
2	B	1	10	0.025
3	B	5	100	0.0125
4	B	1	50	0.005
5	B	1	100	0.0025
6	2	1	50	0.0005

**Sample Preparation**

1. Weigh 125 ± 5 mg, recorded to the nearest 0.01 mg, of sample (equilibrated for moisture) in a 25 mL volumetric flask.
2. Dilute to volume with the diluent solution.
3. If necessary, stir, shake or sonicate the solution until completely dissolved. This will make an approximately 5.0 mg/mL sample.
4. Samples are run in triplicate.

**INSTURMENT CONDITIONS**

Table 4: Instrument conditions for LC

Column:	Zorbax NH2, 4.6x250mm, 5µm
Temperature	30°C
Isocratic Mobile Phase:	13% buffer, 87% acetonitrile
Flow Rate:	1.5 mL/min
Injection:	15 µL
Detection:	UV at 210 nm (4 nm bandwidth), Reference: 260 nm (100 nm bandwidth)
Run Time:	75 min
Autosampler Temperature:	Ambient
Sample Concentration	5000 mg/L

 <b>GLG LIFE TECH CORPORATION</b>	Issued Date: 08/08/2010
<b>DETERMINATION OF REBAUDIOSIDE A AND RELATED STEVIOL GLYCOSIDES BY HPLC</b>	
File No: GLG-QA-STD-HPLC-03	PAGE 5 / 7

### **ANALYSIS PROCEDURE**

#### **A. System Suitability**

1. Perform a Detector Sensitivity Check by injecting stevioside standard 6.
2. Verify that the peak to noise ratio of the stevioside peak is  $\geq 3$ . If not investigate the instrument and take corrective action before continuing. Record any necessary corrective actions.

#### **B. Assay Sequence**

1. Inject the system suitability detector sensitivity check.
2. Inject all rebaudioside A standards in increasing concentrations.
3. Inject stevioside standards 5 through B in increasing concentrations.
4. Inject the samples.
5. Bracket the samples with standards by re-injecting the 5.0 mg/mL standard after a maximum of 12 sample injections and once at the end of the sequence.

#### **C. Integration Parameters**

1. The integration is done using the software tools. An example chromatogram is provided in appendix C for reference on how the integration should occur.

#### **D. Standard Curve Calculation and Acceptance Criteria**

1. Prepare full fit linear regression standard curve by plotting rebaudioside A or stevioside concentration in mg/L on the ordinate scale versus its respective area counts on the abscissa scale. Alternatively, the data acquisition software may be used to prepare the calibration curve.

#### **Acceptance Criteria for rebaudioside A**

1. For all rebaudioside A concentration levels to be acceptable for use in the calibration curve, the standard recoveries (see appendix A) must be within 100.0  $\pm$  3%.
2. The correlation coefficient for the standard curve is acceptable if it is greater than 0.9900.
3. Calculate the tailing factor, T, using the rebaudioside A peak of the 5.0 mg/mL injection of the rebaudioside A standard. Tailing factor should be  $0.8 \geq T \geq 2$  (see appendix B).
4. Calculate the System Drift (see appendix B).
  - a. Determining the area counts for all of the 5.0 mg/mL rebaudioside A standard that was used to bracket the sample injections.

 <b>GLG LIFE TECH CORPORATION</b>	Issued Date: 08/08/2010
<b>DETERMINATION OF REBAUDIOSIDE A AND RELATED STEVIOL GLYCOSIDES BY HPLC</b>	
File No: GLG-QA-STD-HPLC-03	PAGE 6 / 7

- b. Calculate the % RSD (see appendix B) for the rebaudioside A standard that was used to bracket the sample injections. The %RSD must be  $\leq 2.0\%$ .
5. If the standard curve fails the acceptance criteria notify your supervisor and investigate the problem.

#### Acceptance Criteria for Stevioside

1. The stevioside curve is comprised of stevioside standards 5 through B. For all stevioside concentration levels to be acceptable for use in the calibration curve, the standard recoveries must be within  $100.0 \pm 10\%$ .
2. The correlation coefficient for the standard curve is acceptable if it is  $\geq 0.9900$ .
3. Calculate signal to noise for the stevioside curve. The Limit of Quantitation (LOQ) is 0.05%. The signal to noise must be  $\geq 10$  for this concentration. The Limit of Detection (LOD) is 0.01%. The signal to noise must be  $\geq 3$ .
4. If the standard curve fails the acceptance criteria notify your supervisor and investigate the problem.

#### **E. Analyte Calculation and Acceptance Criteria**

1. Identify analytes of interest by matching retention time with standards if available. When analyte standards are not available, determine unknown peaks based on relative retention times (see appendix A) to standards.
2. Determine the area counts of the analyte peaks as well as any measurable peaks (except for solvent peaks) from the standards and samples.
3. Using the equation given below from the linear regression of the standard curve, calculate the concentration in mg/mL of the analytes. Calculate rebaudioside A using the rebaudioside A curve and all other analytes using the stevioside curve. Alternatively, use the data acquisition software to calculate the concentrations of the analytes based on the calibration curves prepared using the software.

$$\text{Conc. (mg/mL)} = \text{Area Response} \times \text{slope} + \text{y-intercept}$$

4. Correct the concentrations of each analyte in the samples by multiplying the concentration of each known glycoside by its correction factor (see appendix D). This corrects for the difference in molecular weight between analyte of interest and stevioside. No correction is needed for rebaudioside B because it and stevioside have the same molecular weight.

 <b>GLG LIFE TECH CORPORATION</b>	Issued Date: 08/08/2010
<b>DETERMINATION OF REBAUDIOSIDE A AND RELATED STEVIOL GLYCOSIDES BY HPLC</b>	
File No: GLG-QA-STD-HPLC-03	PAGE 7 / 7

5. Calculate the w/w % of rebaudioside A and each known glycoside in the samples as follows:

$$w/w\% = \text{Conc. of the Analyte (mg/mL)} \times 100 / \text{Sample Conc (mg/mL)}$$

6. Correct rebaudioside A and all known glycosides, above the LOQ, for moisture and solvents (if applicable) by multiplying the wt/wt% by the following factor (F). All analytes should be reported on a dry basis.

$$F = 100 / (100 - \% \text{ Moisture and Solvents in Samples})$$

## **APPENDIX C**

### **HPLC Chromatograms for Anysweet RA50**

C-1 HPLC Chromatogram for Batch 20130801

C-2 HPLC Chromatogram for Batch 20130804

C-3 HPLC Chromatogram for Batch 20130807

C-4 HPLC Chromatogram for Batch 20130810

C-5 HPLC Chromatogram for Batch 20130814

### C-1 HPLC CHROMATOGRAM FOR BATCH 20130801

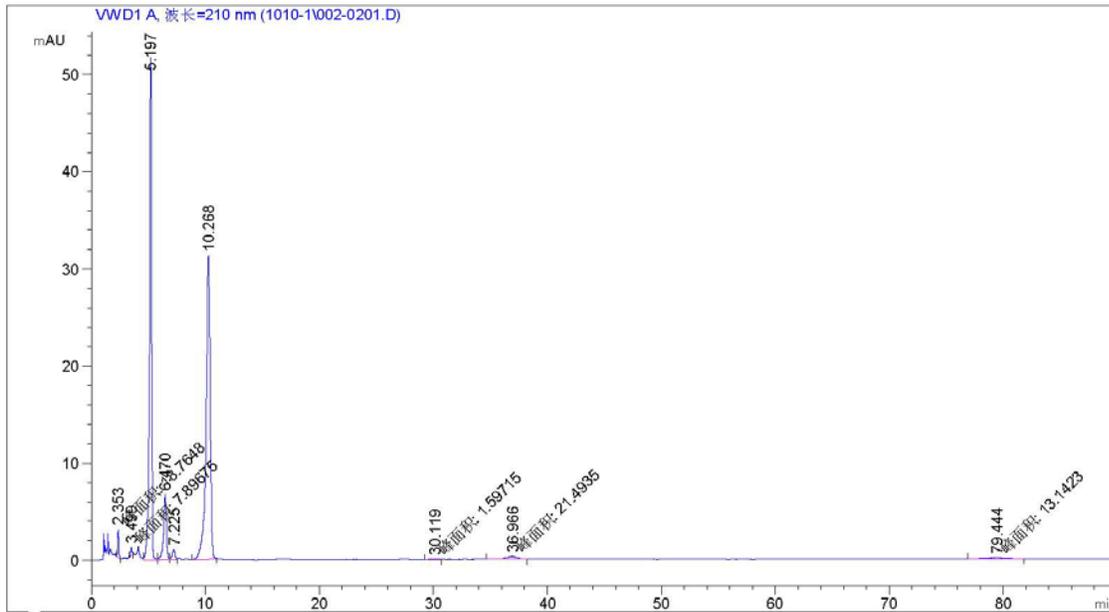
Data File E:\DATA\2013\0809-1\001-0501.D

```

=====
Operator       : sun hongkai                Line       : 1
Instrument     : Instrument 1                Location   : 5
Injection Date : 2013-08-09 17:00:18        Inj        : 1
                                           Inj Volum  : 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-09 16:49:04 sun hongkai
                (modified after loading )
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-09 18:54:33 sun hongkai
                (modified after loading )

Sample Info   : GLG-RA50Plus-20130801
    
```



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	2.353	MM	0.1084	18.76484	2.88585	1.1358	Rub
2	3.499	MM	0.1523	7.89675	8.64301e-1	0.4780	Dul A
3	5.197	BB	0.1933	670.14307	51.73523	40.5627	Stv
4	6.470	BV	0.2528	114.74002	6.68100	6.9450	Reb C
5	7.225	VV	0.2695	18.80674	1.03835	1.1383	Reb F
6	10.268	VB	0.3749	785.53381	31.23750	47.5471	Reb A
7	30.119	MM	0.6706	1.59715	3.96933e-2	0.0967	Reb D

Instrument 1 2013-08-09 18:55:12sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File E:\DATA\2013\0809-1\001-0501.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
8	36.966	MM	1.2718	21.49355	2.81672e-1	1.3010	Sbio
9	79.444	MM	2.4303	13.14232	9.01288e-2	0.7955	Reb B

Total :                    1652.11824    94.85373

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

## C-2 HPLC CHROMATOGRAM FOR BATCH 20130804

Data File: E:\DATA\2013\0813-1\008-0401.D

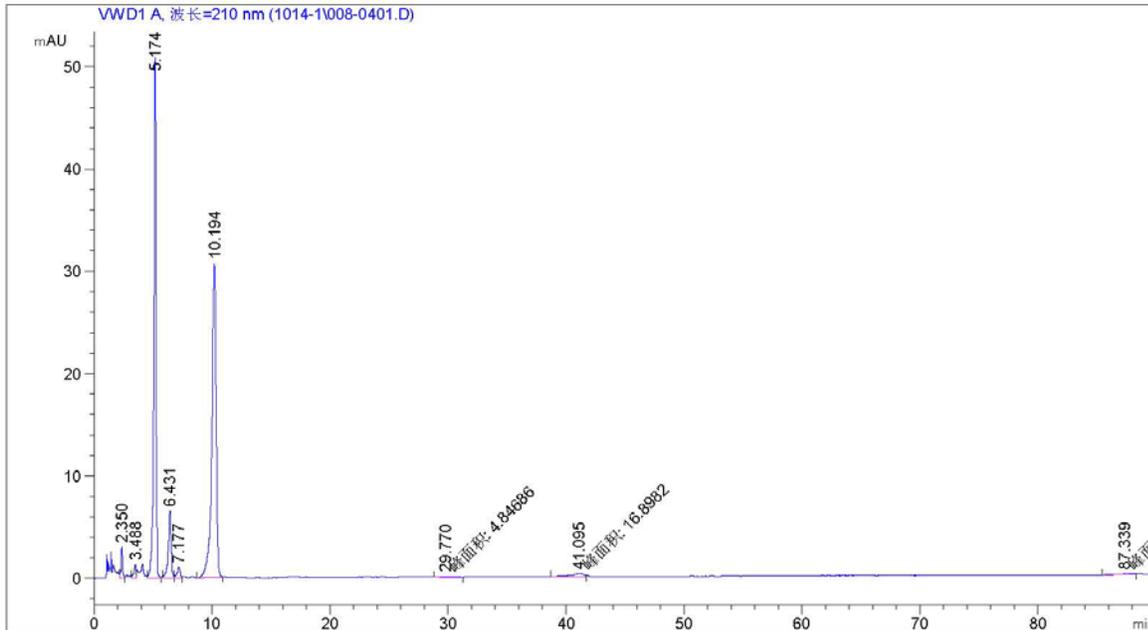
```

=====
Operator       : sun hongkai                Line   :    8
Instrument     : Instrument 2                Location:    4
Injection Date : 2013-08-13 18:38:34        Inj    :    1
                                           Inj Volum: 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-13 16:32:04 sun hongkai
                ( modified after loading )

Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-13 20:30:31 sun hongkai
                ( modified after loading )

Sample Info   : GLG-RA50Plus-20130804
  
```



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	2.350	VV	0.1187	25.88362	3.07201	1.5876	Rub
2	3.488	VV	0.1765	16.60459	1.30151	1.0185	Dul A
3	5.174	VB	0.1949	658.60388	50.81796	40.3968	Stv
4	6.431	BV	0.2557	112.80370	6.56797	6.9190	Reb C

Instrument 2 2013-08-13 20:30:42 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
 High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File: E:\DATA\2013\0813-1\008-0401.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
5	7.177	VV	0.2707	19.27894	1.05886	1.1825	Reb F
6	10.194	VB	0.3776	771.16998	30.69021	47.3012	Reb A
7	29.770	MM	1.4546	4.84686	5.55356e-2	0.2973	Reb D
8	41.095	MM	1.2114	16.89820	2.32480e-1	1.0365	Sbio
9	87.339	MM	1.2098	4.24844	5.85302e-2	0.2606	Reb B

Total: 1630.33820 93.85507

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

### C-3 HPLC CHROMATOGRAM FOR BATCH 20130807

Data File: E:\DATA\2013\0815-1\001-0201.D

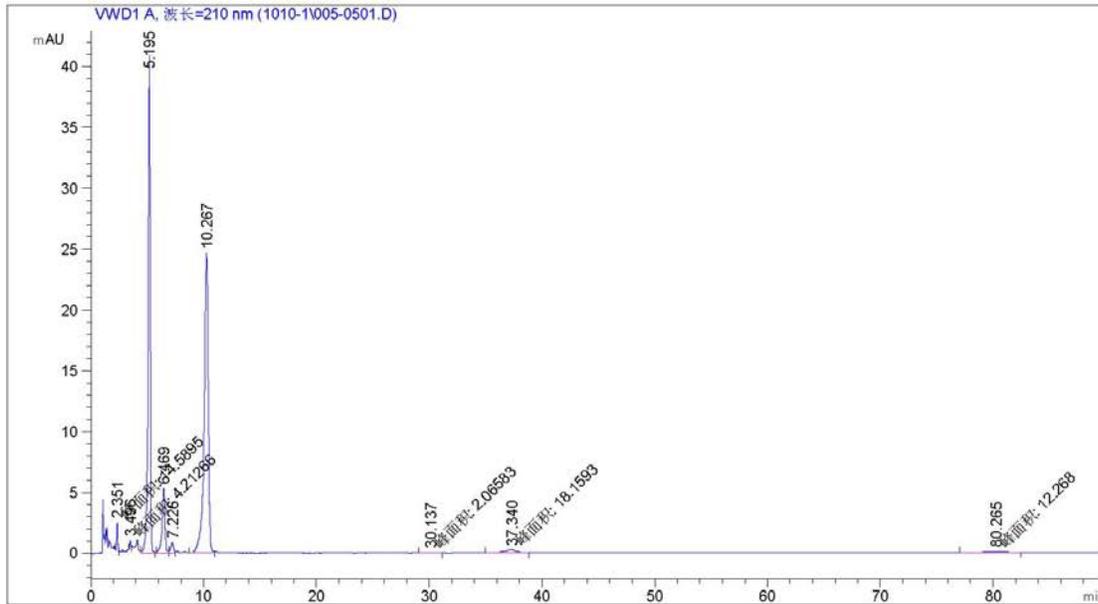
```

=====
Operator       : sun hongkai                Line   : 1
Instrument     : Instrument 1              Location: 2
Injection Date : 2013-08-15 10:22:10      Inj    : 1
                                           Inj Volum: 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_IC.M
Last Changed  : 2013-08-15 8:55:04 sun hongkai
                ( modified after loading )

Analysis Method: D:\CHEM32\1\METHODS\C18_IC.M
Last Changed  : 2013-08-15 12:30:31 sun hongkai
                ( modified after loading )

Sample Info   : GLG-RA50Plus-20130807
=====
  
```



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	2.351	MM	0.1070	14.58948	2.27221	1.1162	Rub
2	3.496	MM	0.1158	4.21266	6.06546e-1	0.3223	Dul A
3	5.195	VB	0.1933	529.13348	40.86843	40.4830	Stv
4	6.469	BB	0.2551	91.57797	5.31007	7.0065	Reb C
5	7.226	BV	0.2632	14.65597	8.46320e-1	1.1213	Reb F
6	10.267	VB	0.3784	620.38757	24.62567	47.4647	Reb A
7	30.137	MM	0.8856	2.06583	3.88783e-2	0.1581	Reb D

Instrument 1 2013-08-15 12:30:40 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

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Data File: E:\DATA\2013\0815-1\001-0201.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
8	37.340	MM	1.3409	18.15933	2.25714e-1	1.3893	Sbio
9	80.265	MM	2.8262	12.26804	7.23462e-2	0.9386	Reb B

Total: 1307.05034 74.86618

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

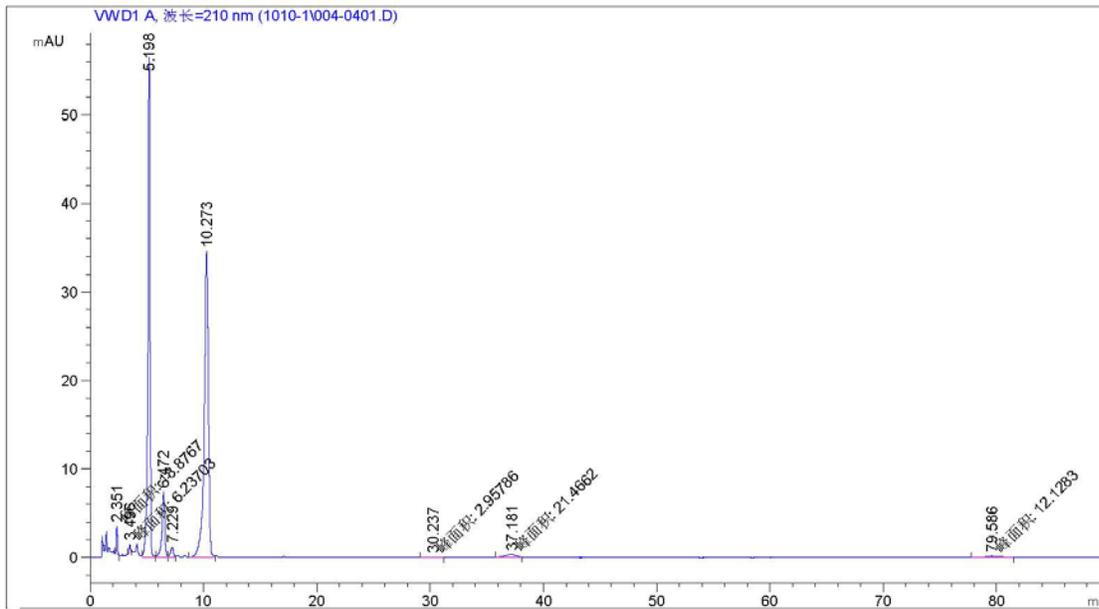
### C-4 HPLC CHROMATOGRAM FOR BATCH 20130810

Data File: E:\DATA\2013\0817-1\001-0401.D

```

=====
Operator       : sun hongkai                      Line       :    1
Instrument     : Instrument 1                      Location   :    4
Injection Date : 2013-08-17 9:32:10              Inj        :    1
                                                    Inj Volum  : 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-17 8:49:04 sun hongkai
                ( modified after loading )
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-17 11:30:31 sun hongkai
                ( modified after loading )
Sample Info   : GLG-RA50Plus-20130810
  
```



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	2.351	MM	0.1016	18.87670	3.09778	1.0420	Rub
2	3.496	MM	0.1192	6.23703	8.72294e-1	0.3443	Dul A
3	5.198	BB	0.1948	730.93616	56.41349	40.3462	Stv
4	6.472	BV	0.2528	126.64555	7.37481	6.9906	Reb C
5	7.229	VV	0.2723	20.89677	1.15475	1.1535	Reb F
6	10.273	VB	0.3787	871.51733	34.55788	48.1060	Reb A
7	30.237	MM	0.9241	2.95786	5.33466e-2	0.1633	Reb D

Instrument 1 2013-08-17 11:30:40 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File: E:\DATA\2013\0817-1\001-0401.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
8	37.181	MM	1.2059	21.46620	2.96676e-1	1.1849	Sbio
9	79.586	MM	1.6188	12.12831	1.24870e-1	0.6695	Reb B

Total : 1811.66191 103.94589

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

### C-5 HPLC CHROMATOGRAM FOR BATCH 20130814

Data File: E:\DATA\2013\0822-1\001-0201.D

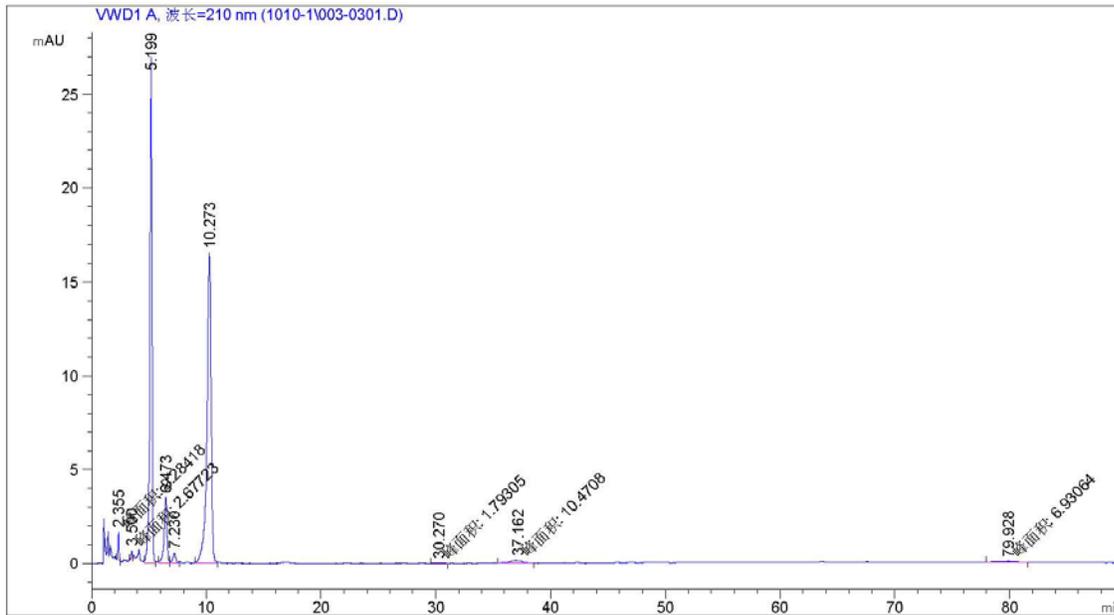
```

=====
Operator       : sun hongkai                Line   : 1
Instrument     : Instrument 1              Location: 2
Injection Date : 2013-08-22 18:38:34      Inj    : 1
                                           Inj Volum: 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-22 16:32:04 sun hongkai
                ( modified after loading )

Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-22 20:30:31 sun hongkai
                ( modified after loading )

Sample Info   : GLG-RA50Plus-20130814
  
```



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	2.355	MM	0.1040	9.28418	1.48755	1.0757	Rub
2	3.500	MM	0.1155	2.67723	3.86271e-1	0.3102	Dul A
3	5.199	BB	0.1926	347.14264	26.93512	40.2223	Stv
4	6.473	BV	0.2527	59.33045	3.50628	6.8744	Reb C
5	7.230	VB	0.2756	9.52694	5.18309e-1	1.1039	Reb F
6	10.273	BB	0.3781	415.90366	16.52412	48.1894	Reb A
7	30.270	MM	0.8691	1.79305	3.43860e-2	0.2078	Reb D

Instrument 1 2013-08-22 20:30:40sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File: E:\DATA\2013\0822-1\001-0201.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
8	37.162	MM	1.2445	10.47083	1.40232e-1	1.2132	Sbio
9	79.928	MM	2.0723	6.93064	5.57411e-2	0.8030	Reb B

Total: 863.05963 49.58801

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

## **APPENDIX D**

### **HPLC Chromatograms for Anysweet RA60**

D-1 HPLC Chromatogram for Batch 20130801

D-2 HPLC Chromatogram for Batch 20130803

D-3 HPLC Chromatogram for Batch 20130806

D-4 HPLC Chromatogram for Batch 20130809

D-5 HPLC Chromatogram for Batch 20130811

## D-1 HPLC CHROMATOGRAM FOR BATCH 20130801

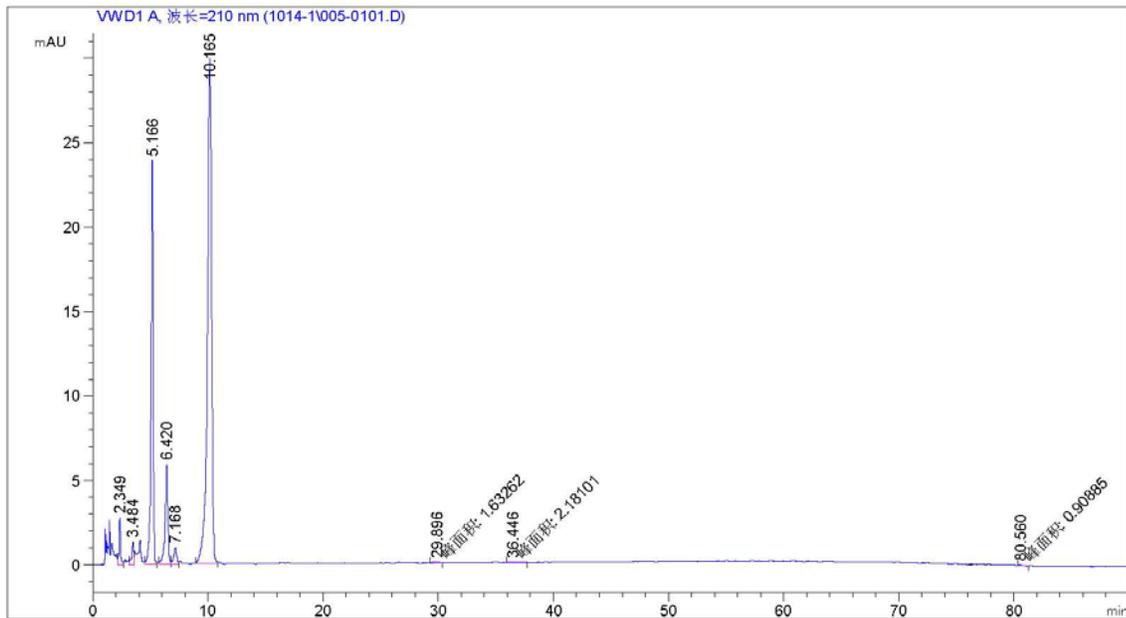
Data File: E:\DATA\2013\0811-1\005-0101.D

```

=====
Operator       : sun hongkai                Line   :    5
Instrument     : Instrument 2              Location:    1
Injection Date : 2013-08-11 08:38:34      Inj    :    1
                                           Inj Volum: 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-11 08:32:04 sun hongkai
                ( modified after loading )
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-11 08:30:31 sun hongkai
                ( modified after loading )

Sample Info   : GLG-RA60Plus-20130801
=====
  
```



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	2.349	VB	0.1206	23.41890	2.76605	1.9225	Rub
2	3.484	VV	0.1712	16.48043	1.33992	1.3529	Dul A
3	5.166	BB	0.1932	309.05209	23.88936	25.3702	Stv
4	6.420	BV	0.2521	101.14768	5.90874	8.3033	Reb C

Instrument 2 2013-08-11 10:30:36

1/2

GRAS Assessment – GLG Life Tech Corporation  
 High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File: E:\DATA\2013\0811-1\005-0101.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
5	7.168	VV	0.2646	17.11528	9.67396e-1	1.4050	Reb F
6	10.165	BB	0.3731	746.23236	29.86091	61.2585	Reb A
7	29.896	MM	0.6867	1.63262	3.96242e-2	0.1340	Reb D
8	36.446	MM	0.9122	2.18101	3.98485e-2	0.1790	Sbio
9	80.560	MM	0.5787	9.08850e-1	2.61768e-2	0.0746	Reb B

Total: 1218.16923 64.83803

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

Instrument 2 2013-08-11 10:30:36

2/2

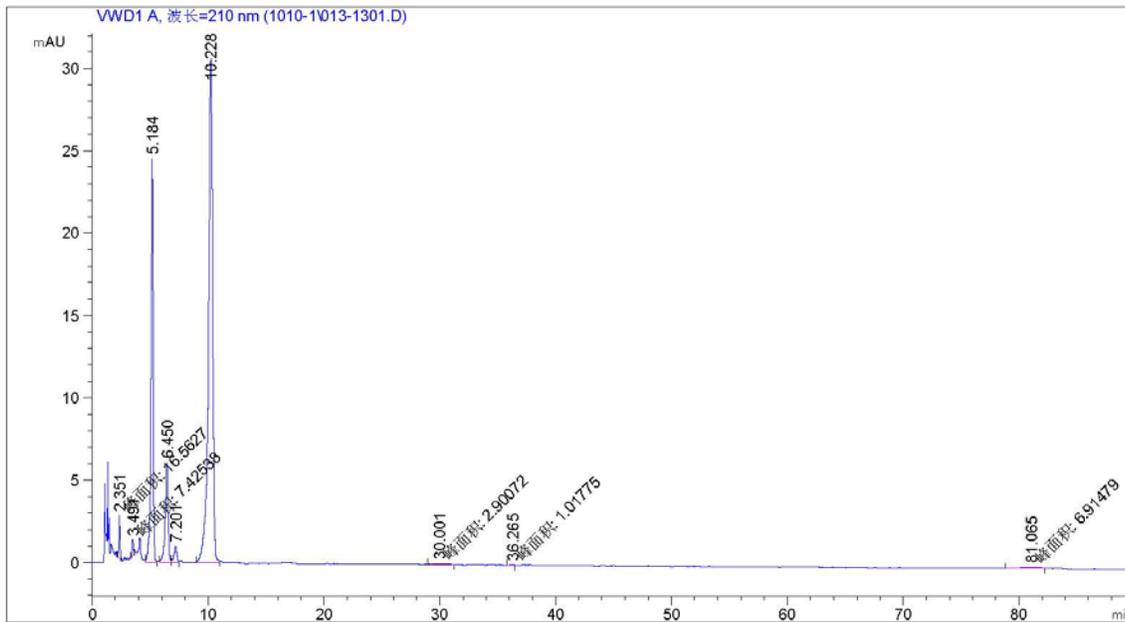
## D-2 HPLC CHROMATOGRAM FOR BATCH 20130803

Data File: E:\DATA\2013\0812-1\001-0701.D

```

=====
Operator       : sun hongkai                Line       :    1
Instrument     : Instrument 1              Location   :    7
Injection Date : 2013-08-12 9:23:10      Inj        :    1
                                           Inj Volum  : 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-12 8:50:34 sun hongkai
                ( modified after loading )
Analysis Method: D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-12 11:25:11 sun hongkai
                ( modified after loading )
Sample Info   : GLG-RA60Plus-20130803
  
```



External Standard Report

```

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

Signal: VWD1 A, Sig=210 nm

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
1	2.351	MM	0.1063	16.56269	2.59785	1.3348	Rub
2	3.491	MM	0.1345	7.42538	9.20027e-1	0.5984	Dul A
3	5.184	BB	0.1945	316.77029	24.50628	25.5289	Stv
4	6.450	BV	0.2557	104.29505	6.07357	8.4052	Reb C
5	7.201	VV	0.2673	17.69776	9.87814e-1	1.4263	Reb F
6	10.228	BB	0.3744	767.24805	30.56489	61.8333	Reb A
7	30.001	MM	1.0624	2.90072	4.55071e-2	0.2338	Reb D

Instrument 1 2013-08-12 11:28:40 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File: E:\DATA\2013\0812-1\001-0701.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU	Area *s	Peak Height [mAU]	Amt/Area %	Name
8	36.265	MM	0.4899	1.01775	3.46271e-2	0.0820		Sbio
9	81.065	MM	1.9686	6.91479	5.85409e-2	0.5573		Reb B

Total :                                   1240.83249   65.78910

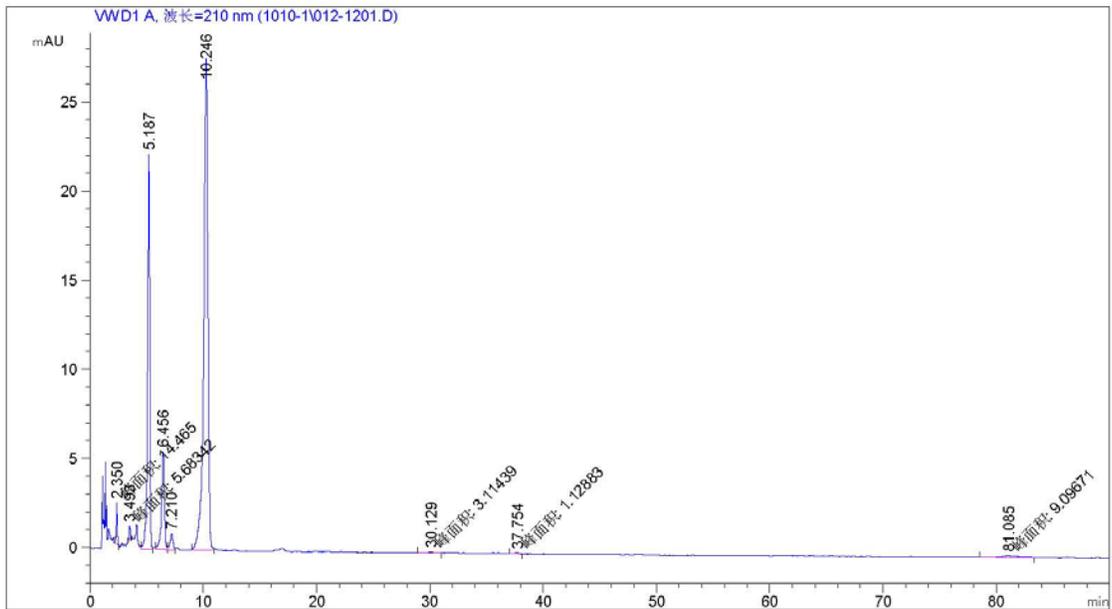
=====  
\*\*\* End of report\*\*\*

### D-3 HPLC CHROMATOGRAM FOR BATCH 20130806

Data File: E:\DATA\2013\0814-1\001-0901.D

```

=====
Operator       : sun hongkai                Line       : 1
Instrument     : Instrument 1              Location   : 9
Injection Date : 2013-08-14 11:43:10      Inj        : 1
                                           Inj Volum : 20.0 µl
Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed   : 2013-08-14 8:52:28 sun hongkai
                ( modified after loading )
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed   : 2013-08-14 13:20:41 sun hongkai
                ( modified after loading )
Sample Info    : GLG-RA60Plus-20130806
=====
  
```



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	2.350	MM	0.1042	14.46499	2.31306	1.2842	Rub
2	3.493	MM	0.1208	5.68342	7.84226e-1	0.5046	Dul A
3	5.187	VB	0.1941	288.62482	22.16464	25.6236	Stv
4	6.456	BV	0.2530	94.20277	5.47790	8.3631	Reb C
5	7.210	VV	0.2684	15.94109	8.84857e-1	1.4152	Reb F
6	10.246	BB	0.3751	694.14716	27.58511	61.6251	Reb A
7	30.129	MM	0.8239	3.11439	6.30039e-2	0.2765	Reb D

Instrument 1 2013-08-14 13:21:40 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

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Data File: E:\DATA\2013\0814-1\001-0901.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU	Area *s	Peak Height [mAU]	Amt/Area %	Name
8	37.754	MM	0.5748	1.12883		3.27319e-2	0.1002	Sbio
9	81.085	MM	1.9257	9.09671		7.87318e-2	0.8076	Reb B

Total :                                    1126.40417    59.38428

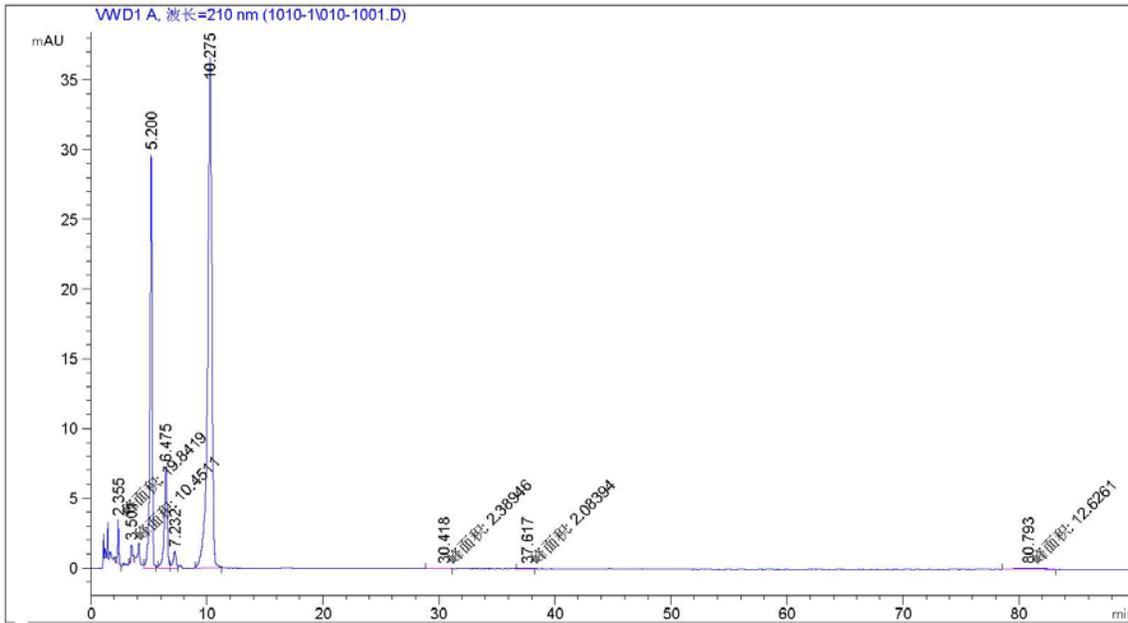
=====  
\*\*\* End of report\*\*\*

### D-4 HPLC CHROMATOGRAM FOR BATCH 20130809

Data File E:\DATA\2013\0817-1\001-0601.D

```

=====
Operator       : sun hongkai                Line       : 1
Instrument     : Instrument 1                Location   : 6
Injection Date : 2013-08-17 17:00:18        Inj        : 1
                                           Inj Volum  : 20.0 µl
Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-17 16:49:04 sun hongkai
                ( modified after loading )
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-17 18:40:33 sun hongkai
                ( modified after loading )
Sample Info   : GLG-RA60Plus-20130809
  
```



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	2.355	MM	0.1056	19.84189	3.13257	1.3179	Rub
2	3.501	MM	0.1526	10.45110	1.14133	0.6942	Dul A
3	5.200	VB	0.1942	386.24799	29.65779	25.6548	Stv
4	6.475	BV	0.2537	125.94335	7.30153	8.3652	Reb C
5	7.232	VV	0.2735	21.52967	1.18292	1.4300	Reb F
6	10.275	BB	0.3796	924.44556	36.53561	61.4021	Reb A
7	30.418	MM	0.8861	2.38946	4.49454e-2	0.1587	Reb D

Instrument 1 2013-08-17 18:41:30 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File E:\DATA\2013\0817-1\001-0601.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU *s	Peak Height [mAU ]	Amt/Area %	Name
8	37.617	MM	0.7956	2.08394	4.36562e-2	0.1384	Sbio
9	80.793	MM	2.2011	12.62608	9.56063e-2	0.8386	Reb B

Total : 1505.55903 79.13596

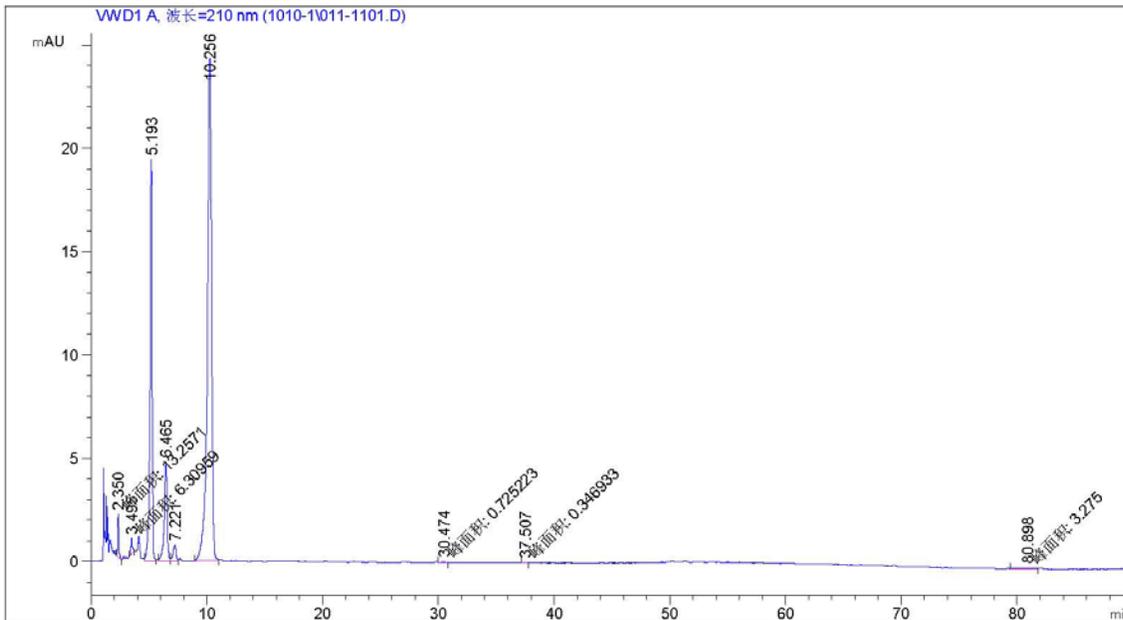
-----  
\*\*\* End of report\*\*\*

## D-5 HPLC CHROMATOGRAM FOR BATCH 20130811

Data File E:\DATA\2013\0819-1\002-0201.D

```

=====
Operator       : sun hongkai                Line       : 2
Instrument     : Instrument 1                Location   : 2
Injection Date : 2013-08-19 8:00:18          Inj        : 1
                                           Inj Volum  : 20.0 µl
Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-19 7:49:04 sun hongkai
                ( modified after loading )
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-19 9:40:33 sun hongkai
                ( modified after loading )
Sample Info   : GLG-RA60Plus-20130811
    
```



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	2.350	MM	0.1076	13.25707	2.05367	1.3494	Rub
2	3.495	MM	0.1445	6.30959	7.27874e-1	0.6422	Dul A
3	5.193	BB	0.1947	251.85249	19.45114	25.6347	Stv
4	6.465	BV	0.2538	82.86881	4.80151	8.4348	Reb C
5	7.221	VV	0.2744	14.22029	7.78019e-1	1.4474	Reb F
6	10.256	BB	0.3772	609.61334	24.29528	62.0491	Reb A
7	30.474	MM	0.3480	7.25223e-1	3.47372e-2	0.0738	Reb D

Instrument 1 2013-08-19 9:40:40 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File E:\DATA\2013\0819-1\002-0201.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU	Area *s	Peak Height [mAU]	Amt/Area %	Name
8	37.507	MM	0.2898	3.46933e-1		1.99520e-2	0.0353	Sbio
9	80.898	MM	1.4098	3.27500		3.87171e-2	0.3333	Reb B

Total :                                    982.46875    52.20090

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

## **APPENDIX E**

### **HPLC Chromatograms for Rebsweet RA80**

E-1 HPLC Chromatogram for Batch 20130801

E-2 HPLC Chromatogram for Batch 20130805

E-3 HPLC Chromatogram for Batch 20130807

E-4 HPLC Chromatogram for Batch 20130810

E-5 HPLC Chromatogram for Batch 20130815

## E-1 HPLC CHROMATOGRAM FOR BATCH 20130801

Date File : E:\DATA\2013\0809-1\001-1401.D

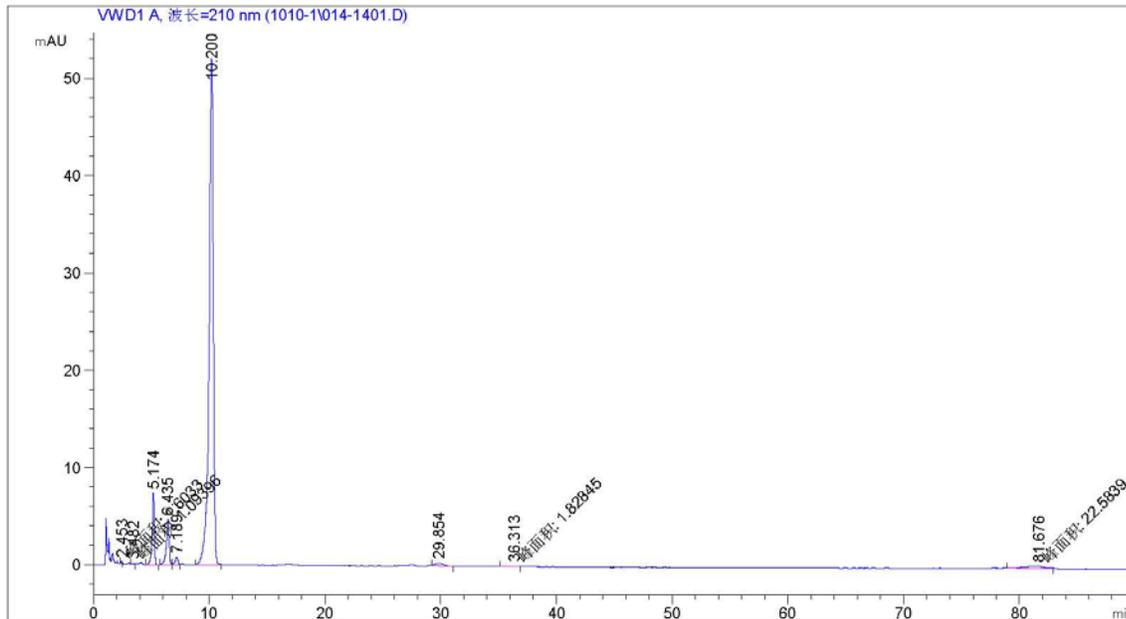
```

=====
Operator       : sun hongkai                Line :    1
Instrument     : Instrument 1              Location:   14
Injection Date : 2013-08-09 12:35:10      Inj :    1
                                           Inj Volum: 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-09 7:49:04 sun hongkai
                ( modified after loading )

Analysis Method: D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-09 14:30:05 sun hongkai
                ( modified after loading )

Sample Info   : GLG-RA80-20130801
  
```



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	2.453	MM	0.1635	1.60330	1.63478e-1	0.1045	Rub
2	3.482	MM	0.1782	1.09396	1.02327e-1	0.0713	Dul A
3	5.174	VB	0.1951	95.92639	7.39258	6.2506	Stv

Instrument 1 2013-08-09 14:30:20 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
 High Purity Steviol Glycosides (≥ 95%) – GLG-SG

Date File : E:\DATA\2013\0809-1\001-1401.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
4	6.435	BV	0.2528	79.95422	4.65575	5.2098	Reb C
5	7.189	VV	0.2633	14.33031	8.03856e-1	0.9338	Reb F
6	10.200	BB	0.3735	1302.15845	52.03192	84.8489	Reb A
7	29.854	BB	0.6391	15.19986	2.94961e-1	0.9904	Reb D
8	36.313	MM	0.8306	1.82845	3.66886e-2	0.1191	Sbio
9	81.676	MM	2.1625	22.58386	1.74053e-1	1.4716	Reb B

Total : 1534.67880 65.65561

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

## E-2 HPLC CHROMATOGRAM FOR BATCH 20130805

Data File: E:\DATA\2013\0810-1\006-0201.D

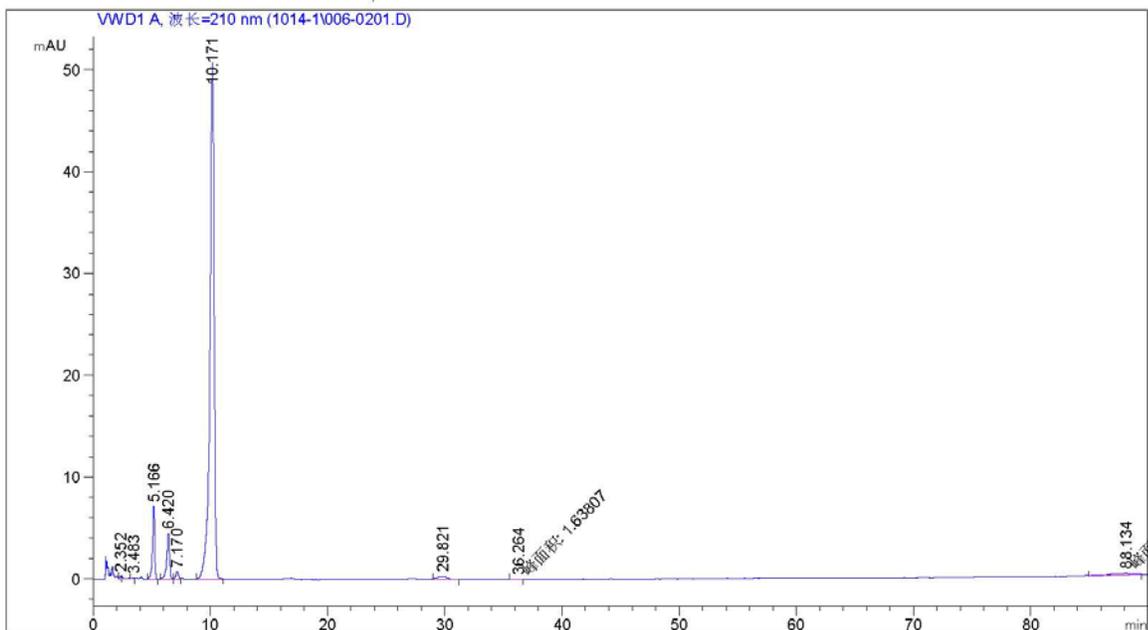
```

=====
Operator       : sun hongkai                Line   :    6
Instrument     : Instrument 2              Location:    2
Injection Date : 2013-08-10 12:25:26      Inj    :    1
                                           Inj Volum: 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-10 11:25:01 sun hongkai
                ( modified after loading )

Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-10 16:10:20 sun hongkai
                ( modified after loading )

Sample Info   : GLG-RA80-20130805
=====
  
```



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	2.352	VV	0.1340	3.21399	3.21660e-1	0.2144	Rub
2	3.483	VV	0.1592	1.30161	1.16766e-1	0.0868	Dul A
3	5.166	BB	0.1902	90.96425	7.17125	6.0693	Stv
4	6.420	BV	0.2509	77.03345	4.52850	5.1398	Reb C

GRAS Assessment – GLG Life Tech Corporation  
 High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File: E:\DATA\2013\0810-1\006-0201.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
5	7.170	VV	0.2536	12.99529	7.76094e-1	0.8671	Reb F
6	10.171	BB	0.3773	1273.92212	50.74876	84.9986	Reb A
7	29.821	BB	0.6871	15.16763	2.70122e-1	1.0120	Reb D
8	36.264	MM	0.7206	1.63807	3.78869e-2	0.1093	Sbio
9	88.134	MM	2.1958	22.52085	1.70941e-1	1.5026	Reb B

Total :                           1498.75726   64.14197

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

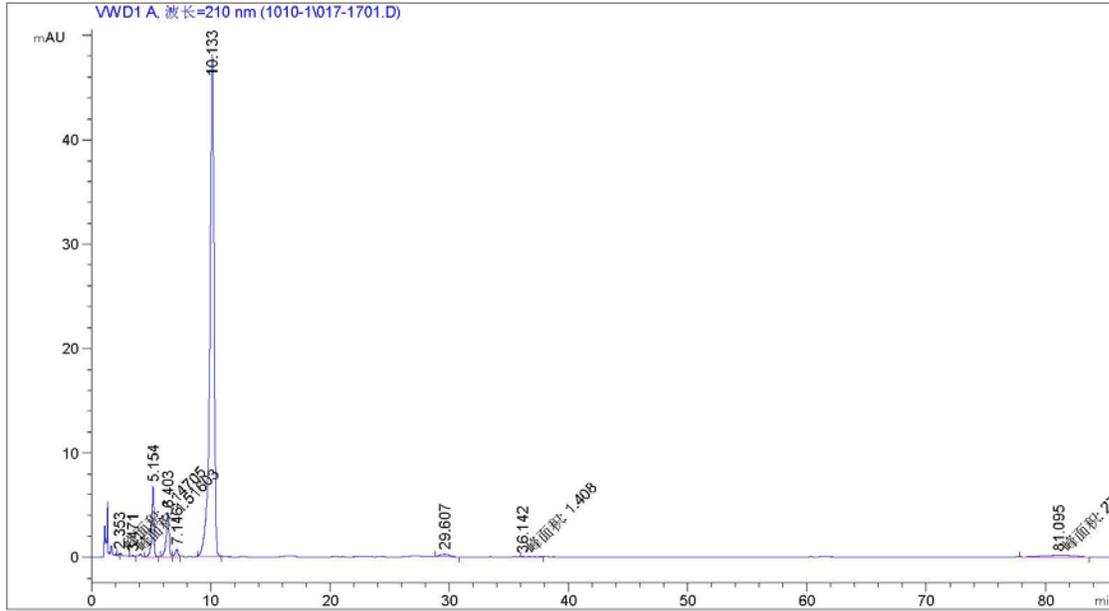
### E-3 HPLC CHROMATOGRAM FOR BATCH 20130807

Data File: E:\DATA\2013\0811-1\017-1701.D

```

=====
Operator       : sun hongkai                Line   :   17
Instrument     : Instrument 1              Location:   17
Injection Date : 2013-08-11 15:55:14      Inj     :    1
                                           Inj Volum: 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed   : 2013-08-11 12:32:15 sun hongkai
                  ( modified after loading )
Analysis Method: D:\CHEM32\1\METHODS\C18_LC.M
Last Changed   : 2013-08-11 18:11:23 sun hongkai
                  ( modified after loading )
Sample Info    : GLG-RA80-20130807
  
```



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	2.353	MM	0.0918	1.14705	2.08195e-1	0.0810	Rub
2	3.471	MM	0.2636	1.51603	9.58368e-2	0.1071	Dul A
3	5.154	VB	0.1946	88.04715	6.80701	6.2194	Stv

Instrument 1 2013-08-11 18:12:05 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File: E:\DATA\2013\0811-1\017-1701.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
4	6.403	BV	0.2518	73.22519	4.28387	5.1724	Reb C
5	7.146	VV	0.2644	13.36795	7.46051e-1	0.9443	Reb F
6	10.133	BB	0.3713	1194.75415	48.09740	84.3942	Reb A
7	29.607	BB	0.6760	14.79746	2.66880e-1	1.0453	Reb D
8	36.142	MM	0.9681	1.40800	2.42410e-2	0.0995	Sbio
9	81.095	MM	2.5184	27.42011	1.81462e-1	1.9369	Reb B

Total :                                    1415.68309    60.71095

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

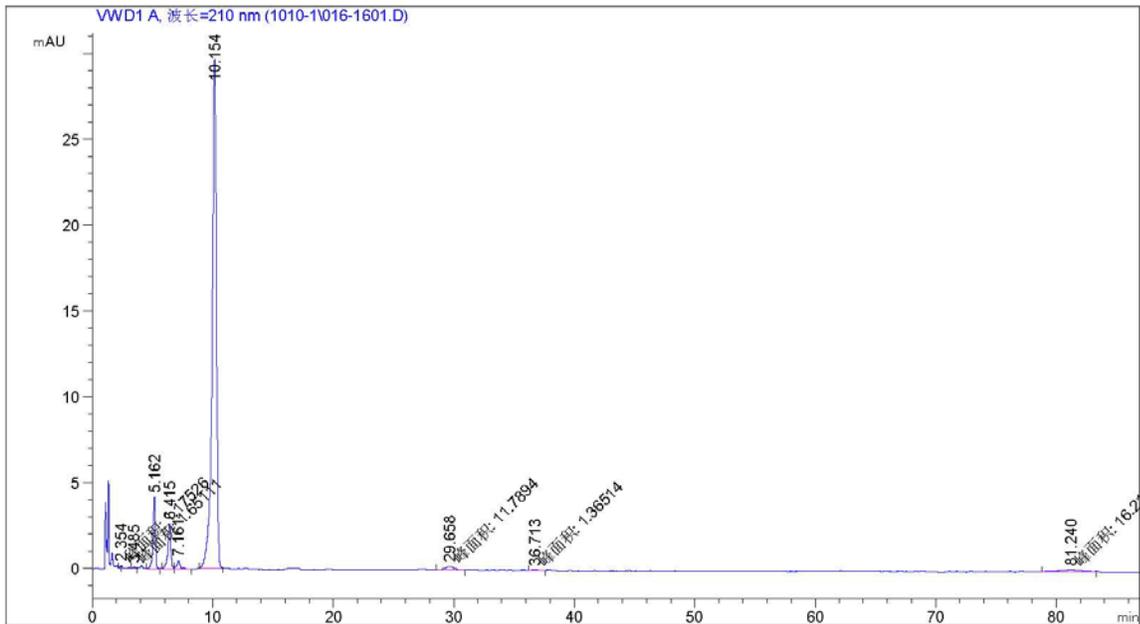
## E-4 HPLC CHROMATOGRAM FOR BATCH 20130810

Data File: E:\DATA\2013\0814-1\016-1601.D

```

=====
Operator       : sun hongkai                Line   :   16
Instrument     : Instrument 1              Location:   16
Injection Date : 2013-08-14 14:26:26      Inj     :    1
                                           Inj Volum: 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-14 12:49:04 sun hongkai
                ( modified after loading )
Analysis Method: D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-14 16:23:15 sun hongkai
                ( modified after loading )
Sample Info   : GLG-RA80-20130810
  
```



### 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	2.354	MM	0.1287	1.17526	1.52249e-1	0.1337	Rub
2	3.485	MM	0.2746	1.65111	1.00201e-1	0.1879	Dul A
3	5.162	VB	0.1941	54.56785	4.19245	6.2085	Stv

Instrument 1 2013-08-14 16:24:37 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
 High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File: E:\DATA\2013\0814-1\016-1601.D

Peak #	Ret [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
4	6.415	BV	0.2565	45.43475	2.63464	5.1693	Reb C
5	7.161	VB	0.2975	9.68992	4.72445e-1	1.1025	Reb F
6	10.154	BB	0.3713	737.04254	29.67714	83.8569	Reb A
7	29.658	MM	0.9789	11.78940	2.00727e-1	1.3413	Reb D
8	36.713	MM	0.6189	1.36514	3.67595e-2	0.1553	Sbio
9	81.240	MM	2.2966	16.21270	1.17655e-1	1.8446	Reb B

Total : 878.92867 37.58427

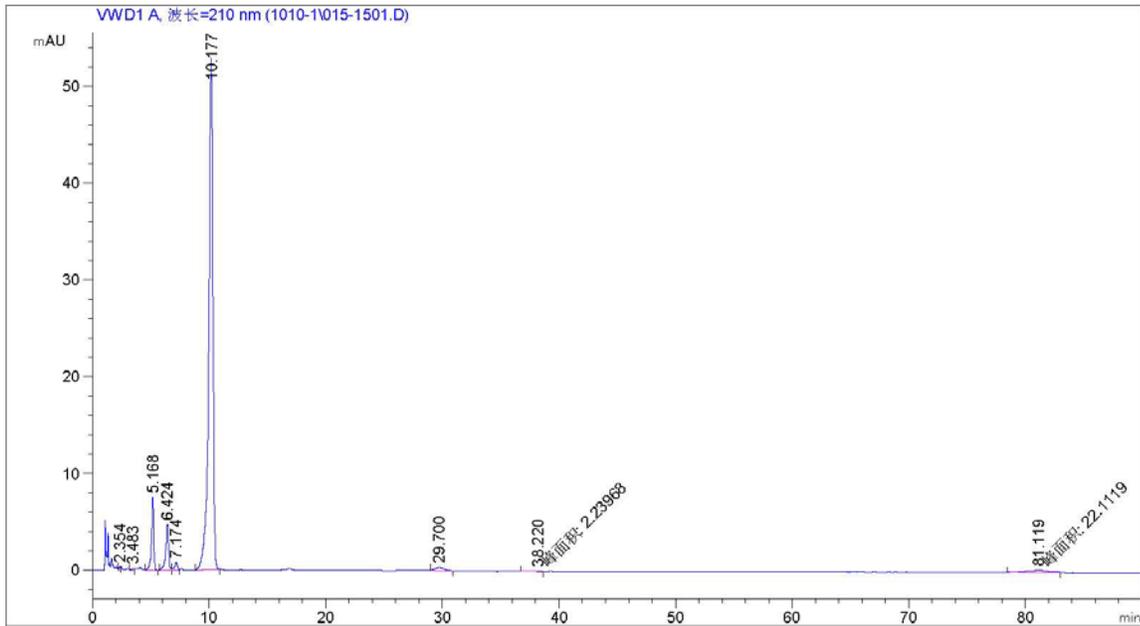
=====  
 \*\*\* End of report \*\*\*

### E-5 HPLC CHROMATOGRAM FOR BATCH 20130815

Data File: E:\DATA\2013\0818\015-1501.D

```

=====
Operator       : sun hongkai                Line   : 15
Instrument     : Instrument 1                Location: 15
Injection Date : 2013-08-18 12:54:35       Inj    : 1
                                           Inj Volum: 20.0 µl
Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-18 11:50:11 sun hongkai
                                           ( modified after loading )
Analysis Method: D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-18 14:10:05 sun hongkai
                                           ( modified after loading )
Sample Info   : GLG-RA80-20130815
=====
  
```



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	2.354	VV	0.1360	3.74107	3.68300e-1	0.2395	Rub
2	3.483	VV	0.2036	2.56335	1.72862e-1	0.1641	Dul A
3	5.168	VB	0.1932	97.29013	7.52053	6.2284	Stv

Instrument 1 2013-08-18 14:10:20 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

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Data File: E:\DATA\2013\0818\015-1501.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
4	6.424	BV	0.2534	81.33167	4.72168	5.2067	Reb C
5	7.174	VV	0.2568	13.91811	8.05934e-1	0.8910	Reb F
6	10.177	BB	0.3764	1322.25769	52.84078	84.6492	Reb A
7	29.700	BB	0.6672	16.59062	3.01969e-1	1.0621	Reb D
8	38.220	MM	1.1620	2.23968	3.21237e-2	0.1434	Sbio
9	81.119	MM	1.9431	22.11191	1.89661e-1	1.4156	Reb B

Total :                                    1562.04423    66.95384

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

## **APPENDIX F**

### **HPLC Chromatograms for Rebsweet RA85**

F-1 HPLC Chromatogram for Batch 20130802

F-2 HPLC Chromatogram for Batch 20130806

F-3 HPLC Chromatogram for Batch 20130808

F-4 HPLC Chromatogram for Batch 20130812

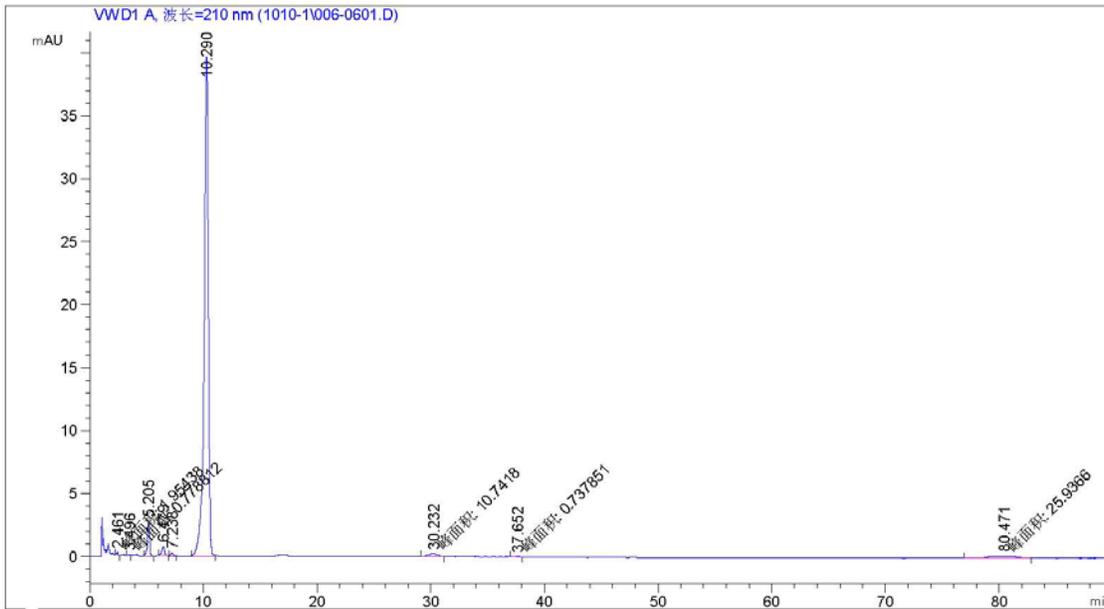
F-5 HPLC Chromatogram for Batch 20130816

## F-1 HPLC CHROMATOGRAM FOR BATCH 20130802

Data File E:\DATA\2013\0809-1\001-0501.D

```

=====
Operator       : sun hongkai                Line       : 1
Instrument     : Instrument 1                Location   : 5
Injection Date : 2013-08-09 9:00:18          Inj        : 1
                                           Inj Volum  : 20.0 µl
Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed   : 2013-08-09 8:48:04 sun hongkai
                ( modified after loading )
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed   : 2013-08-09 10:54:33 sun hongkai
                ( modified after loading )
Sample Info    : GLG-RA85-20130802
=====
  
```



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	Peak Height [mAU]	Amt/Area %	Name
1	2.461	MM	0.1494	1.95438	2.18072e-1	0.1792	Rub
2	3.496	MM	0.1664	7.78812e-1	7.79864e-2	0.0714	Dul A
3	5.205	BB	0.1906	35.02429	2.75428	3.2109	Stv
4	6.479	BB	0.2543	12.86146	7.54001e-1	1.1791	Reb C
5	7.238	BB	0.2382	3.85002	2.45599e-1	0.3530	Reb F
6	10.290	BB	0.3754	998.89160	39.65220	91.5762	Reb A
7	30.232	MM	0.8633	10.74184	2.07375e-1	0.9848	Reb D

Instrument 1 2013-08-09 10:55:03 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File E:\DATA\2013\0809-1\001-0501.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU	Peak Height [mAU]	Amt/Area %	Name
8	37.652	MM	0.4340	7.37851e-1	2.83365e-2	0.0676	Sbio
9	80.471	MM	2.8688	25.93658	1.50680e-1	2.3778	Reb B

Total :                            1090.77684    44.08853

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

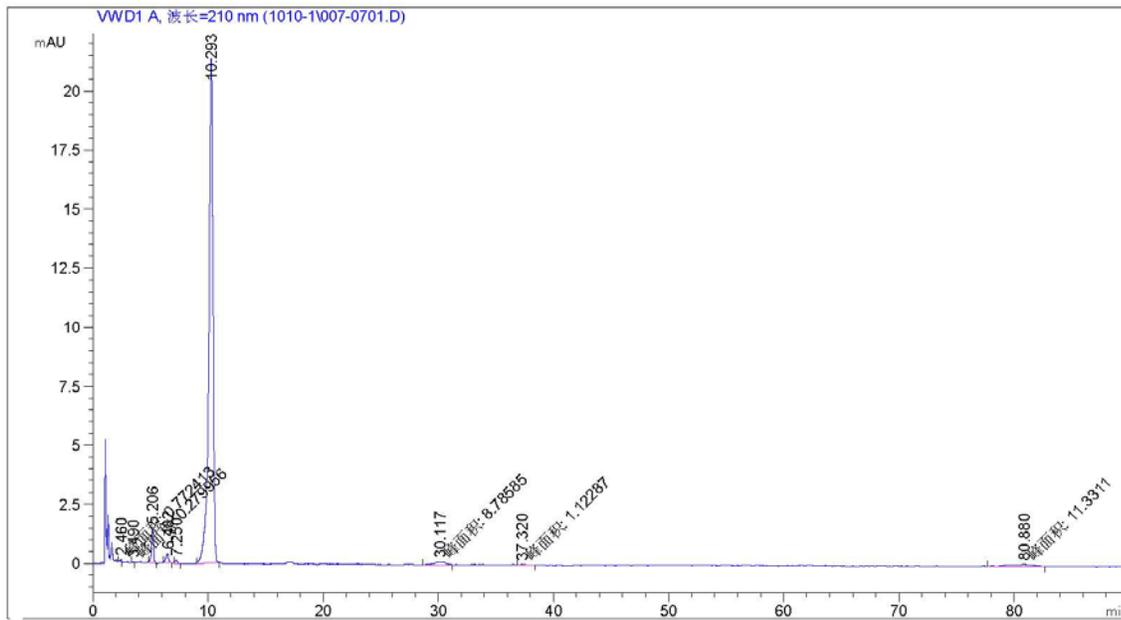
## F-2 HPLC CHROMATOGRAM FOR BATCH 20130806

Data File: E:\DATA\2013\0811-1\003-0401.D

```

=====
Operator       : sun hongkai                      Line    : 3
Instrument     : Instrument 1                    Location: 4
Injection Date : 2013-08-11 10:02:08           Inj     : 1
                                           Inj Volum: 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-11 8:14:04 sun hongkai
                ( modified after loading )
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-11 13:34:33 sun hongkai
                ( modified after loading )
Sample Info   : GLG-RA85-20130806
  
```



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	2.460	MM	0.1473	7.72413e-1	8.73907e-2	0.1322	Rub
2	3.490	MM	0.1231	2.79956e-1	3.79133e-2	0.0479	Dul A
3	5.206	BB	0.1858	18.01677	1.46393	3.0836	Stv
4	6.482	BB	0.2347	6.48022	4.04944e-1	1.1091	Reb C
5	7.250	BB	0.2187	1.92995	1.33058e-1	0.3303	Reb F
6	10.293	BB	0.3744	535.55878	21.33655	91.6616	Reb A
7	30.117	MM	1.2107	8.78585	1.20950e-1	1.5037	Reb D

Instrument 1 2013-08-11 13:34:54 sun hongkai

1/2



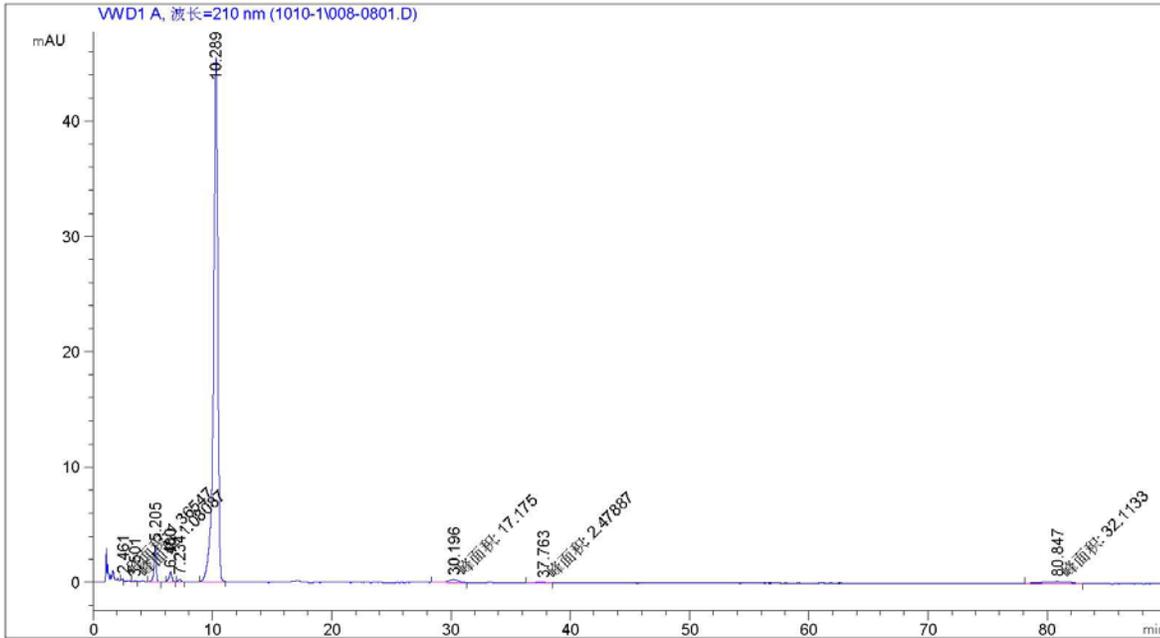
### F-3 HPLC CHROMATOGRAM FOR BATCH 20130808

Data File: E:\DATA\2013\0814-1\002-0401.D

```

=====
Operator       : sun hongkai                Line       : 2
Instrument     : Instrument 1                Location    : 4
Injection Date : 2013-08-14 8:30:12         Inj         : 1
                                           Inj Volum  : 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-14 8:04:04 sun hongkai
                ( modified after loading )
Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-14 10:49:43 sun hongkai
                ( modified after loading )
Sample Info   : GLG-RA85-20130808
  
```



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	Area *s	Peak Height [mAU]	Amt/Area %	Name
1	2.461	MM	0.1374	1.36547		1.65664e-1	0.1083	Rub
2	3.501	MM	0.2160	1.08087		8.34057e-2	0.0857	Dul A
3	5.205	VB	0.1927	40.62661		3.14961	3.2231	Stv
4	6.480	BB	0.2517	14.82988		6.68282e-1	1.1765	Reb C
5	7.234	BB	0.2478	4.94278		3.04252e-1	0.3921	Reb F
6	10.289	BB	0.3757	1145.88196		45.45295	90.9073	Reb A
7	30.196	MM	1.0431	17.17499		2.74421e-1	1.3626	Reb D

Instrument 1 2013-08-14 10:50:52 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

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Data File: E:\DATA\2013\0814-1\002-0401.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU	Area *s	Peak Height [mAU]	Amt/Area %	Name
8	37.763	MM	1.0769	2.47887		3.83660e-2	0.1967	Sbio
9	80.847	MM	2.7686	32.11327		1.93319e-1	2.5477	Reb B

Total :                                    1260.49469    50.53027

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

### F-4 HPLC Chromatogram for Batch 20130812

Data File: E:\DATA\2013\0815-1\001-0801.D

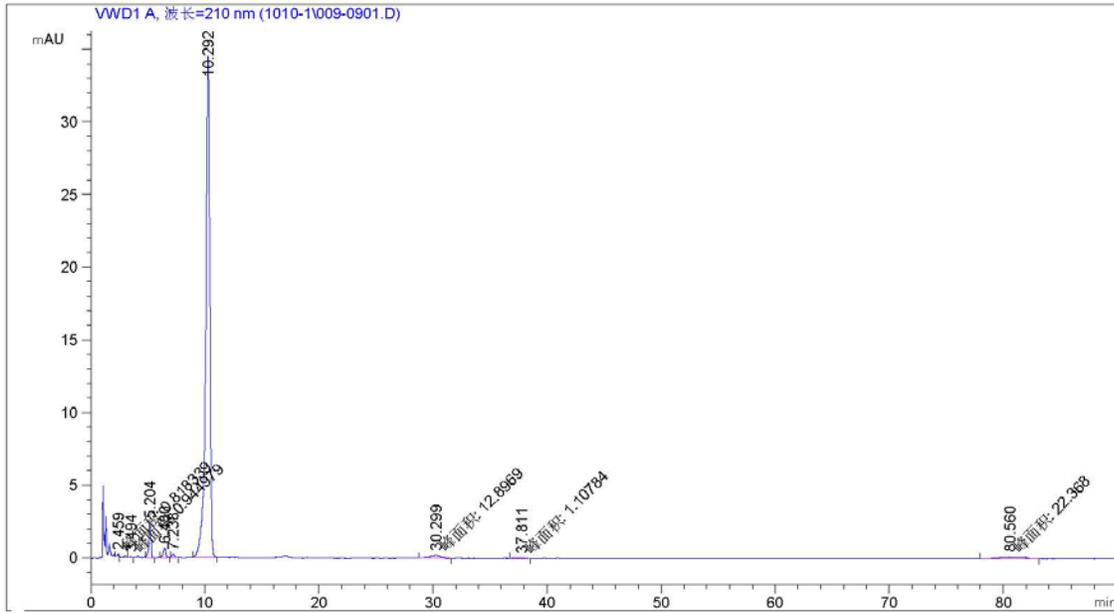
```

=====
Operator       : sun hongkai           Line       : 1
Instrument     : Instrument 1          Location   : 8
Injection Date : 2013-08-15 14:00:08 Inj        : 1
                                           Inj Volum : 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-15 10:29:04 sun hongkai
                ( modified after loading )

Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-15 16:14:42 sun hongkai
                ( modified after loading )

Sample Info   : GLG-RA85-20130812
  
```



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	Peak Height [mAU]	Amt/Area %	Name
1	2.459	MM	0.1342	8.18339e-1	1.01661e-1	0.0858	Rub
2	3.494	MM	0.2330	9.44979e-1	6.75960e-2	0.0990	Dul A
3	5.204	BB	0.1947	31.31650	2.41964	3.2820	Stv
4	6.480	BB	0.2640	11.97974	6.69654e-1	1.2555	Reb C
5	7.238	BB	0.2626	4.42868	2.49308e-1	0.4641	Reb F
6	10.292	BB	0.3781	868.32935	34.49722	91.0017	Reb A
7	30.299	MM	1.0536	12.89691	2.04009e-1	1.3516	Reb D

Instrument 1 2013-08-15 16:15:50 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
High Purity Steviol Glycosides (≥ 95%) – GLG-SG

---

Data File: E:\DATA\2013\0815-1\001-0801.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU	Peak Height [mAU]	Amt/Area %	Name
8	37.811	MM	0.7466	1.10784	2.47312e-2	0.1161	Sbio
9	80.560	MM	2.6463	22.36804	1.40877e-1	2.3442	Reb B

Total : 954.19038 38.37469

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

## F-5 HPLC CHROMATOGRAM FOR BATCH 20130816

Data File: E:\DATA\2013\0819-1\007-0301.D

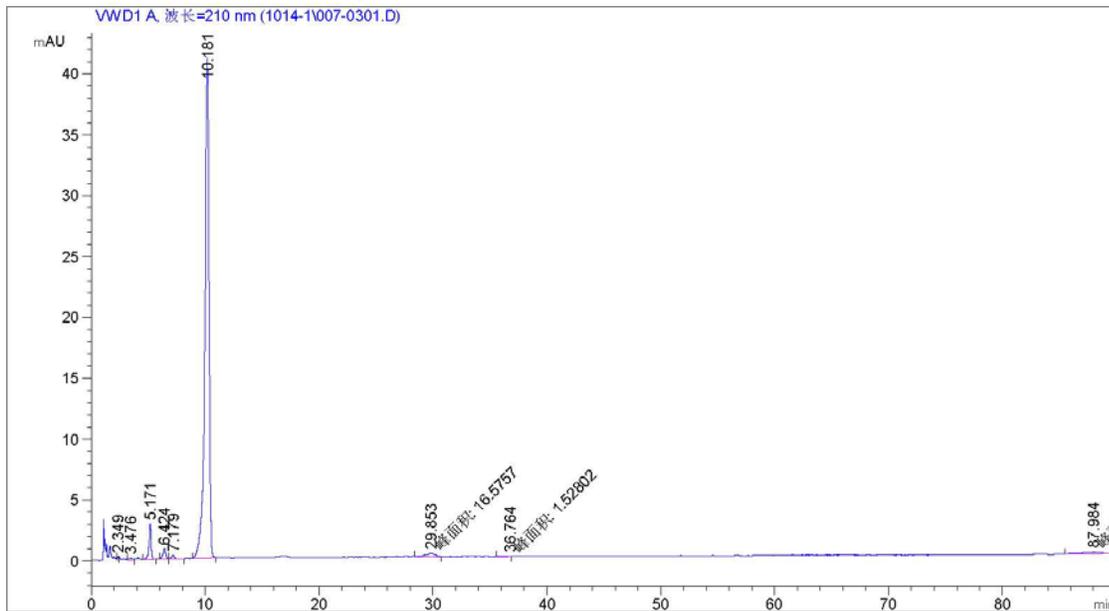
```

=====
Operator       : sun hongkai                      Line   :    7
Instrument     : Instrument 2                      Location:    3
Injection Date : 2013-08-19 19:26:38             Inj    :    1
                                                    Inj Volum: 20.0 µl

Acq.Method    : D:\CHEM32\1\METHODS\SUGAR-D_LC.M
Last Changed  : 2013-08-19 16:21:24 sun hongkai
                ( modified after loading )

Analysis Method : D:\CHEM32\1\METHODS\C18_LC.M
Last Changed  : 2013-08-19 18:30:31 sun hongkai
                ( modified after loading )

Sample Info   : GLG-RA85-20130816
=====
  
```



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	2.349	VV	0.0972	1.88857	2.76217e-1	0.1677	Rub
2	3.476	VV	0.2813	2.99456	1.37741e-1	0.2660	Dul A
3	5.171	VB	0.1960	38.02344	2.88560	3.3769	Stv
4	6.424	BV	0.2632	14.62438	8.15170e-1	1.2988	Reb C

Instrument 2 2013-08-19 18:30:59 sun hongkai

1/2

GRAS Assessment – GLG Life Tech Corporation  
 High Purity Steviol Glycosides (≥ 95%) – GLG-SG

Data File: E:\DATA\2013\0819-1\007-0301.D

Peak #	Ret Time [min]	Type	Peak Width [min]	Area mAU*s	Peak Height [mAU]	Amt/Area %	Name
5	7.179	VB	0.2628	5.40154	2.95389e-1	0.4797	Reb F
6	10.181	BB	0.3762	1026.69629	41.05019	91.1829	Reb A
7	29.853	MM	1.0412	16.57572	2.65323e-1	1.4721	Reb D
8	36.764	MM	1.0810	1.52802	2.35592e-2	0.1357	Sbio
9	87.984	MM	2.4642	18.24220	1.23383e-1	1.6201	Reb B

Total :                                    1125.97473    45.87257

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

## **APPENDIX G**

### **Certificates of Analysis for Multiple Production Batches of Anysweet RA50**

- G-1 Certificate of Analysis for Batch 20130801
- G-2 Certificate of Analysis for Batch 20130804
- G-3 Certificate of Analysis for Batch 20130807
- G-4 Certificate of Analysis for Batch 20130810
- G-5 Certificate of Analysis for Batch 20130814

## G-1 CERTIFICATE OF ANALYSIS FOR BATCH 20130801



### Certificate of Analysis

GLG Life Tech Corporation  
 www.glglifitech.com  
 GLG-QA-COA-22-1

**Product:** Anysweet RA50 Plus  
**Lot Number:** GLG-RA50 Plus-20130801  
**Manufacturing Date:** Aug. 4th, 2013  
**Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Anysweet™ RA50 plus is a purified extract containing rebaudioside A (MW: 967.03) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

**Distributed By:** GLG Life Tech Corporation  
 Suite 2168-1050 West Pender Street  
 Vancouver, B.C. V6E 3S7  
 Canada  
 Phone: 1.604.669.2602  
 Fax: 1.604.662.8858  
 Email: sales@glglifitech.com  
 Web: www.glglifitech.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:** Aug. 9th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Total Steviol Glycosides	≥ 95.0%	95.58%	JECFA HPLC
Rebaudioside A	≥ 50.0%	51.05%	JECFA HPLC
Stevioside	≥ 25.0%	32.25%	JECFA HPLC
Specific Rotation (α) <sub>D20</sub>	-30° – -38°	-35°	Polarimeter
Loss on Drying	≤ 4.0%	2.68%	USP
pH	4.5 - 7.0	5.68	USP
Residue on Ignition	< 1.0%	0.07%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.04mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.062%	USP
– Methanol	≤ 0.02%	0.004%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10 cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

**Conclusion:** Qualified

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

**Analyzed by:** (b) (6) **Date:** 09/08/2013  
**Checked by:** (b) (6) **Date:** 09/08/2013  
**Approved by:** (Quality Manager) **Date:** 09/08/2013

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## G-2 CERTIFICATE OF ANALYSIS FOR BATCH 20130804



### Certificate of Analysis

GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-22-1

**Product:** Anysweet RA50 Plus  
**Lot Number:** GLG-RA50 Plus-20130804  
**Manufacturing Date:** Aug. 8th, 2013  
**Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Anysweet™ RA50 plus is a purified extract containing rebaudioside A (MW: 967.03) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

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

**Manufacturing By:** Qingdao Runde Biotechnology Co., Ltd  
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 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:** Aug. 13th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Total Steviol Glycosides	≥ 95.0%	96.12%	JECFA HPLC
Rebaudioside A	≥ 50.0%	51.17%	JECFA HPLC
Stevioside	≥ 25.0%	33.12%	JECFA HPLC
Specific Rotation (α) <sub>D20</sub>	-30° – -38°	-35°	Polarimeter
Loss on Drying	≤ 4.0%	2.56%	USP
pH	4.5 - 7.0	5.48	USP
Residue on Ignition	< 1.0%	0.08%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.03mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.055%	USP
– Methanol	≤ 0.02%	0.007%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10 cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

**Conclusion:** Qualified

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

**Analyzed by:** (b) (6) **Date:** 13/08/2013  
**Checked by:** (b) (6) **Date:** 13/08/2013  
**Approved by:** (Quality Manager) **Date:** 13/08/2013

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### G-3 CERTIFICATE OF ANALYSIS FOR BATCH 20130807



GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-22-1

## Certificate of Analysis

**Product:** Anysweet RA50 Plus **Manufacturing Date:** Aug. 10th, 2013  
**Lot Number:** GLG-RA50 Plus-20130807 **Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Anysweet™ RA50 plus is a purified extract containing rebaudioside A (MW: 967.03) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

**Distributed By:** GLG Life Tech Corporation  
 Suite 2168-1050 West Pender Street  
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 Canada  
 Phone: 1.604.669.2602  
 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: +86.532.83181836

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

**Date of Analysis:** Aug. 15th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Total Steviol Glycosides	≥ 95.0%	95.96%	JECFA HPLC
Rebaudioside A	≥ 50.0%	51.38%	JECFA HPLC
Stevioside	≥ 25.0%	33.27%	JECFA HPLC
Specific Rotation (α) <sub>D25</sub>	-30° – -38°	-35°	Polarimeter
Loss on Drying	≤ 4.0%	2.39%	USP
pH	4.5 - 7.0	5.51	USP
Residue on Ignition	< 1.0%	0.05%	USP
Lead (Pb)	< 1mg/kg	0.02mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.04mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.072%	USP
– Methanol	≤ 0.02%	0.004%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10 cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

**Conclusion:** Qualified

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

**Analyzed by:** (b) (6) **Date:** 15/08/2013  
**Checked by:** (b) (6) **Date:** 15/08/2013  
**Approved by:** (b) (6) (Quality Manager) **Date:** 15/08/2013

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## G-4 CERTIFICATE OF ANALYSIS FOR BATCH 20130810



### Certificate of Analysis

GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-22-1

**Product:** Anysweet RA50 Plus      **Manufacturing Date:** Aug. 12th, 2013  
**Lot Number:** GLG-RA50 Plus-20130810      **Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Anysweet™ RA50 plus is a purified extract containing rebaudioside A (MW: 967.03) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

**Distributed By:** GLG Life Tech Corporation      Phone: 1.604.669.2602  
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**Manufacturing By:** Qingdao Runde Biotechnology Co., Ltd      Phone: +86.532.83181169  
 Lingshanwei Town, Jiaonan County      Fax: +86.532.83181836  
 Qingdao, Shandong, China 266427

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

**Date of Analysis:** Aug. 17th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Total Steviol Glycosides	≥ 95.0%	95.75%	JECFA HPLC
Rebaudioside A	≥ 50.0%	50.86%	JECFA HPLC
Stevioside	≥ 25.0%	34.41%	JECFA HPLC
Specific Rotation (α) <sub>D20</sub>	-30° — -38°	-34°	Polarimeter
Loss on Drying	≤ 4.0%	2.66%	USP
pH	4.5 - 7.0	5.58	USP
Residue on Ignition	< 1.0%	0.08%	USP
Lead (Pb)	< 1mg/kg	0.02mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.03mg/kg	AAS
Residual Solvents -- Ethanol	≤ 0.5%	0.068%	USP
-- Methanol	≤ 0.02%	0.006%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10 cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

**Conclusion:** Quality Control

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

**Analyzed by:** (b) (6)      **Date:** 17/08/2013  
**Checked by:** (b) (6)      **Date:** 17/08/2013  
**Approved by:** (b) (6) (Quality Manager)      **Date:** 17/08/2013

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## G-5 CERTIFICATE OF ANALYSIS FOR BATCH 20130814



### Certificate of Analysis

GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-22-1

**Product:** Anysweet RA50 Plus  
**Lot Number:** GLG-RA50 Plus-20130814  
**Manufacturing Date:** Aug. 17th, 2013  
**Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Anysweet™ RA50 plus is a purified extract containing rebaudioside A (MW: 967.03) from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

**Distributed By:** GLG Life Tech Corporation  
 Suite 2168-1050 West Pender Street  
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 Phone: 1.604.669.2602  
 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: +86.532.83181836

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

**Date of Analysis:** Aug. 22nd, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Total Steviol Glycosides	≥ 95.0%	95.54%	JECFA HPLC
Rebaudioside A	≥ 50.0%	51.56%	JECFA HPLC
Stevioside	≥ 25.0%	32.98%	JECFA HPLC
Specific Rotation (α) <sub>D</sub> <sup>20</sup>	-30° – -38°	-34°	Polarimeter
Loss on Drying	≤ 4.0%	2.82%	USP
pH	4.5 - 7.0	5.63	USP
Residue on Ignition	< 1.0%	0.06%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.04mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.045%	USP
– Methanol	≤ 0.02%	0.007%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10 cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

**Conclusion:** *Quality Free*

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

**Analyzed by:** (b) (6) **Date:** 22/08/2013  
**Checked by:** (b) (6) **Date:** 22/08/2013  
**Approved by:** (Quality Manager) **Date:** 22/08/2013

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## **APPENDIX H**

### **Certificates of Analysis for Multiple Production Batches of Anysweet RA60**

- H-1 Certificate of Analysis for Batch 20130801
- H-2 Certificate of Analysis for Batch 20130803
- H-3 Certificate of Analysis for Batch 20130806
- H-4 Certificate of Analysis for Batch 20130809
- H-5 Certificate of Analysis for Batch 20130811

## H-1 CERTIFICATE OF ANALYSIS FOR BATCH 20130801



### Certificate of Analysis

GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-15-1

**Product:** Anysweet RA60 Plus  
**Lot Number:** GLG-RA60 plus- 20130801  
**Manufacturing Date:** Aug. 6th, 2013  
**Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Anysweet RA60 Plus is extracted from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages

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 Canada  
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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: +86.532.83181836

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

**Date of Analysis:** Aug. 11th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Total Steviol Glycosides	≥ 95.0%	96.34%	JECFA HPLC
Rebaudioside A	≥ 60.0%	61.63%	JECFA HPLC
Stevioside	≥ 15.0%	22.43%	JECFA HPLC
Specific Rotation (α) <sub>25°</sub>	-30°— -38°	-34°	Polarimeter
Loss on Drying	≤ 4.0%	2.55%	USP
pH	4.5 - 7.0	5.42	USP
Residue on Ignition	< 1.0%	0.05%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.02mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.016%	USP
– Methanol	≤ 0.02%	0.004%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Qualified

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

**Analyzed by:** (b) (6) **Date:** 11/08/2013  
**Checked by:** [Redacted] **Date:** 11/08/2013  
**Approved by:** [Redacted] (Quality Manager) **Date:** 11/08/2013

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## H-2 CERTIFICATE OF ANALYSIS FOR BATCH 20130803



### Certificate of Analysis

GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-15-1

**Product:** Anysweet RA60 Plus  
**Lot Number:** GLG-RA60 plus-20130803  
**Manufacturing Date:** Aug. 7th, 2013  
**Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Anysweet RA60 Plus is extracted from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages

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**Manufacturing By:** Qingdao Runde Biotechnology Co., Ltd  
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 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:** Aug. 12th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Total Steviol Glycosides	≥ 95.0%	95.78%	JECFA HPLC
Rebaudioside A	≥ 60.0%	60.89%	JECFA HPLC
Stevioside	≥ 15.0%	22.98%	JECFA HPLC
Specific Rotation (α) <sub>D20</sub>	-30° – -38°	-33°	Polarimeter
Loss on Drying	≤ 4.0%	2.92%	USP
pH	4.5 - 7.0	5.40	USP
Residue on Ignition	< 1.0%	0.07%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.03mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.032%	USP
– Methanol	≤ 0.02%	0.005%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Qualified

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

**Analyzed by:** (b) (6) **Date:** 12/08/2013  
**Checked by:** (b) (6) **Date:** 12/08/2013  
**Approved by:** (b) (6) Quality Manager **Date:** 12/08/2013

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### H-3 CERTIFICATE OF ANALYSIS FOR BATCH 20130806



## Certificate of Analysis

GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-15-1

**Product:** Anysweet RA60 Plus      **Manufacturing Date:** Aug. 9th, 2013  
**Lot Number:** GLG-RA60 plus-20130806      **Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Anysweet RA60 Plus is extracted from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages

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**Manufacturing By:** Qingdao Runde Biotechnology Co., Ltd      Phone: +86.532.83181169  
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 Qingdao, Shandong, China 266427

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

**Date of Analysis:** Aug. 14th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Total Steviol Glycosides	≥ 95.0%	96.40%	JECFA HPLC
Rebaudioside A	≥ 60.0%	61.56%	JECFA HPLC
Stevioside	≥ 15.0%	23.16%	JECFA HPLC
Specific Rotation (α) <sub>D25</sub>	-30° – -38°	-33°	Polarimeter
Loss on Drying	≤ 4.0%	2.72%	USP
pH	4.5 - 7.0	5.40	USP
Residue on Ignition	< 1.0%	0.07%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.04mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.029%	USP
– Methanol	≤ 0.02%	0.004%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Qualified

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

**Analyzed by:** (b) (6)      **Date:** 14/08/2013  
**Checked by:** (b) (6)      **Date:** 14/08/2013  
**Approved by:** (b) (6) (Quality Manager)      **Date:** 14/08/2013

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## H-4 CERTIFICATE OF ANALYSIS FOR BATCH 20130809



GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-15-1

### Certificate of Analysis

**Product:** Anysweet RA60 Plus  
**Lot Number:** GLG-RA60 plus-20130809  
**Manufacturing Date:** Aug. 12th, 2013  
**Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Anysweet RA60 Plus is extracted from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages

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**Manufacturing By:** Qingdao Runde Biotechnology Co., Ltd  
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 Phone: +86.532.83181169  
 Fax: +86.532.83181836

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**Date of Analysis:** Aug. 17th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Total Steviol Glycosides	≥ 95.0%	96.51%	JECFA HPLC
Rebaudioside A	≥ 60.0%	61.45%	JECFA HPLC
Stevioside	≥ 15.0%	23.73%	JECFA HPLC
Specific Rotation (α) <sub>D20</sub>	-30° – -38°	-33°	Polarimeter
Loss on Drying	≤ 4.0%	2.86%	USP
pH	4.5 - 7.0	5.49	USP
Residue on Ignition	< 1.0%	0.07%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.05mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.023%	USP
– Methanol	≤ 0.02%	0.005%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

**Conclusion:** Qualified

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

**Analyzed by:** (b) (6) **Date:** 17/08/2013  
**Checked by:** (b) (6) **Date:** 17/08/2013  
**Approved by:** (b) (6) (Quality Manager) **Date:** 17/08/2013

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## H-5 CERTIFICATE OF ANALYSIS FOR BATCH 20130811



### Certificate of Analysis

GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-15-1

**Product:** Anysweet RA60 Plus      **Manufacturing Date:** Aug. 14th, 2013  
**Lot Number:** GLG-RA60 plus-20130811      **Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Anysweet RA60 Plus is extracted from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages

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

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**Date of Analysis:** Aug. 19th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Total Steviol Glycosides	≥ 95.0%	96.13%	JECFA HPLC
Rebaudioside A	≥ 60.0%	61.06%	JECFA HPLC
Stevioside	≥ 15.0%	22.37%	JECFA HPLC
Specific Rotation (α) <sub>D20</sub>	-30° – -38°	-33°	Polarimeter
Loss on Drying	≤ 4.0%	3.10%	USP
pH	4.5 - 7.0	5.52	USP
Residue on Ignition	< 1.0%	0.08%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.03mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.035%	USP
– Methanol	≤ 0.02%	0.005%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Qualified

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

**Analyzed by:** (b) (6)      **Date:** 19/08/2013  
**Checked by:** (b) (6)      **Date:** 19/08/2013  
**Approved by:** (b) (6) (Quality Manager)      **Date:** 19/08/2013

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## **APPENDIX I**

### **Certificates of Analysis for Multiple Production Batches of Rebsweet RA80**

- I-1 Certificate of Analysis for Batch 20130801
- I-2 Certificate of Analysis for Batch 20130805
- I-3 Certificate of Analysis for Batch 20130807
- I-4 Certificate of Analysis for Batch 20130810
- I-5 Certificate of Analysis for Batch 20130815

## I-1 CERTIFICATE OF ANALYSIS FOR BATCH 20130801



### Certificate of Analysis



Research and Development  
 GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-08

**Product:** Rebsweet™ RA80      **Manufacturing Date:** Aug. 4th, 2013  
**Lot Number:** GLG-RA80-20130801      **Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Rebsweet™ RA80 is a highly purified extract containing rebaudioside A from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

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**Manufacturing By:** Qingdao Runde Biotechnology Co., Ltd      Phone: +86.532.83181169  
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 Qingdao, Shandong, China 266427

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**Date of Analysis:** Aug. 9th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 80%	80.85%	JECFA HPLC
Total Steviol Glycosides	≥ 95%	96.12%	JECFA HPLC
pH	4.5 - 7.0	5.69	USP
Residue on Ignition	< 1.0%	0.05%	USP
Loss on Drying	≤ 4.0%	2.86%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.03mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.081%	USP
– Methanol	≤ 0.02%	0.005%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Qualified

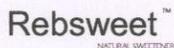
Note: This product should be stored sealed in a cool, dry place.

**Analyzed by:** (b) (6)      **Date:** 09/08/2013  
**Checked by:** \_\_\_\_\_      **Date:** 09/08/2013  
**Approved by:** \_\_\_\_\_ (Quality Manager)      **Date:** 09/08/2013

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## I-2 CERTIFICATE OF ANALYSIS FOR BATCH 20130805



### Certificate of Analysis



Research and Development  
 GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-08

**Product:** Rebsweet™ RA80      **Manufacturing Date:** Aug. 5th, 2013  
**Lot Number:** GLG-RA80-20130805      **Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Rebsweet™ RA80 is a highly purified extract containing rebaudioside A from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

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**Manufacturing By:** Qingdao Runde Biotechnology Co., Ltd      Phone: +86.532.83181169  
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 Qingdao, Shandong, China 266427

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

**Date of Analysis:** Aug. 10th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 80%	80.98%	JECFA HPLC
Total Steviol Glycosides	≥ 95%	96.05%	JECFA HPLC
pH	4.5 - 7.0	5.46	USP
Residue on Ignition	< 1.0%	0.06%	USP
Loss on Drying	≤ 4.0%	2.98%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.02mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.038%	USP
– Methanol	≤ 0.02%	0.006%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

**Conclusion:** Qualified



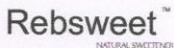
Note: This product should be stored sealed in a cool, dry place.

**Analyzed by:** (b) (6)      **Date:** 10/08/2013  
**Checked by:** \_\_\_\_\_      **Date:** 10/08/2013  
**Approved by:** \_\_\_\_\_ (Quality Manager)      **Date:** 10/08/2013

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### I-3 CERTIFICATE OF ANALYSIS FOR BATCH 20130807



## Certificate of Analysis



Research and Development  
 GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-08

**Product:** Rebsweet™ RA80      **Manufacturing Date:** Aug. 6th, 2013  
**Lot Number:** GLG-RA80-20130807      **Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Rebsweet™ RA80 is a highly purified extract containing rebaudioside A from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

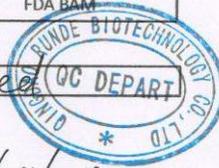
**Distributed By:** GLG Life Tech Corporation      Phone: 1.604.669.2602  
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**Manufacturing By:** Qingdao Runde Biotechnology Co., Ltd      Phone: +86.532.83181169  
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 Qingdao, Shandong, China 266427

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

**Date of Analysis:** Aug. 11th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 80%	81.46%	JECFA HPLC
Total Steviol Glycosides	≥ 95%	96.63%	JECFA HPLC
pH	4.5 - 7.0	5.54	USP
Residue on Ignition	< 1.0%	0.07%	USP
Loss on Drying	≤ 4.0%	2.52%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.02mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.052%	USP
– Methanol	≤ 0.02%	0.006%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Qualified 

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

**Analyzed by:** (b) (6)      **Date:** 11/08/2013  
**Checked by:** (b) (6)      **Date:** 11/08/2013  
**Approved by:** (b) (6) (Quality Manager)      **Date:** 11/08/2013

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## I-4 CERTIFICATE OF ANALYSIS FOR BATCH 20130810

**Rebsweet™**  
NATURAL SWEETENER

### Certificate of Analysis



Research and Development  
 GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-08

**Product:** Rebsweet™ RA80  
**Lot Number:** GLG-RA80-20130810

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

**Product Description:** Rebsweet™ RA80 is a highly purified extract containing rebaudioside A from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

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 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:** Aug. 14th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 80%	81.35%	JECFA HPLC
Total Steviol Glycosides	≥ 95%	96.08%	JECFA HPLC
pH	4.5 - 7.0	5.58	USP
Residue on Ignition	< 1.0%	0.08%	USP
Loss on Drying	≤ 4.0%	2.94%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.02mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.046%	USP
– Methanol	≤ 0.02%	0.005%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

**Conclusion:** Qualified 

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

**Analyzed by:** (b) (6) **Date:** 14/08/2013  
**Checked by:** (b) (6) **Date:** 14/08/2013  
**Approved by:** (b) (6) (Quality Manager) **Date:** 14/08/2013

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## I-5 CERTIFICATE OF ANALYSIS FOR BATCH 20130815

**Rebsweet™**  
NATURAL SWEETENER

### Certificate of Analysis



Research and Development  
 GLG Life Tech Corporation  
 www.glglifotech.com  
 GLG-QA-COA-08

**Product:** Rebsweet™ RA80      **Manufacturing Date:** Aug. 13th, 2013  
**Lot Number:** GLG-RA80-20130815      **Country of Origin:** China  
**Shelf Life:** 2 years

**Product Description:** Rebsweet™ RA80 is a highly purified extract containing rebaudioside A from Stevia rebaudiana Bertoni leaf. It is a white hygroscopic powder that is used as a high potency sweetener for food and beverages.

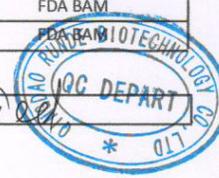
**Distributed By:**      GLG Life Tech Corporation      Phone: 1.604.669.2602  
 Suite 2168-1050 West Pender Street      Fax: 1.604.662.8858  
 Vancouver, B.C. V6E 3S7      Email: sales@glglifotech.com  
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**Manufacturing By:**      Qingdao Runde Biotechnology Co., Ltd      Phone: +86.532.83181169  
 Lingshanwei Town, Jiaonan County      Fax: +86.532.83181836  
 Qingdao, Shandong, China 266427

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**Date of Analysis:** Aug. 18th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 80%	81.02%	JECFA HPLC
Total Steviol Glycosides	≥ 95%	95.93%	JECFA HPLC
pH	4.5 - 7.0	5.52	USP
Residue on Ignition	< 1.0%	0.08%	USP
Loss on Drying	≤ 4.0%	2.76%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.04mg/kg	AAS
Residual Solvents – Ethanol	≤ 0.5%	0.052%	USP
– Methanol	≤ 0.02%	0.004%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Qualified 

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

**Analyzed by:** (b) (6)      **Date:** 18/08/2013  
**Checked by:** (b) (6)      **Date:** 18/08/2013  
**Approved by:** (b) (6) (Quality Manager)      **Date:** 18/08/2013

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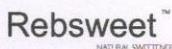
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## **APPENDIX J**

### **Certificates of Analysis for Multiple Production Batches of Rebsweet RA85**

- J-1 Certificate of Analysis for Batch 20130802
- J-2 Certificate of Analysis for Batch 20130806
- J-3 Certificate of Analysis for Batch 20130808
- J-4 Certificate of Analysis for Batch 20130812
- J-5 Certificate of Analysis for Batch 20130816

## J-1 CERTIFICATE OF ANALYSIS FOR BATCH 20130802



### Certificate of Analysis



Research and Development  
 GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-59

**Product:** Rebsweet™ RA85      **Manufacturing Date:** Aug. 4th, 2013  
**Lot Number:** GLG-RA85-20130802      **Country of Origin:** China  
**Shelf Life:** 2 years

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

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

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

**Date of Analysis:** Aug. 9th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 85.0%	86.78%	JECFA HPLC
Total Steviol Glycosides	≥ 95.0%	96.85%	JECFA HPLC
pH	4.5 - 7.0	5.51	USP
Residue on Ignition	< 1.0%	0.06%	USP
Loss on Drying	≤ 4.0%	2.50%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.05mg/kg	AAS
Residual Solvent-ethanol	≤ 0.5%	0.035%	USP
--Methanol	≤ 0.02%	0.005%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Search Passed

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

**Analyzed by:** (b) (6)      **Date:** 09/08/2013  
**Checked by:** (b) (6)      **Date:** 09/08/2013  
**Approved by:** (b) (6) (Quality Manager)      **Date:** 09/08/2013

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## J-2 CERTIFICATE OF ANALYSIS FOR BATCH 20130806

**Rebsweet™**  
NATURAL SWEETENER

### Certificate of Analysis



Research and Development  
 GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-59

**Product:** Rebsweet™ RA85  
**Lot Number:** GLG-RA85-20130806

**Manufacturing Date:** Aug. 7th, 2013  
**Country of Origin:** China  
**Shelf Life:** 2 years

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

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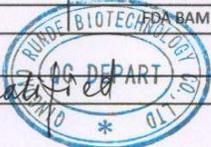
**Manufacturing By:** Qingdao Runde Biotechnology Co., Ltd  
 Lingshanwei Town, Jiaonan County  
 Qingdao, Shandong, China 266427  
 Phone: +86.532.83181169  
 Fax: +86.532.83181836

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

**Date of Analysis:** Aug. 11th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 85.0%	86.36%	JECFA HPLC
Total Steviol Glycosides	≥ 95.0%	96.98%	JECFA HPLC
pH	4.5 - 7.0	5.49	USP
Residue on Ignition	< 1.0%	0.08%	USP
Loss on Drying	≤ 4.0%	2.86%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.04mg/kg	AAS
Residual Solvent-ethanol	≤ 0.5%	0.028%	USP
–Methanol	≤ 0.02%	0.004%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Qualified



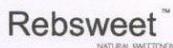
Note: This product should be stored sealed in a cool, dry place.

**Analyzed by:** (b) (6)  **Date:** 11/08/2013  
**Checked by:**  **Date:** 11/08/2013  
**Approved by:**  Quality Manager) **Date:** 11/08/2013

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### J-3 CERTIFICATE OF ANALYSIS FOR BATCH 20130808



## Certificate of Analysis



Research and Development  
 GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-59

**Product:** Rebsweet™ RA85  
**Lot Number:** GLG-RA85-20130808

**Manufacturing Date:** Aug. 9th, 2013  
**Country of Origin:** China  
**Shelf Life:** 2 years

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

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

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**Date of Analysis:** Aug. 14th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 85.0%	86.34%	JECFA HPLC
Total Steviol Glycosides	≥ 95.0%	96.64%	JECFA HPLC
pH	4.5 - 7.0	5.42	USP
Residue on Ignition	< 1.0%	0.07%	USP
Loss on Drying	≤ 4.0%	2.78%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.03mg/kg	AAS
Residual Solvent-ethanol	≤ 0.5%	0.021%	USP
--Methanol	≤ 0.02%	0.006%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion

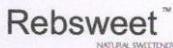
Note: This product should be stored sealed in a cool, dry place.

**Analyzed by:** (b) (6) **Date:** 14/08/2013  
**Checked by:** (b) (6) **Date:** 14/08/2013  
**Approved by:** (b) (6) (Quality Manager) **Date:** 14/08/2013

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## J-4 CERTIFICATE OF ANALYSIS FOR BATCH 20130812



### Certificate of Analysis



Research and Development  
 GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-59

**Product:** Rebsweet™ RA85      **Manufacturing Date:** Aug. 10th, 2013  
**Lot Number:** GLG-RA85-20130812      **Country of Origin:** China  
**Shelf Life:** 2 years

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

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

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**Date of Analysis:** Aug. 15th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 85.0%	86.23%	JECFA HPLC
Total Steviol Glycosides	≥ 95.0%	97.01%	JECFA HPLC
pH	4.5 - 7.0	5.50	USP
Residue on Ignition	< 1.0%	0.07%	USP
Loss on Drying	≤ 4.0%	2.96%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.04mg/kg	AAS
Residual Solvent-ethanol	≤ 0.5%	0.032%	USP
--Methanol	≤ 0.02%	0.004%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Qualified

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

**Analyzed by:** (b) (6)      **Date:** 15/08/2013  
**Checked by:** (b) (6)      **Date:** 15/08/2013  
**Approved by:** (b) (6) (Quality Manager)      **Date:** 15/08/2013

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## J-5 CERTIFICATE OF ANALYSIS FOR BATCH 20130816



### Certificate of Analysis



Research and Development  
 GLG Life Tech Corporation  
 www.glglifetech.com  
 GLG-QA-COA-59

**Product:** Rebsweet™ RA85  
**Lot Number:** GLG-RA85-20130816

**Manufacturing Date:** Aug. 14th, 2013  
**Country of Origin:** China  
**Shelf Life:** 2 years

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

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 Canada  
 Phone: 1.604.669.2602  
 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: +86.532.83181836

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

**Date of Analysis:** Aug. 19th, 2013

INSPECTION ITEM	SPECIFICATION	RESULT	METHOD
Appearance	White powder	White powder	Visual
Rebaudioside A	≥ 85.0%	86.52%	JECFA HPLC
Total Steviol Glycosides	≥ 95.0%	96.58%	JECFA HPLC
pH	4.5 - 7.0	5.53	USP
Residue on Ignition	< 1.0%	0.06%	USP
Loss on Drying	≤ 4.0%	2.60%	USP
Lead (Pb)	< 1mg/kg	0.01mg/kg	AAS
Arsenic (As)	< 1mg/kg	0.03mg/kg	AAS
Residual Solvent-ethanol	≤ 0.5%	0.041%	USP
--Methanol	≤ 0.02%	0.005%	USP
Total Plate Count	≤ 1,000 cfu/g	< 10cfu/g	FDA BAM
Yeast and Mold	≤ 100 cfu/g	< 10cfu/g	FDA BAM
E. Coli	Negative	Negative	FDA BAM
Salmonella	Negative	Negative	FDA BAM
Staphylococcus aureus	Negative	Negative	FDA BAM

Conclusion: Qualified 

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

**Analyzed by:** (b) (6) **Date:** 19/08/2013  
**Checked by:** (b) (6) **Date:** 19/08/2013  
**Approved by:** (b) (6) (Quality Manager) **Date:** 19/08/2013

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## **APPENDIX K**

### **Pesticide Analytical Reports from SGS-CSTC Standards Technical Services, Co. Ltd. Qingdao, China**

- K-1 Test Report for Pesticides GLG RA60 Lot 20130501
- K-2 Test Report for Pesticides GLG RA80 Lot 20130401

## K-1 TEST REPORT FOR PESTICIDES GLG RA60 LOT 20130501



**Test Report**                      **No.:** QDAFF130602465-4                      **Date:** Jun 21, 2013

Client name: Qingdao Runde Biotechnology Co., Ltd  
 Client address: Lingshanwei Town, Jiaonan City Qingdao, Shandong, China 266427

The following sample(s) was/were submitted by/ on behalf of the client as (except SGS reference No. & SGS job No. & Date of receipt & Testing period):

Code	SGS job No.	Sample name	Batch No./Date	Manufacture
#4	QDAFF13060224565-4	Steviol Glycosides	GLG-RA60 PLUS-20130501	Qingdao Runde Biotechnology Co., Ltd

SGS reference No.: TAOFD1301816601  
 Date of receipt: Jun 14, 2013  
 Testing period: Jun 14, 2013 ~ Jun 21, 2013

**TEST(S) REQUESTED:**  
 Selected test(s) as requested by applicant:  
 -- Pesticide Residues Quantification Content (194 items)

**TEST METHOD(S):**  
 US FDA PAM: 1999 Pesticide Analytical Manual, §64 LFGB L 00.00-34 (2010) Modular Multiple Analytical Method for the Determination of Pesticide Residues in Foodstuffs

**TEST RESULT(S):**  
 Please refer to next page.

- Remark:**
1. The results shown in this test report refer only to the sample(s) tested, and for clients internal use only.
  2. N = Not detected
  3. NR = Not recovered



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Test Report

No.: QDAFF130602465-4

Date: Jun 21, 2013

TEST RESULT(S):

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
1	2-phenyl-phenol 邻苯基苯酚	90-43-7	mg/kg	0.01	N
2	benalaxyl & benalaxyl-M 苯霜灵和精苯霜灵	71626-11-4&98243-83-5	mg/kg	0.01	N
3	Benfluralin 乙丁氟灵	1861-40-1	mg/kg	0.01	N
4	Bifenthrin 联苯菊酯	82657-04-3	mg/kg	0.01	N
5	Bromopropylate 溴螨酯	18181-80-1	mg/kg	0.01	N
6	Butachlor 丁草胺	23184-66-9	mg/kg	0.01	N
7	Cadusafos 硫线磷	95465-99-9	mg/kg	0.01	N
8	Captan 克菌丹	133-06-2	mg/kg	0.05	N
9	Chlordane 克氯丹	57-74-9	mg/kg	0.01	N
10	Chlorfenapyr 虫螨腈	122453-73-0	mg/kg	0.01	N
11	Chlorfenvinphos 毒虫畏	470-90-6	mg/kg	0.01	N
12	Chlorpropham 氯苯胺灵	101-21-3	mg/kg	0.01	N
13	Chlorpyrifos 毒死蜱	2921-88-2	mg/kg	0.01	N
14	Chlorpyrifos-methyl 甲基毒死蜱	5598-13-0	mg/kg	0.01	N
15	Cyfluthrin 氟氰菊酯	68359-37-5	mg/kg	0.01	N
16	Cyhalothrin & λ-Cyhalothrin 氯氟菊酯和高效氯氟菊酯	68085-85-8&91465-08-6	mg/kg	0.01	N
17	Cypermethrin & Z-Cypermethrin 氯氰菊酯和氯 氰菊酯 (Z)	52315-07-8	mg/kg	0.01	N
18	Cyprodinil 啉菌环胺	121552-61-2	mg/kg	0.01	N
19	Deltamethrin & Tralomethrin 溴氰菊酯和四溴菊酯	52918-63-5&66841-25-6	mg/kg	0.01	N
20	Diazinon 二嗪磷	333-41-5	mg/kg	0.01	N
21	Dicloran 氯硝胺	99-30-9	mg/kg	0.01	N
22	Dicofol 三氯杀螨醇	115-32-2	mg/kg	0.01	N

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Test Report

No.: QDAFF130602465-4

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
23	Dichlofluanid 抑菌灵	1085-98-9	mg/kg	0.05	N
24	Edifenphos 敌瘟磷	17109-49-8	mg/kg	0.01	N
25	Endosulfan-sulfate 硫丹硫酸酯	1031-07-8	mg/kg	0.01	N
26	α-endosulfan α-硫丹	959-98-8	mg/kg	0.01	N
27	β-endosulfan β-硫丹	33213-65-9	mg/kg	0.01	N
28	Ethion 乙硫磷	563-12-2	mg/kg	0.01	N
29	Ethoprophos 灭线磷	13194-48-4	mg/kg	0.01	N
30	Etofenprox 醚菊酯	80844-07-1	mg/kg	0.01	N
31	Etrimfos 乙啶硫磷	38260-54-7	mg/kg	0.01	N
32	Fenarimol 氟苯嘧啶磷	60168-88-9	mg/kg	0.01	N
33	Fenitrothion 杀螟硫磷/杀螟松	122-14-5	mg/kg	0.01	N
34	Fenpropathrin 甲氰菊酯	64257-84-7	mg/kg	0.01	N
35	Fenthion 倍硫磷	55-38-9	mg/kg	0.01	N
36	Fenvalerate & Esfenvalerate 氟戊菊酯和高效氟戊菊酯	51630-58-1&66230-04-4	mg/kg	0.01	N
37	Fipronil 氟虫腴	120068-37-3	mg/kg	0.01	N
38	Flucythrinate 氟氰戊菊酯	70124-77-5	mg/kg	0.01	N
39	Flusilazole 氟硅唑	85509-19-9	mg/kg	0.01	N
40	Tau-fluvalinate 氟胺氟菊酯	102851-06-9	mg/kg	0.05	N
41	HCH-γ 六六六/林丹-γ	58-89-9	mg/kg	0.01	N
42	Heptenophos 庚烯磷	23560-59-0	mg/kg	0.01	N
43	Isocarbophos 水胺硫磷	24353-61-5	mg/kg	0.01	N
44	Isofenphos 异柳磷	25311-71-1	mg/kg	0.01	N
45	Isofenphos-methyl 甲基异柳磷	99675-03-3	mg/kg	0.01	N
46	Myclobutanil 灭克落/腈菌唑	88671-89-0	mg/kg	0.01	N

Page 3 of 10

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Test Report

No.: QDAFF130602465-4

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
47	Napropamide 敌草胺	15299-99-7	mg/kg	0.01	N
48	Nitrothal-Isopropyl 酞菌酯	10552-74-6	mg/kg	0.01	N
49	o, p'-DDD o, p'-滴滴滴	53-19-0	mg/kg	0.01	N
50	o, p'-DDE o, p'-滴滴伊	3424-82-6	mg/kg	0.01	N
51	o, p'-DDT o, p'-滴滴涕	789-02-6	mg/kg	0.01	N
52	p, p'-DDD p, p'-滴滴滴	72-54-8	mg/kg	0.01	N
53	p, p'-DDE p, p'-滴滴伊	72-55-9	mg/kg	0.01	N
54	p, p'-DDT p, p'-滴滴涕	50-29-3	mg/kg	0.01	N
55	Paclobutrazol 多效唑	76738-62-0	mg/kg	0.01	N
56	Parathion 对硫磷	56-38-2	mg/kg	0.01	N
57	Parathion Methyl 甲基对硫磷	298-00-0	mg/kg	0.01	N
58	Penconazole 戊菌唑	66246-88-6	mg/kg	0.01	N
59	Pendimethalin 二甲戊乐灵	40487-42-1	mg/kg	0.01	N
60	Permethrin 氯菊酯	52645-53-1	mg/kg	0.01	N
61	Phenthoate 稻丰散	2597-03-7	mg/kg	0.01	N
62	Phorate 甲拌磷	298-02-2	mg/kg	0.01	N
63	Phorate-sulfoxide 甲拌磷亚砷	2588-03-6	mg/kg	0.05	N
64	Phorate-sulfone 甲拌磷砷	2588-04-7	mg/kg	0.05	N
65	Phosphamidone 磷胺	13171-21-6	mg/kg	0.03	N
66	Pirimiphos-Ethyl 嘧啶磷	23505-41-1	mg/kg	0.01	N
67	Pirimiphos-Methyl 甲基嘧啶磷	29232-93-7	mg/kg	0.01	N
68	Procymidone 腐霉利	32809-16-8	mg/kg	0.01	N
69	Profenophos 丙溴磷	41198-08-7	mg/kg	0.01	N
70	Prometryn 扑草净	7287-19-6	mg/kg	0.01	N

Page 4 of 10

RAND: 22031146

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Test Report

No.: QDAFF130602465-4

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
71	Propham 苯胺灵	122-42-9	mg/kg	0.01	N
72	Propiconazole 丙环唑	60207-90-1	mg/kg	0.01	N
73	Propyzamide 炔苯酰草胺	23950-58-5	mg/kg	0.01	N
74	Pyrazophos 吡菌磷	13457-18-6	mg/kg	0.01	N
75	Quinalphos 啶硫磷	13593-03-8	mg/kg	0.01	N
76	Quintozene 五氯硝基苯	82-68-8	mg/kg	0.01	N
77	S-421 八氯二苯醚	127-90-2	mg/kg	0.01	N
78	Tetrachlorvinphos 杀虫畏	22248-79-9	mg/kg	0.01	N
79	Tetradifon 三氯杀螨砜	116-29-0	mg/kg	0.01	N
80	Tolclofos-methyl 甲基立枯磷	57018-04-9	mg/kg	0.01	N
81	Tolyfluanid 甲苯氟磺胺	731-27-1	mg/kg	0.05	N
82	Triadimefon 三唑酮	43121-43-3	mg/kg	0.02	N
83	Triazophos 三唑磷	24017-47-8	mg/kg	0.01	N
84	Trifluralin 氟乐灵	1582-09-8	mg/kg	0.01	N
85	Vinclozoline 乙炔菌核利	50471-44-8	mg/kg	0.01	N
86	Famoxadone 噁唑菌酮	131807-57-3	mg/kg	0.05	N
87	HCH-α 六六六-α	319-84-6	mg/kg	0.01	N
88	HCH-β 六六六-β	319-85-7	mg/kg	0.01	N
89	BHC-δ 六六六-δ	319-86-8	mg/kg	0.01	N
90	Acephate 乙酰甲胺磷	30560-19-1	mg/kg	0.01	N
91	Acetamiprid 啉虫脲	135410-20-7	mg/kg	0.01	N
92	Acetochlor 乙草胺	34256-82-1	mg/kg	0.01	N
93	Aldicarb 涕灭威	116-06-3	mg/kg	0.01	N
94	Aldicarb-sulfoxid 涕灭威亚砜	1646-87-3	mg/kg	0.01	N

Page 5 of 10

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Test Report

No.: QDAFF130602465-4

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
95	Aldicarb-sulfone 涕灭威砒	1646-88-4	mg/kg	0.01	N
96	Atrazine 莠去津	1912-24-9	mg/kg	0.01	N
97	Azinphos-methyl 保棉磷	86-50-0	mg/kg	0.01	N
98	Azoxystrobin 嘧菌酯	131860-33-8	mg/kg	0.01	N
99	Bendiocarb 恶虫威	22781-23-3	mg/kg	0.01	N
100	Benfuracarb 丙硫克百威	82560-54-1	mg/kg	0.01	N
101	Benoxacor 解草嗪	98730-04-2	mg/kg	0.01	N
102	Bensulfuron-methyl 苄嘧磺隆	83055-99-6	mg/kg	0.01	N
103	Boscalid 啮酰菌胺	188425-85-6	mg/kg	0.01	N
104	Bupirimate 乙嘧酚磺酸酯	41483-43-6	mg/kg	0.01	N
105	Buprofezin 噻嗪酮	69327-76-0	mg/kg	0.01	N
106	Butocarboxim 丁酮威	034681-10-2	mg/kg	0.01	NR
107	Carbaryl 甲萘威	63-25-2	mg/kg	0.01	N
108	Carbendazim 多菌灵	10605-21-7	mg/kg	0.01	N
109	Carbofuran 虫螨威 (克百威)	1563-66-2	mg/kg	0.01	N
110	Carbofuran-3-hydroxy 3-羟基虫螨威	16655-82-6	mg/kg	0.01	N
111	Carbosulfan 丁硫克百威	55285-14-8	mg/kg	0.01	N
112	Chlorbenzuron 灭幼脲	57160-47-1	mg/kg	0.01	N
113	Clethodim 烯草酮	99129-21-2	mg/kg	0.01	NR
114	Clothianidin 噻虫胺/可尼丁	210880-92-5	mg/kg	0.01	N
115	Cyanazine 氰草津	21725-46-2	mg/kg	0.01	N
116	Cyflufenamid 环氟菌胺	180409-60-3	mg/kg	0.01	N
117	Cymoxanil 霜脲氰	57966-95-7	mg/kg	0.01	N
118	Cyromazine 灭蝇胺	66215-27-8	mg/kg	0.01	N

Page 6 of 10

RAND: 22031146

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Test Report

No.: QDAFF130602465-4

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
119	Dichlorvos 敌敌畏	62-73-7	mg/kg	0.01	N
120	Diethofencarb 乙霉威	87130-20-9	mg/kg	0.01	N
121	Difenoconazole 苯醚甲环唑	119446-68-3	mg/kg	0.01	N
122	Dimethoate 乐果	60-51-5	mg/kg	0.01	N
123	Dimethomorph 烯啶吗啉	110488-70-5	mg/kg	0.01	N
124	Diniconazole 烯啶醇	83657-24-3	mg/kg	0.01	N
125	Emamectin benzoate 甲胺基阿维菌素苯甲酸盐	155569-91-8	mg/kg	0.01	N
126	Ethiofencarb 乙硫甲威	29973-13-5	mg/kg	0.01	NR
127	Fenhexamid 环酰菌胺	126833-17-8	mg/kg	0.01	N
128	Fenobucarb 仲丁威	3766-81-2	mg/kg	0.01	N
129	Fenoxycarb 苯醚威	79127-80-3	mg/kg	0.01	N
130	Fenpropimorph 丁苯吗啉	67564-91-4	mg/kg	0.01	N
131	Fenpyroximate 啞螨酯	111812-58-9	mg/kg	0.01	N
132	Fluazifop-butyl & Fluazifop-p-butyl 吡氟禾草灵和 精吡氟禾草灵	69806-50-4&79241-46-6	mg/kg	0.01	N
133	Flufenoxuron 氟虫脲	101463-69-8	mg/kg	0.01	N
134	Furathiocarb 呋线威	65907-30-4	mg/kg	0.01	N
135	Hexythiazox 啞螨酮	78587-05-0	mg/kg	0.01	N
136	Imazalil 抑霉唑/烯菌灵	35554-44-0	mg/kg	0.01	N
137	Imidacloprid 吡虫啉	138261-41-3	mg/kg	0.01	N
138	Indoxacarb 茚虫威 (安打)	173584-44-6	mg/kg	0.01	N
139	Iprodione 异菌脲	36734-19-7	mg/kg	0.01	N
140	Iprovalicarb 丙森锌	140923-17-7	mg/kg	0.01	N
141	Isoprocarb 异丙威	2631-40-5	mg/kg	0.01	N

Page 7 of 10

RAND: 22031146

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QDFD



Test Report

No.: QDAFF130602465-4

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
142	Isoprothiolane 稻瘟灵	50512-35-1	mg/kg	0.01	N
143	Isoproturon 异丙隆	34123-59-6	mg/kg	0.01	N
144	Kresoxim-methyl 苯氧菊酯/亚胺菌	143390-89-0	mg/kg	0.01	N
145	Linuron 利谷隆	330-55-2	mg/kg	0.01	N
146	Malathion 马拉硫磷	121-75-5	mg/kg	0.01	N
147	Metalaxyl & Metalaxyl-M 甲霜灵和精甲霜灵	57837-19-1&70630-17-0	mg/kg	0.01	N
148	Metamitron 灭它通	41394-05-2	mg/kg	0.01	N
149	Methamidophos 甲胺磷	10265-92-6	mg/kg	0.01	N
150	Methidathion 杀扑磷	950-37-8	mg/kg	0.01	N
151	Methiocarb 灭虫威	2032-65-7	mg/kg	0.01	N
152	Methomyl 灭多威	16752-77-5	mg/kg	0.01	N
153	Methoxyfenozide 甲氧虫酰肼	161050-58-4	mg/kg	0.01	N
154	Metolachlor & S-Metolachlor 异丙甲草胺&精-异丙甲草胺	51218-45-2&87392-12-9	mg/kg	0.01	N
155	Mevinphos 速灭磷	7786-34-7	mg/kg	0.01	N
156	Monocrotophos 久效磷	6923-22-4	mg/kg	0.01	N
157	Nicosulfuron 烟嘧磺隆	111991-09-4	mg/kg	0.01	N
158	Omethoate 氧化乐果	1113-02-6	mg/kg	0.01	N
159	Oxadiazon 恶草酮	19666-30-9	mg/kg	0.01	N
160	Oxadixyl 恶霜灵	77732-09-3	mg/kg	0.01	N
161	Oxydemeton-methyl 砒吸磷	301-12-2	mg/kg	0.01	N
162	Phosalone 伏杀硫磷	2310-17-0	mg/kg	0.01	N
163	Phosmet 亚胺硫磷	732-11-6	mg/kg	0.01	N
164	Phoxim 辛硫磷	14816-18-3	mg/kg	0.01	N
165	Pirimicarb 抗蚜威	23103-98-2	mg/kg	0.01	N

Page 8 of 10

RAND: 22031146

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QDFD



Test Report

No.: QDAFF130602465-4

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
166	Prochloraz 咪鲜胺	67747-09-5	mg/kg	0.01	N
167	Promecarb 猛杀威	2631-37-0	mg/kg	0.01	N
168	Propamocarb 霜霉威	24579-73-5	mg/kg	0.01	N
169	Propargite 炔螨特	2312-35-8	mg/kg	0.01	N
170	Propoxur 残杀威	114-26-1	mg/kg	0.01	N
171	Pymetrozine 吡蚜酮/吡嗪酮	123312-89-0	mg/kg	0.01	N
172	Pyridaben 哒螨灵/哒螨酮	96489-71-3	mg/kg	0.01	N
173	Pyridaphenthion 哒嗪硫磷	119-12-0	mg/kg	0.01	N
174	Pyrimethanil 啞霉胺	53112-28-0	mg/kg	0.01	N
175	Quizalofop-ethyl & Quizalofop-p-ethyl 啞禾灵& 精啞禾灵	76578-14-8&100646-51-3	mg/kg	0.01	N
176	Rimsulfuron 虱嗪磺隆	122931-48-0	mg/kg	0.01	N
177	Simazine 西玛津	122-34-9	mg/kg	0.01	N
178	Spinosad 艾克敌(多杀菌素)	168316-95-8	mg/kg	0.01	N
179	Spiroxamine 螺环菌胺	118134-30-8	mg/kg	0.01	N
180	Tebuconazole 戊唑醇	107534-96-3	mg/kg	0.01	N
181	Tebufenozide 虫酰肼	112410-23-8	mg/kg	0.01	N
182	Thiabendazole 噻菌灵	148-79-8	mg/kg	0.01	N
183	Thiacloprid 噻虫啉	111988-49-9	mg/kg	0.01	N
184	Thiamethoxam 噻虫嗪	153719-23-4	mg/kg	0.01	N
185	Thifensulfuron-methyl 阔叶散	79277-27-3	mg/kg	0.01	N
186	Thiodicarb 硫双威	59669-26-0	mg/kg	0.01	N
187	Thiofanox-sulfon 久效威砒	39184-59-3	mg/kg	0.01	N
188	Thiofanox-sulfoxid 久效威亚砒	39184-27-5	mg/kg	0.01	N

Page 9 of 10

RAND: 22031146

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Q.D.F.D



Test Report

No.: QDAFF130602465-4

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
189	Triadimenol 三唑醇	55219-65-3	mg/kg	0.01	N
190	Triasulfuron 醚苯磺隆	82097-50-5	mg/kg	0.01	N
191	Trichlorphon 敌百虫	52-68-6	mg/kg	0.01	N
192	Triflumizole 氟菌唑	68694-11-1	mg/kg	0.01	N
193	Triflurosulfuron-methyl 氟胺磺隆	126535-15-7	mg/kg	0.01	N
194	Vamidotion 完灭硫磷	2275-23-2	mg/kg	0.01	N

Sample description: Sample in bag

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

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K-2 Test Report for Pesticides GLG RA80 Lot 20130401



**Test Report**                      **No.:** QDAFF130602465-1                      **Date:** Jun 21, 2013

Client name: Qingdao Runde Biotechnology Co., Ltd  
 Client address: Lingshanwei Town, Jiaonan City Qingdao, Shandong, China 266427

The following sample(s) was/were submitted by/ on behalf of the client as (except SGS reference No. & SGS job No. & Date of receipt & Testing period):

Code	SGS job No.	Sample name	Batch No./Date	Manufacture
#1	QDAFF130602465-1	Steviol Glycosides	GLG-RA80-20130401	Qingdao Runde Biotechnology Co., Ltd

SGS reference No.: TAOFD1301816601  
 Date of receipt: Jun 14, 2013  
 Testing period: Jun 14, 2013 ~ Jun 21, 2013

**TEST(S) REQUESTED:**

Selected test(s) as requested by applicant:  
 -- Pesticide Residues Quantification Content (194 items)

**TEST METHOD(S):**

US FDA PAM: 1999 Pesticide Analytical Manual, §64 LFGB L 00.00-34 (2010) Modular Multiple Analytical Method for the Determination of Pesticide Residues in Foodstuffs

**TEST RESULT(S):**

Please refer to next page.

**Remark:**

1. The results shown in this test report refer only to the sample(s) tested, and for clients internal use only.
2. N = Not detected
3. NR = Not recovered

Signed for and on behalf of SGS



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QDFD



Test Report

No.: QDAFF130602465-1

Date: Jun 21, 2013

TEST RESULT(S):

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
1	2-phenyl-phenol 邻苯基苯酚	90-43-7	mg/kg	0.01	N
2	benalaxyl & benalaxyl-M 苯霜灵和精苯霜灵	71626-11-4&98243-83-5	mg/kg	0.01	N
3	Benfluralin 乙丁氟灵	1861-40-1	mg/kg	0.01	N
4	Bifenthrin 联苯菊酯	82657-04-3	mg/kg	0.01	N
5	Bromopropylate 溴螨酯	18181-80-1	mg/kg	0.01	N
6	Butachlor 丁草胺	23184-66-9	mg/kg	0.01	N
7	Cadusafos 硫线磷	95465-99-9	mg/kg	0.01	N
8	Captan 克菌丹	133-06-2	mg/kg	0.05	N
9	Chlordane 克氯丹	57-74-9	mg/kg	0.01	N
10	Chlorfenapyr 虫螨腈	122453-73-0	mg/kg	0.01	N
11	Chlorfenvinphos 毒虫畏	470-90-6	mg/kg	0.01	N
12	Chlorpropham 氯苯胺灵	101-21-3	mg/kg	0.01	N
13	Chlorpyrifos 毒死蜱	2921-88-2	mg/kg	0.01	N
14	Chlorpyrifos-methyl 甲基毒死蜱	5598-13-0	mg/kg	0.01	N
15	Cyfluthrin 氟氯氰菊酯	68359-37-5	mg/kg	0.01	N
16	Cyhalothrin & λ-Cyhalothrin 氯氟氰菊酯和高效氯氟氰菊酯	68085-85-8&91465-08-6	mg/kg	0.01	N
17	Cypermethrin & Z-Cypermethrin 氯氰菊酯和氯 氰菊酯 (Z)	52315-07-8	mg/kg	0.01	N
18	Cyprodinil 啉菌环胺	121552-61-2	mg/kg	0.01	N
19	Deltamethrin & Tralomethrin 溴氰菊酯和四溴菊酯	52918-63-5&66841-25-6	mg/kg	0.01	N
20	Diazinon 二嗪磷	333-41-5	mg/kg	0.01	N
21	Dicloran 氯硝胺	99-30-9	mg/kg	0.01	N
22	Dicofol 三氯杀螨醇	115-32-2	mg/kg	0.01	N

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Test Report

No.: QDAFF130602465-1

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
23	Dichlofluaniid 抑菌灵	1085-98-9	mg/kg	0.05	N
24	Edifenphos 敌瘟磷	17109-49-8	mg/kg	0.01	N
25	Endosulfan-sulfate 硫丹硫酸酯	1031-07-8	mg/kg	0.01	N
26	α-endosulfan α-硫丹	959-98-8	mg/kg	0.01	N
27	β-endosulfan β-硫丹	33213-65-9	mg/kg	0.01	N
28	Ethion 乙硫磷	563-12-2	mg/kg	0.01	N
29	Ethoprophos 灭线磷	13194-48-4	mg/kg	0.01	N
30	Etofenprox 醚菊酯	80844-07-1	mg/kg	0.01	N
31	Etrimfos 乙啉硫磷	38260-54-7	mg/kg	0.01	N
32	Fenarimol 氯苯嘧啶磷	60168-88-9	mg/kg	0.01	N
33	Fenitrothion 杀螟硫磷/杀螟松	122-14-5	mg/kg	0.01	N
34	Fenpropathrin 甲氰菊酯	64257-84-7	mg/kg	0.01	N
35	Fenthion 倍硫磷	55-38-9	mg/kg	0.01	N
36	Fenvalerate & Esfenvalerate 氟戊菊酯和高效氟戊菊酯	51630-58-1&66230-04-4	mg/kg	0.01	N
37	Fipronil 氟虫腴	120068-37-3	mg/kg	0.01	N
38	Flucythrinate 氟氰戊菊酯	70124-77-5	mg/kg	0.01	N
39	Flusilazole 氟硅唑	85509-19-9	mg/kg	0.01	N
40	Tau-fluvalinate 氟胺氰菊酯	102851-06-9	mg/kg	0.05	N
41	HCH-γ 六六六/林丹-γ	58-89-9	mg/kg	0.01	N
42	Heptenophos 庚烯磷	23560-59-0	mg/kg	0.01	N
43	Isocarbophos 水胺硫磷	24353-61-5	mg/kg	0.01	N
44	Isofenphos 异柳磷	25311-71-1	mg/kg	0.01	N
45	Isofenphos-methyl 甲基异柳磷	99675-03-3	mg/kg	0.01	N
46	Myclobutanil 灭克落/腈菌唑	88671-89-0	mg/kg	0.01	N

Page 3 of 10

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QDFD



Test Report

No.: QDAFF130602465-1

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
47	Napropamide 敌草胺	15299-99-7	mg/kg	0.01	N
48	Nitrothal-Isopropyl 酞菌酯	10552-74-6	mg/kg	0.01	N
49	o, p'-DDD o, p'-滴滴滴	53-19-0	mg/kg	0.01	N
50	o, p'-DDE o, p'-滴滴伊	3424-82-6	mg/kg	0.01	N
51	o, p'-DDT o, p'-滴滴涕	789-02-6	mg/kg	0.01	N
52	p, p'-DDD p, p'-滴滴滴	72-54-8	mg/kg	0.01	N
53	p, p'-DDE p, p'-滴滴伊	72-55-9	mg/kg	0.01	N
54	p, p'-DDT p, p'-滴滴涕	50-29-3	mg/kg	0.01	N
55	Paclobutrazol 多效唑	76738-62-0	mg/kg	0.01	N
56	Parathion 对硫磷	56-38-2	mg/kg	0.01	N
57	Parathion Methyl 甲基对硫磷	298-00-0	mg/kg	0.01	N
58	Penconazole 戊菌唑	66246-88-6	mg/kg	0.01	N
59	Pendimethalin 二甲戊乐灵	40487-42-1	mg/kg	0.01	N
60	Permethrin 氯菊酯	52645-53-1	mg/kg	0.01	N
61	Phenthoate 稻丰散	2597-03-7	mg/kg	0.01	N
62	Phorate 甲拌磷	298-02-2	mg/kg	0.01	N
63	Phorate-sulfoxide 甲拌磷亚砷	2588-03-6	mg/kg	0.05	N
64	Phorate-sulfone 甲拌磷砷	2588-04-7	mg/kg	0.05	N
65	Phosphamidone 磷胺	13171-21-6	mg/kg	0.03	N
66	Pirimiphos-Ethyl 啞啞磷	23505-41-1	mg/kg	0.01	N
67	Pirimiphos-Methyl 甲基啞啞磷	29232-93-7	mg/kg	0.01	N
68	Procyimdone 腐霉利	32809-16-8	mg/kg	0.01	N
69	Profenophos 丙溴磷	41198-08-7	mg/kg	0.01	N
70	Prometryn 扑草净	7287-19-6	mg/kg	0.01	N

Page 4 of 10

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QDFD



Test Report

No.: QDAFF130602465-1

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
71	Propham 苯胺灵	122-42-9	mg/kg	0.01	N
72	Propiconazole 丙环唑	60207-90-1	mg/kg	0.01	N
73	Propyzamide 炔苯酰草胺	23950-58-5	mg/kg	0.01	N
74	Pyrazophos 吡菌磷	13457-18-6	mg/kg	0.01	N
75	Quinalphos 喹硫磷	13593-03-8	mg/kg	0.01	N
76	Quintozene 五氯硝基苯	82-68-8	mg/kg	0.01	N
77	S-421 八氯二苯醚	127-90-2	mg/kg	0.01	N
78	Tetrachlorvinphos 杀虫畏	22248-79-9	mg/kg	0.01	N
79	Tetradifon 三氯杀螨砜	116-29-0	mg/kg	0.01	N
80	Tolclofos-methyl 甲基立枯磷	57018-04-9	mg/kg	0.01	N
81	Tolyfluanid 甲苯氟磺胺	731-27-1	mg/kg	0.05	N
82	Triadimefon 三唑酮	43121-43-3	mg/kg	0.02	N
83	Triazophos 三唑磷	24017-47-8	mg/kg	0.01	N
84	Trifluralin 氟乐灵	1582-09-8	mg/kg	0.01	N
85	Vinclozoline 乙烯菌核利	50471-44-8	mg/kg	0.01	N
86	Famoxadone 噁唑菌酮	131807-57-3	mg/kg	0.05	N
87	HCH-α 六六六-α	319-84-6	mg/kg	0.01	N
88	HCH-β 六六六-β	319-85-7	mg/kg	0.01	N
89	BHC-δ 六六六-δ	319-86-8	mg/kg	0.01	N
90	Acephate 乙酰甲胺磷	30560-19-1	mg/kg	0.01	N
91	Acetamidrid 啉虫脲	135410-20-7	mg/kg	0.01	N
92	Acetochlor 乙草胺	34256-82-1	mg/kg	0.01	N
93	Aldicarb 涕灭威	116-06-3	mg/kg	0.01	N
94	Aldicarb-sulfoxid 涕灭威亚砷	1646-87-3	mg/kg	0.01	N

Page 5 of 10

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Test Report

No.: QDAFF130602465-1

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
95	Aldicarb-sulfone 涕灭威砒	1646-88-4	mg/kg	0.01	N
96	Atrazine 莠去津	1912-24-9	mg/kg	0.01	N
97	Azinphos-methyl 保棉磷	86-50-0	mg/kg	0.01	N
98	Azoxystrobin 啞菌酯	131860-33-8	mg/kg	0.01	N
99	Bendiocarb 悉虫威	22781-23-3	mg/kg	0.01	N
100	Benfuracarb 丙硫克百威	82560-54-1	mg/kg	0.01	N
101	Benoxacor 解草嗪	98730-04-2	mg/kg	0.01	N
102	Bensulfuron-methyl 苜嘧磺隆	83055-99-6	mg/kg	0.01	N
103	Boscalid 啞酰菌胺	188425-85-6	mg/kg	0.01	N
104	Bupirimate 乙啞酚磷酸酯	41483-43-6	mg/kg	0.01	N
105	Buprofezin 啞嗪酮	69327-76-0	mg/kg	0.01	N
106	Butocarboxim 丁酮威	034681-10-2	mg/kg	0.01	NR
107	Carbaryl 甲萘威	63-25-2	mg/kg	0.01	N
108	Carbendazim 多菌灵	10605-21-7	mg/kg	0.01	N
109	Carbofuran 虫螨威 (克百威)	1563-66-2	mg/kg	0.01	N
110	Carbofuran-3-hydroxy 3-羟基虫螨威	16655-82-6	mg/kg	0.01	N
111	Carbosulfan 丁硫克百威	55285-14-8	mg/kg	0.01	N
112	Chlorbenzuron 灭幼脲	57160-47-1	mg/kg	0.01	N
113	Clethodim 烯草酮	99129-21-2	mg/kg	0.01	NR
114	Clothianidin 啞虫胺/可尼丁	210880-92-5	mg/kg	0.01	N
115	Cyanazine 氰草津	21725-46-2	mg/kg	0.01	N
116	Cyflufenamid 环氟菌胺	180409-60-3	mg/kg	0.01	N
117	Cymoxanil 霜脲氰	57966-95-7	mg/kg	0.01	N
118	Cyromazine 灭蝇胺	66215-27-8	mg/kg	0.01	N

Page 6 of 10

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Test Report

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Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
119	Dichlorvos 敌敌畏	62-73-7	mg/kg	0.01	N
120	Diethofencarb 乙霉威	87130-20-9	mg/kg	0.01	N
121	Difenoconazole 苯醚甲环唑	119446-68-3	mg/kg	0.01	N
122	Dimethoate 乐果	60-51-5	mg/kg	0.01	N
123	Dimethomorph 烯酰吗啉	110488-70-5	mg/kg	0.01	N
124	Diniconazole 烯啶醇	83657-24-3	mg/kg	0.01	N
125	Emamectin benzoate 甲胺基阿维菌素苯甲酸盐	155569-91-8	mg/kg	0.01	N
126	Ethiofencarb 乙硫甲威	29973-13-5	mg/kg	0.01	NR
127	Fenhexamid 环酰菌胺	126833-17-8	mg/kg	0.01	N
128	Fenobucarb 仲丁威	3766-81-2	mg/kg	0.01	N
129	Fenoxycarb 苯醚威	79127-80-3	mg/kg	0.01	N
130	Fenpropimorph 丁苯吗啉	67564-91-4	mg/kg	0.01	N
131	Fenpyroximate 啞螨酯	111812-58-9	mg/kg	0.01	N
132	Fluazifop-butyl & Fluazifop-p-butyl 吡氟禾草灵和精吡氟禾草灵	69806-50-4&79241-46-6	mg/kg	0.01	N
133	Flufenoxuron 氟虫脲	101463-69-8	mg/kg	0.01	N
134	Furathiocarb 呋线威	65907-30-4	mg/kg	0.01	N
135	Hexythiazox 噻螨酮	78587-05-0	mg/kg	0.01	N
136	Imazalil 抑霉唑/烯菌灵	35554-44-0	mg/kg	0.01	N
137	Imidacloprid 吡虫啉	138261-41-3	mg/kg	0.01	N
138	Indoxacarb 茚虫威 (安打)	173584-44-6	mg/kg	0.01	N
139	Iprodione 异菌脲	36734-19-7	mg/kg	0.01	N
140	Iprovalicarb 丙森锌	140923-17-7	mg/kg	0.01	N
141	Isoprocarb 异丙威	2631-40-5	mg/kg	0.01	N

Page 7 of 10

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Test Report

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Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
142	Isoprothiolane 稻瘟灵	50512-35-1	mg/kg	0.01	N
143	Isoproturon 异丙隆	34123-59-6	mg/kg	0.01	N
144	Kresoxim-methyl 苯氧菊酯/亚胺菌	143390-89-0	mg/kg	0.01	N
145	Linuron 利谷隆	330-55-2	mg/kg	0.01	N
146	Malathion 马拉硫磷	121-75-5	mg/kg	0.01	N
147	Metalaxyl & Metalaxyl-M 甲霜灵和精甲霜灵	57837-19-1&70630-17-0	mg/kg	0.01	N
148	Metamitron 灭它通	41394-05-2	mg/kg	0.01	N
149	Methamidophos 甲胺磷	10265-92-6	mg/kg	0.01	N
150	Methidathion 杀扑磷	950-37-8	mg/kg	0.01	N
151	Methiocarb 灭虫威	2032-65-7	mg/kg	0.01	N
152	Methomyl 灭多威	16752-77-5	mg/kg	0.01	N
153	Methoxyfenozide 甲氧虫酰肼	161050-58-4	mg/kg	0.01	N
154	Metolachlor & S-Metolachlor 异丙甲草胺&精-异丙甲草胺	51218-45-2&87392-12-9	mg/kg	0.01	N
155	Mevinphos 速灭磷	7786-34-7	mg/kg	0.01	N
156	Monocrotophos 久效磷	6923-22-4	mg/kg	0.01	N
157	Nicosulfuron 烟嘧磺隆	111991-09-4	mg/kg	0.01	N
158	Omethoate 氧化乐果	1113-02-6	mg/kg	0.01	N
159	Oxadiazon 恶草酮	19666-30-9	mg/kg	0.01	N
160	Oxadixyl 恶霜灵	77732-09-3	mg/kg	0.01	N
161	Oxydemeton-methyl 虱吸磷	301-12-2	mg/kg	0.01	N
162	Phosalone 伏杀硫磷	2310-17-0	mg/kg	0.01	N
163	Phosmet 亚胺硫磷	732-11-6	mg/kg	0.01	N
164	Phoxim 辛硫磷	14816-18-3	mg/kg	0.01	N
165	Pirimicarb 抗蚜威	23103-98-2	mg/kg	0.01	N

Page 8 of 10

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Test Report

No.: QDAFF130602465-1

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
166	Prochloraz 咪鲜胺	67747-09-5	mg/kg	0.01	N
167	Promecarb 猛杀威	2631-37-0	mg/kg	0.01	N
168	Propamocarb 霜霉威	24579-73-5	mg/kg	0.01	N
169	Propargite 炔螨特	2312-35-8	mg/kg	0.01	N
170	Propoxur 残杀威	114-26-1	mg/kg	0.01	N
171	Pymetrozine 吡蚜酮/吡嗪酮	123312-89-0	mg/kg	0.01	N
172	Pyridaben 哒螨灵/哒螨酮	96489-71-3	mg/kg	0.01	N
173	Pyridaphenthion 哒嗪硫磷	119-12-0	mg/kg	0.01	N
174	Pyrimethanil 啞霉胺	53112-28-0	mg/kg	0.01	N
175	Quizalofop-ethyl & Quizalofop-p-ethyl 啞禾灵 & 精啞禾灵	76578-14-8&100646-51-3	mg/kg	0.01	N
176	Rimsulfuron 啞啞磺隆	122931-48-0	mg/kg	0.01	N
177	Simazine 西玛津	122-34-9	mg/kg	0.01	N
178	Spinosad 艾克敌(多杀菌素)	168316-95-8	mg/kg	0.01	N
179	Spiroxamine 螺环菌胺	118134-30-8	mg/kg	0.01	N
180	Tebuconazole 戊唑醇	107534-96-3	mg/kg	0.01	N
181	Tebufenozide 虫酰肼	112410-23-8	mg/kg	0.01	N
182	Thiabendazole 噻菌灵	148-79-8	mg/kg	0.01	N
183	Thiacloprid 噻虫啉	111988-49-9	mg/kg	0.01	N
184	Thiamethoxam 噻虫嗪	153719-23-4	mg/kg	0.01	N
185	Thifensulfuron-methyl 阔叶散	79277-27-3	mg/kg	0.01	N
186	Thiodicarb 硫双威	59669-26-0	mg/kg	0.01	N
187	Thiofanox-sulfon 久效威砒	39184-59-3	mg/kg	0.01	N
188	Thiofanox-sulfoxid 久效威亚砒	39184-27-5	mg/kg	0.01	N

Page 9 of 10

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Test Report

No.: QDAFF130602465-1

Date: Jun 21, 2013

No.	Test Item(s)	CAS	Unit(s)	Method detected limit(s)	Test result(s)
189	Triadimenol 三唑醇	55219-65-3	mg/kg	0.01	N
190	Triasulfuron 醚苯磺隆	82097-50-5	mg/kg	0.01	N
191	Trichlorphon 敌百虫	52-68-6	mg/kg	0.01	N
192	Triflumizole 氟菌唑	68694-11-1	mg/kg	0.01	N
193	Triflusalufuron-methyl 氟胺磺隆	126535-15-7	mg/kg	0.01	N
194	Vamidotion 完灭硫磷	2275-23-2	mg/kg	0.01	N

Sample description: Sample in bag

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

Page 10 of 10

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## APPENDIX L

### Summary of Regulatory & Expert Body Safety Reviews

#### 1. Summary of JECFA Reviews

At an early review at its 51<sup>st</sup> meeting, JECFA (WHO, 2000) expressed the following reservations about the safety data available at that time for steviol glycosides:

The Committee noted several shortcomings in the information available on stevioside. In some studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was 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 63<sup>rd</sup> meeting (WHO, 2006):

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

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

The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg/kg bw was established for

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

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

In 2007, at its 68<sup>th</sup> meeting, JECFA (WHO, 2007) concluded that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008. Subsequently, sufficient data had been received by JECFA to revise and finalize food additive specifications for steviol glycosides (FAO, 2007a). The Chemical and Technical Assessment report, written after the 2007 meeting, explained the Committee's thinking, which resulted in flexibility in the identity specifications (FAO, 2007b).

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

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

In 2008, based on additional clinical studies, at its 69<sup>th</sup> meeting, JECFA finalized the evaluation of steviol glycosides (WHO, 2008), 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 95<sup>th</sup> percentile. A correction was considered to be necessary for the consumption of non-alcoholic flavored drinks (soft drinks) by children, and the corrected exposure estimate at the 95<sup>th</sup> percentile for children ranged from 1.0 to 12.7 mg/kg bw/day. For adults, the mean and 97.5<sup>th</sup> percentile intakes were estimated to range from 1.9 to 2.3 and 5.6 to 6.8 mg/kg bw/day, respectively. Non-alcoholic flavored drinks (soft drinks) are the main contributors to the total anticipated exposure to steviol glycosides for both consumer categories. For high consumers, EFSA noted that revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg/kg bw (steviol equivalent).

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.

## APPENDIX M

### 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 in the following sections.

#### Acute Toxicity Studies

The oral LD<sub>50</sub> 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<sub>50</sub> 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 (Toskulkaio *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 M-1.

**Table M-1. Mutagenicity & Genotoxicity Studies on Steviol**

	<i>In Vivo/In Vitro</i>	SYSTEM	TEST SAMPLE PURITY	AUTHOR CONCLUSION	RESULTS AND REMARKS
Sekihashi et al., 2002 <sup>a</sup>	<i>In Vivo/In Vitro</i>	Comet Assay	Not reported	Negative	In <i>in vitro</i> study, steviol at 62.5, 125, 250 and 500 µg/ml did not damage DNA of TK6 and WTK1 cells in presence or absence of S9 mix. In <i>in vivo</i> study, mice sacrificed 3 or 24 hours after one-time oral administration of 250, 500, 1000 or 2000 mg/kg of steviol. Stomach, colon, kidneys, testis and liver DNA not damaged. An identical <i>in vivo</i> experiment with stevia extract performed, which also gave negative results.
Oh et al., 1999 <sup>b</sup>	<i>In Vivo?</i>	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 <sup>c</sup>	<i>In Vivo?</i>	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 <sup>a</sup>	<i>In Vitro</i>	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 <sup>c</sup>	<i>In Vitro</i>	Bacterial Mutagenicity	Not Reported	Positive	Mutagenic effects of steviol and/or metabolites found in <i>S.typhimurium</i> TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene. Magnitude of increase of these mutations over the control not reported.
Klongpanichpak et al., 1997 <sup>c</sup>	<i>In Vitro</i>	Bacterial Mutagenicity	Not Reported	Negative	Steviol and stevioside inactive in TA strains of <i>S. typhimurium</i> , <i>e. coli</i> WP2, <i>uvrA/PKM101</i> and rec assay using <i>B. subtilis</i> even when microsomal activated fraction present. Magnitude of increase of these mutations over the control not reported.
Matsui et al., 1996 <sup>a</sup>	<i>In Vitro</i>	Bacterial Mutagenicity	Not Reported	Negative	Testing of Southern Blot technique with probe for gpt gene DNA of <i>E. coli</i> . The chromosomal DNA of TM677 and steviol-induced TM677 mutants digested by restriction enzymes and probed. No significant differences found in fragment length between wild-type and mutant DNA.
Matsui et al., 1996 <sup>a</sup>	<i>In Vitro</i>	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 <sup>c</sup>	<i>In Vitro</i>	Bacterial Mutagenicity	Not Reported	Negative	The direct mutagenic activity of 15-oxo-steviol was refuted.

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

<sup>a</sup> Abstract only. <sup>b</sup> As reported in WHO, 2006. <sup>c</sup> As reviewed by Geuns, 2003. <sup>d</sup> Full article.

## APPENDIX N

### Studies on Steviol Glycosides Preparations that Are Primarily Stevioside

This section 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  $\alpha$ -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  $\alpha$ -glucosylated steviol glycosides follows that of non-modified steviol glycosides. Due to the similarities in metabolic fate, the safety of  $\alpha$ -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.

## 2. Acute Toxicity Studies

The oral LD<sub>50</sub> studies of stevioside (purity, 96%) following administration of a single dose to rodents are summarized in Table N-1. 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 N-1. Acute Toxicity of Stevioside (Purity 96%) Given Orally to Rodents**

SPECIES	SEX	LD <sub>50</sub> (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<sub>0</sub>, F<sub>1</sub>, and F<sub>2</sub> generations. These studies are summarized in Table N-2. 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.<sup>1</sup> 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

<sup>1</sup> A thorough review of the subject study is provided in Appendix E.

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 suggest 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 suggests that differences noted in hematological and chemistry data are probably random, nonspecific, and not toxicologically significant.

**Table N-2. 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 <sup>a</sup>	F344 rat/ 10 females and 10 males in each of 6 groups	Stevioside/ Not reported	0, 0.31, 0.62, 1.25, 2.5, 5% in diet/13 weeks	Not reported	No effects observed on mortality, body weight or food consumption. Clinical chemistry investigation revealed increased LDH levels and histopathological investigation indicated increased incidence of single-cell liver necrosis in all male treated groups, but not in a clear dose-response relationship. Investigators did not consider these changes to be treatment related due to the small magnitude and low severity of changes, the lack of a clear dose relationship and the limitation to males only. Organ weights, urine chemistry and gross necropsy not discussed. Authors concluded that 5% stevioside in diet is a tolerable dose for a 2 year study.
Yodyingyuad and Bunyawong, 1991 <sup>a</sup>	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 <sub>0</sub> , F <sub>1</sub> and F <sub>2</sub> generations in reproductive study were dosed for 90 days. Histological examination showed no effect at any dose. Weights of organs, blood analysis, urine chemistry and gross necropsy not discussed. The F <sub>1</sub> and F <sub>2</sub> hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents).
Mitsubishi, 1976 <sup>b</sup>	Rat (strain not reported)	Stevioside/ Not reported	Dietary concentrations up to 7%/ 3 months	Not reported	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy and histopathology not discussed.
Akashi and Yokoyama, 1975 <sup>b</sup>	Rat (strain not reported)	Stevioside/ Not reported	Oral doses up to 2500 mg/kg bw/3 months	2500	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy and histopathology not discussed.
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 the hematological parameters, significant changes were noted in all except WBCs, RBCs, and PCV% and in all clinical chemistry parameters except proteins, total lipids, ATL and AST.

<sup>a</sup> Abstract only. <sup>b</sup> As reported by Geuns, 2003.

#### 4. Chronic Toxicity Studies

Chronic effects of stevioside have been studied in three separate studies (Table N-3). 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 N-3. Summary of Chronic Toxicity Studies on Stevioside**

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

<sup>a</sup> 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; Schwartzman et al., 1977). In experimental studies in rats, crude stevia leaf extract has been shown to inhibit fertility (Planas and Kuc, 1968). Reproductive toxicity studies have been conducted with orally administered purified stevioside as tabulated in Table 15. 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 (Yodyingyud 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 N-4.

**Table N-4. Summary of Reproductive Toxicity Studies on Steviol Glycosides**

STUDY	ANIMAL MODEL/ GROUP SIZE	TEST SAMPLE PURITY STEVIOSIDE (UNLESS OTHERWISE NOTED)	DOSES / DURATION	AUTHOR ASSIGNED NOAEL (mg/kg bw/day)	RESULTS AND REMARKS
Kumar and Oommen, 2008	Swiss albino mice/ 4 groups of 5 females	Not reported	500 and 800 mg/kg bw/15 days	800	Stevioside and stevia extract (purity and composition not reported) did not have any effect on reproductive parameters in mice when administered to female mice before or during pregnancy. No changes seen in number of implantations or uterine resorptions. No gross anatomical or histopathologic effects seen in 16-day embryos.
Usami et al., 1995 <sup>a</sup>	Wistar Rat/4 groups of 25 or 26 pregnant rats	95.6% <sup>b</sup>	0, 250, 500, 1000 mg/kg bw/10 days	1000	Pregnant rats given doses of stevioside by gavage once a day on days 6-15 of gestation and were sacrificed on day 20 of gestation. Fetuses were examined for malformations in addition to maternal and fetal body weight, number of live fetuses, sex distribution, and numbers of resorptions or dead fetuses. No treatment-related effects observed. Authors concluded that orally administered stevioside is not teratogenic in rats.
Yodyingyuad and Bunyawong, 1991	Hamster/ 10 male, 10 female per group (40 total)	90%	0, 500, 1000, 2500 mg/kg bw/day/ duration unclear/ 3 months	2500	Males from each group were mated to females from respective dose group. Each female was allowed to bear 3 litters during the course of experiment. Stevioside had no effect on pregnancies of females at any dose. The F <sub>1</sub> and F <sub>2</sub> hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents); showed normal growth and fertility. Histological examination showed no effect on reproductive organs at any dose.

Oliveira-Filho et al., 1989 <sup>a</sup>	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 <sup>3</sup> H-methyltrienolone (a specific ligand of androgen receptors) to prostate cytosol; zinc content of prostate, testis, submandibular salivary gland, and pancreas; water content of testes and prostate; body-weight gain; and final weights of testes, prostate, seminal vesicle, submandibular salivary gland, and adrenal. Only difference due to treatment was seminal vesicle weight, which fell to 60% compared to control.
Mori et al., 1981	Rat/11 male, 11 female per group (44 total)	96%	0, 0.15, 0.75 or 3 % of feed/60 days	2000	Males given stevioside dose in diet for 60 days before and during mating with females who received same diet (as mated male) 14 days before mating and 7 days during gestation. No effect due to treatment on fertility or mating performance, and no effect of fetal development. Rats of each sex had slightly decreased body weight gain at highest dose with non-significant increase in number of dead and resorbed fetuses at highest dose.
Planas and Kuc, 1968 <sup>c</sup>	Rat/14 per group (28 total)	Not reported (Crude stevia extract)	0 or 5% Crude stevia extract /18 days	Not reported	Extract given orally to adult female rats for 12 days, who were mated with untreated males during the last 6 days. Fertility reduced to 21% of fertility in control rats and remained reduced in a 50-60 day recovery. Histological examination, weights of organs, blood analysis, urine chemistry and gross necropsy not discussed.

<sup>a</sup> Only abstract available. <sup>b</sup> 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 N-5. 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 was reviewed and considered adequate to support the safety of its use as a sweetener in foods (Urban et al., 2013).

**Table N-5. Mutagenicity & Genotoxicity Studies on Stevia Extracts & Stevioside**

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCENTRATION / DOSE	RESULT	REFERENCE
<i>In Vitro</i>						
Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Stevioside	83	5 mg/plate <sup>a</sup> 1 mg/plate <sup>b</sup>	Negative	Matsui et al. (1996)
Reverse mutation	<i>S. typhimurium</i> TA98, TA100	Stevioside	99	50 mg/plate	Negative <sup>c</sup>	Suttajit et al. (1993)
Reverse mutation	<i>S. typhimurium</i> TA98, TA100	Stevioside	NS	50 mg/plate	Negative	Klongpanichpak et al (1997)
Forward mutation	<i>S. typhimurium</i> TM677	Stevioside	83	10 mg/plate	Negative <sup>c</sup>	Matsui et al. (1996)
Forward mutation	<i>S. typhimurium</i> TM677	Stevioside	NS	10 mg/plate	Negative <sup>c</sup>	Pezzuto et al. (1985)
Forward mutation	<i>S. typhimurium</i> TM677	Stevioside	NS	Not specified	Negative <sup>c</sup>	Medon et al. (1982)
Gene mutation	Mouse lymphoma L5178Y cells, TK- locus	Stevioside	NS	5 mg/mL	Negative <sup>c,d</sup>	Oh et al. (1999)
Gene mutation (umu)	<i>S. typhimurium</i> TA1535/pSK1002	Stevioside	83	5 mg/plate	Negative <sup>c</sup>	Matsui et al. (1996)
Gene mutation	<i>B. subtilis</i> H17 rec+, M45 rec-	Stevioside	83	10 mg/disk	Negative <sup>c</sup>	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 <sup>a</sup>	Ishidate et al. (1984)
<i>In Vivo</i>						
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 <sup>e</sup>	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 <sup>e</sup>	Sasaki et al. (2002)
Micronucleus formation	ddY mouse bone marrow and regenerating liver	Stevioside	NS	62.5 - 250 mg/kg bw	Negative	Oh et al. (1999)
Mutation	<i>D. melanogaster</i> Muller 5 strain	Stevioside	NS	2% in feed	Negative	Kerr et al. (1983)

NS = Not specified. <sup>a</sup> Without metabolic activation. <sup>b</sup> As calculated by Williams, 2007. <sup>c</sup> With and without metabolic activation (source not specified in original monograph). <sup>d</sup> Inadequate detail available. <sup>e</sup> 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 II diabetes. These effects were key concerns for JECFA. In 2006, JECFA summarized the available clinical studies of stevioside and further studies were recommended (WHO, 2006). Subsequently, several studies were conducted, and in 2009, JECFA reviewed these new studies (WHO, 2009). JECFA's summaries of the key studies are included below.

### a. Studies Summarized in 2006

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

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

In a follow-up multi-center randomized, double-blind, placebo-controlled trial was conducted in hypertensive Chinese men and women (aged 20–75 years), 85 patients were given capsules containing 500 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 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 ( $73 \pm 2\%$ ), rebaudioside A ( $24 \pm 2\%$ ), 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 II diabetes were given either 1 g of stevioside (stevioside, 91%; other stevia glycosides, 9%) or 1 g of maize starch (control group), which was taken with a standard carbohydrate-rich test meal. Blood samples were drawn at 30 minutes before, and for 240 minutes after, ingestion of the test meal. Stevioside reduced postprandial blood glucose concentrations by an average of 18% and increased the insulinogenic index by an average of 40%, indicating beneficial effects on glucose metabolism. Insulin secretion was not significantly increased. No hypoglycemic or adverse effects were reported by the patients or observed by the investigators. Systolic and diastolic blood pressure was not altered by stevioside administration (Gregersen et al., 2004).

#### **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 68<sup>th</sup> 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).

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 O

### Studies on Rebaudioside A

#### Safety Data on Rebaudioside A<sup>2</sup>

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, two studies found in a recent literature search examined the anti-hyperglycemic activity of rebaudioside A in diabetic rats (Saravanan et al., 2012; Saravanan and Ramachandran, 2013). 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. In the second study, the administration of 200 mg/kg bw reb A to diabetic rats normalized levels of plasma glucose, insulin, lipid peroxidation products, enzymatic, non-enzymatic antioxidants and lipids (Saravanan and Ramachandran, 2013). The doses used are 10-40 times higher than expected from the use of rebaudioside A as a sweetener (Saravanan et al., 2012).

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

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<sup>2</sup> Questions about the safety of rebaudioside A were previously raised by Huxtable (2002), and Kobylewski and Eckhert (2008). Their respective concerns, as well as opposing views supporting the safety of designated food uses of rebaudioside A expressed by Expert Panels, have been outlined in other GRAS notifications that were submitted to FDA. A more detailed account can be found in GRAS notifications 278, 287, 303, and 304. This matter is discussed by the Expert Panel in Section VI.C.

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) 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 ( $\sim 22\%$ ) 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  $\mu\text{g/mL}$ , 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  $\geq 2,000$  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  $\geq 2,000$  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 O-1.

**Table O-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 <i>E. coli</i> 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)
Chromosome 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)

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCENTRATION / DOSE	RESULT	REFERENCE
Unscheduled DNA Synthesis	Unscheduled DNA synthesis in one group of 4 Wistar rats	Reb A	95.6	Up to 2000 mg/kg bw	No increase in unscheduled DNA synthesis	Williams and Burdock (2009)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevioside, 52%; Reb A, 22%	250 - 2000 mg/kg bw	Negative <sup>a</sup>	Sekihashi et al. (2002)
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Reb A	NS	1.2 - 55 mg/mL	Negative <sup>b</sup>	Nakajima (2000a)
Micronucleus formation	BDF1 mouse bone marrow	Reb A	NS	500-2000 mg/kg bw/ day for 2 days	Negative <sup>c</sup>	Nakajima (2000b)
Forward mutation	<i>S. typhimurium</i> TM677	Reb A	NS	10 mg/plate	Negative <sup>b</sup>	Pezzuto et al. (1985)

NS = Not specified.

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

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

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

#### 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<sub>0</sub> or F<sub>1</sub> generations. The survival and general condition of the F<sub>1</sub> and F<sub>2</sub> offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by rebaudioside A treatment. The NOAEL for reproductive effects was 25,000 ppm, and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm, or 2,048 to 2273 mg/kg body weight/day (the highest dose tested).

The results from two unpublished studies with rebaudioside A (Sloter 2008a,b) further support the above described findings from published studies. In a two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were fed either basal diet or the diet containing rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter 2008a). For the F<sub>0</sub> and F<sub>1</sub> generations, rebaudioside A doses were 0, 500, 1,000, and 2,000 mg/kg/day. At initiation of study, F<sub>0</sub> animals were approximately 7 weeks of age. The test diet was offered to the offspring selected to become the F<sub>1</sub> generation following weaning [beginning on postnatal day (PND) 21]. The F<sub>0</sub> and F<sub>1</sub> males continued to receive rebaudioside A

throughout mating, continuing through the day of euthanasia. The F<sub>0</sub> and F<sub>1</sub> females continued to receive rebaudioside A throughout mating, gestation and lactation until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females as evaluated by estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints. Both for parental systemic and reproductive toxicity, a dose level  $\geq 2,000$  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  $\geq 2,000$  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 ( $0.11 \pm 0.06\%$ , mean  $\pm$  standard error) and placebo ( $0.09 \pm 0.05\%$ ; p = 0.355) groups. Similarly, no significant (p > 0.05 for all) changes from baseline for rebaudioside A and placebo, respectively, in fasting glucose ( $7.5 \pm 3.7$  mg/dL and  $11.2 \pm 4.5$  mg/dL), insulin ( $1.0 \pm 0.64$   $\mu$ U/mL and  $3.3 \pm 1.5$   $\mu$ U/mL), and Cpeptide ( $0.13 \pm 0.09$  ng/mL and  $0.42 \pm 0.14$  ng/mL) were noted. No treatment related changes in blood pressure, body weight, and fasting lipids were noted. Rebaudioside A was well-tolerated, and records of hypoglycemic episodes showed no excess versus placebo. Based on these results, the investigators suggested that chronic use of 1,000 mg/person/day rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

**SUBMISSION END**