GRAS ASSOCIATES, LLC

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858

April 24, 2019

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-255)
5001 Campus Drive
College Park, MD 20740-3835

Attention: Dr. Paulette Gaynor
Re: GRAS Notification – High Purity Glucosylated Steviol Glycosides

Dear Dr. Gaynor:

GRAS Associates, LLC, acting as the Agent for Qufu Shengren Pharmaceutical Co., Ltd. ("Qufu", People's Republic of China), Sunwin Stevia International ("Sunwin", USA), and NuNaturals, Inc. ("NuNaturals", USA), is submitting for FDA review Form 3667 and the enclosed CD, free of viruses, containing a GRAS Notification for *High Purity Glucosylated Steviol Glycosides.* Along with Qufu, Sunwin, and NuNaturals's determination of safety, an Expert Panel of qualified persons was assembled to assess the composite safety information of the subject substance with the intended use as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into food in general, other than infant formulas and meat and poultry products. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

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

We look forward to your feedback.

Sincerely,

William J. Rowe, President
Agent for Blue California
GRAS Associates, LLC
27499 Riverview Center Blvd., Suite 212
Bonita Springs, FL 34134
wrowe@nutrasource.ca

Enclosure: GRAS Notification for Qufu, Sunwin, & NuNaturals –*High Purity Glucosylated Steviol Glycosides*

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
000858	4/30/2019
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – 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)*
3. Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: N/A
4. Are you submitting an amendment or supplement submitted in response to a communication from FDA? ☐ Yes ☐ No (If yes, enter the date of communication *(yyyy/mm/dd)*: _____)

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Simon Yi	Vice President

Organization *(if applicable)*: Qufu Shengren Pharmaceutical Co., Ltd

Mailing Address *(number and street)*: No. 6 Shengwang Ave.

City	State or Province	Zip Code/Postal Code	Country
Qufu	Shandong	273100	China

Telephone Number	Fax Number	E-Mail Address
0086 537 4913739	0086 537 4911573	simon@sunwinstevia.com

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

Name of Contact Person	Position or Title
William J. Rowe	President and CEO

Organization *(if applicable)*: GRAS Associates

Mailing Address *(number and street)*: 27499 Riverview Center Blvd., Suite 212

City	State or Province	Zip Code/Postal Code	Country
Bonita Springs	Florida	34134	United States of America

Telephone Number	Fax Number	E-Mail Address
239-444-1724	239-444-1723	wrowe@nutrasource.ca

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

TasteRight High Purity Glucosylated Steviol Glycosides; Enzyme Treated Stevia; Refined Enzyme Treated Stevia

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

☐ Electronic Submission Gateway ☒ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

Number of volumes: _____

Total number of pages: _____

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

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

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

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS 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 conclusions of GRAS status *(Check one)*

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

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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, 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 [illegible] of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

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 and principles, in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☑ Yes ☐ No, you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

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

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Qufu Shengren Pharmaceutical Co., Ltd, Sunwin Stevia International, and NuNaturals, Inc.
(name of notifier)

has concluded that the intended use*(s)* of TasteRight High Purity Glucosylated Steviol Glycosides; Enzyme Treated Stevia; Refined Enzym
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Sunwin Stevia International agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; 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; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

431 Fairway Drive #251, Deerfield Beach, FL, 33441, USA
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance. The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
[redacted]	Katrina Emmel on behalf of William J. Rowe, President	04/24/2019

SECTION G – LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Multiple Appendices – Appendices 1 through 15	

GRAS Notification

of

High Purity Glucosylated Steviol Glycosides

Food Usage Conditions for General Recognition of Safety

on behalf of

Qufu Shengren Pharmaceutical Co., Ltd.
Sunwin Group
Shandong, People's Republic of China
&
Sunwin Stevia International,
Deerfield, FL
&
NuNaturals, Inc.
Eugene, OR

4/25/19

TABLE OF CONTENTS

FOREWORD 5
PART 1. SIGNED STATEMENTS AND CERTIFICATION 5
A. Basis of Exclusion from the Requirement for Premarket Approval Pursuant to Subpart E of 170 5
B. Name and Address of Responsible Parties 6
C. Common Name and Identity of Notified Substance 6
D. Conditions of Intended Use in Food 7
E. Basis for GRAS Conclusion 7
F. Availability of Information 7
PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT 7
A. Chemical Identity of Ingredient 7
B. Manufacturing Processes 11
C. Product Specifications 13
1. JECFA Specifications for Steviol Glycosides 13
2. Specifications for Qufu, Sunwin, and NuNaturals's TasteRight High Purity Glucosylated Steviol Glycosides Preparations and Supporting Methods 15
D. Physical or Technical Effect 18
1. Stability Data on Steviol Glycosides 18
2. Stability Data for Qufu, Sunwin, and NuNaturals's TasteRight Enzyme Treated Stevia Preparations 19
3. Stability Data for Qufu, Sunwin, and NuNaturals's TasteRight Refined Enzyme Treated Stevia Preparations 22
E. Calculation of Steviol Equivalents of TasteRight Glucosylated Steviol Glycosides 25
PART 3. DIETARY EXPOSURE 26
A. Estimate of Dietary Exposure to the Substance 26
B. Estimated Dietary Exposure to Any Other Substance That is Expected to be Formed In or On Food 28
C. Dietary Exposure to Contaminants or Byproducts 28
PART 4. SELF-LIMITING LEVELS OF USE 28
PART 5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958 29
A. Other Information on Dietary Exposure 29
1. History of Traditional Medicinal and Human Food Use 29
B. Summary of Regulatory History of Enzyme Modified Steviol Glycosides 29
1. U.S. Regulatory History 29
2. Canadian Regulatory History 30
3. European Regulatory History 30
4. Asian Regulatory History 31
5. Other Regulatory History 31
PART 6. NARRATIVE 31
A. GRAS Criteria 36
B. Expert Panel Findings on Safety of TasteRight Preparations 38
C. Common Knowledge Elements for GRAS Conclusions 38
1. Public Availability of Scientific Information 38
2. Scientific Consensus 39
D. Conclusion 40
PART 7. LIST OF SUPPORTING DATA AND INFORMATION IN THE GRAS NOTICE 40
A. References 40
B. Appendices 51
APPENDIX 1 SPECIFICATIONS AND CERTIFICATES OF ANALYSES FOR RAW MATERIALS AND PRODUCTION PROCESSING AIDS 52
APPENDIX 2 ANALYTICAL METHOD 79
APPENDIX 3 CERTIFICATES OF ANALYSIS FOR MULTIPLE BATCHES OF TASTERIGHT ENZYME TREATED STEVIA 88

APPENDIX 4 CERTIFICATES OF ANALYSIS FOR MULTIPLE BATCHES OF TASTERIGHT REFINED ENZYME TREATED STEVIA 94
APPENDIX 5 ANALYTICAL CHROMATOGRAMS FOR MULTIPLE PRODUCTION BATCHES OF TASTERIGHT ENZYME TREATED STEVIA 100
APPENDIX 6 ANALYTICAL CHROMATOGRAMS FOR MULTIPLE PRODUCTION BATCHES OF TASTERIGHT REFINED ENZYME TREATED STEVIA 106
APPENDIX 7 PESTICIDE TESTING REPORTS 112
APPENDIX 8 RELATIVE SWEETNESS INTENSITY METHOD 120
APPENDIX 9 TASTERIGHT ENZYME TREATED STEVIA STABILITY REPORT 132
APPENDIX 10 TASTERIGHT REFINED ENZYME TREATED STEVIA STABILITY REPORT 135
APPENDIX 11 ESTIMATED DAILY INTAKE LEVELS OF STEVIOL GLYCOSIDES PREPARATIONS 138
A. Food Uses as Addressed by JECFA, EFSA, FSANZ & Others 138
B. Estimated Daily Intake 138
C. JECFA 138
D. EFSA 138
E. FSANZ 139
F. Merisant 139
G. Cargill 139
H. BioVittoria 140
I. Other Publications 140
APPENDIX 12 STUDIES ON STEVIOL GLYCOSIDES PREPARATIONS 141
PART 1. PREPARATIONS THAT ARE PRIMARILY MIXTURES OF STEVIOSIDE & REBAUDIOSIDE A 141
A. Absorption, Distribution, Metabolism & Excretion (ADME) Studies 141
1. Animal Studies 141
2. Human Studies 141
B. Acute Toxicity Studies 142
C. Subchronic Toxicity Studies 142
D. Chronic Toxicity Studies 143
E. Reproductive & Developmental Toxicity Studies 143
F. Mutagenicity & Genotoxicity Studies 144
G. Clinical Studies & Other Reports in Humans 145
PART 2. PREPARATIONS THAT ARE PRIMARILY REBAUDIOSIDE A 147
A. Absorption, Distribution, Metabolism & Excretion (ADME) Studies 147
1. Animal Studies 147
B. Subchronic Toxicity Studies 149
C. Mutagenicity & Genotoxicity Studies 149
D. Reproductive & Developmental Studies 150
E. Clinical Studies on Rebaudioside A 150
F. Safety of Rebaudioside A 151
PART 3. STUDIES ON PRINCIPAL METABOLITE: STEVIOL 153
A. Acute Toxicity Studies 153
B. Developmental Toxicity Studies 153
C. Mutagenicity & Genotoxicity Studies 153
D. Endocrine Disruption Studies 154
APPENDIX 13 SUMMARY OF THE REGULATORY HISTORY OF STEVIOL GLYCOSIDES 155
A. History of Traditional Medicinal and Human Food Use 155
B. Summary of Regulatory History of Enzyme Modified Steviol Glycosides 155
1. U.S. Regulatory History 155
2. Canadian Regulatory History 156
3. European Regulatory History 157

4. Asian Regulatory History 159
5. Australia and New Zealand Regulation History 159
6. South Africa 160

APPENDIX 14 SUMMARY OF PUBLISHED SAFETY REVIEWS 161
A. Summary of JECFA Reviews 161
B. Summary of FSANZ Review of Steviol Glycosides 161
C. Summary of EFSA Review of Steviol Glycosides 162
D. Other Published Reviews 162

APPENDIX 15 GRAS ASSOCIATES EXPERT PANEL REPORT 164

FIGURES

Figure 1. Chemical Structures of Various Steviol Glycosides[a] 8
Figure 2. Chemical Structures of Various Glucosylated Steviol Glycosides[a] 10
Figure 3. Flow Chart of Manufacturing Process for Qufu, Sunwin, and NuNaturals's TasteRight High Purity Glucosylated Steviol Glycosides Preparations 12

TABLES

Table 1. Components Expected to be Present in Glucosylated Steviol Glycosides[a] 9
Table 2. Specifications for Steviol Glycosides Starting Material 14
Table 3. Typical Levels of Steviol Glycosides in Untreated Stevia Extract Starting Material 15
Table 4. Specifications for Qufu, Sunwin, and NuNaturals's TasteRight Enzyme Treated Stevia Preparation 16
Table 5. Specifications for Qufu, Sunwin, and NuNaturals's TasteRight Refined Enzyme Treated Stevia Preparation 17
Table 6. TasteRight Enzyme Treated Stevia Stability Under Intense Conditions 19
Table 7. TasteRight Enzyme Treated Stevia Accelerated Storage Stability Data 20
Table 8. TasteRight Enzyme Treated Stevia Storage Stability Data 21
Table 9. TasteRight Refined Enzyme Treated Stevia Stability Under Intense Conditions 22
Table 10. TasteRight Refined Enzyme Treated Stevia Accelerated Storage Stability Data 23
Table 11. TasteRight Refined Enzyme Treated Stevia Storage Stability Data 24
Table 12. Steviol Equivalency Factors for Various Steviol Glycosides 25
Table 13. Steviol Equivalency of TasteRight Preparations 25
Table 14. Daily Intake of Sweeteners (in Sucrose Equivalents) & Estimated Daily Intakes of TasteRight Enzyme Treated Stevia 27
Table 15. Daily Intake of Sweeteners (in Sucrose Equivalents) & Estimated Daily Intakes of TasteRight Refined Enzyme Treated Stevia 27

FOREWORD

Qufu Shengren Pharmaceutical Co., Ltd. ("Qufu"), Sunwin Stevia International ("Sunwin"), and NuNaturals, Inc. ("NuNaturals") based our Generally Recognized as Safe (GRAS) assessment on high purity glucosylated steviol glycosides primarily on the composite safety information, i.e., scientific procedures with corroboration from history of use. The safety/toxicity of steviol glycosides, history of use of steviol glycosides, and compositional details, specifications, and method of preparation of the subject ingredient were reviewed. In addition, a search of the scientific and regulatory literature was conducted through March 12, 2019, with particular attention paid to adverse reports, as well as those that supported conclusions of safety. Those references that were deemed pertinent to this review are listed in Part 7. The composite safety/toxicity studies, in concert with dietary exposure information, ultimately provide the specific scientific foundation for the GRAS conclusion.

At Qufu, Sunwin, and NuNaturals's request, GRAS Associates, LLC ("GA") convened an Expert Panel to complete an independent safety evaluation of Qufu, Sunwin, and NuNaturals's high purity enzyme glucosylated steviol glycosides preparations. The purpose of the evaluation is to ascertain whether Qufu, Sunwin, and NuNaturals's conclusion that the intended food uses of high purity glucosylated steviol glycosides as described in Part 3 are generally recognized as safe, i.e., GRAS, under the intended conditions of use. In addition, Qufu, Sunwin, and NuNaturals have asked GA to act as Agent for the submission of this GRAS notification.

PART 1. SIGNED STATEMENTS AND CERTIFICATION

A. Basis of Exclusion from the Requirement for Premarket Approval Pursuant to Subpart E of 170[1]

Qufu, Sunwin, and NuNaturals have concluded that our high purity glucosylated steviol glycosides preparations (≥ 95% total steviol glycosides), referred to as "Enzyme Treated Stevia", "Refined Enzyme Treated Stevia", and "TasteRight", and which meet the specifications described below, are GRAS in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic (FD&C Act) 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 high purity glucosylated steviol glycosides preparations (≥ 95% total steviol glycosides).

[1] See 81 FR 54960, 17 August 2016. Accessible at: https://www.gpo.gov/fdsys/pkg/FR-2016-08-17/pdf/2016-19164.pdf (Accessed 1/16/19).

Signed:

Agent for Qufu, Sunwin, and NuNaturals

William J. Rowe
President
GRAS Associates, LLC
27499 Riverview Center Blvd.
Suite 212
Bonita Springs, FL 34134

Date: 4/22/19

B. Name and Address of Responsible Parties

Qufu Shengren Pharmaceutical Co., Ltd.
No. 6, Sunwin Blvd.
Qufu, Shandong
273100
People's Republic of China

Sunwin Stevia International
431 Fairway Drive #251
Deerfield Beach, FL
33441
USA

NuNaturals, Inc.
2220 W. 2nd Ave.
Eugene, OR
97402
USA

As the Responsible Parties, Qufu, Sunwin, and NuNaturals accept responsibility for the GRAS conclusion that has been made for our high purity glucosylated steviol glycosides preparations (≥ 95% total steviol glycosides) as described in the subject safety evaluation; consequently, the purified steviol glycosides preparations having acceptable steviol glycosides compositions which meet the conditions described herein, are not subject to premarket approval requirements for food ingredients.

C. Common Name and Identity of Notified Substance

The common name of the ingredient to be used on food labels is "Enzyme Treated Stevia" or "Refined Enzyme Treated Stevia." Qufu, Sunwin, and NuNaturals also plan to market our high purity glycoyslated steviol glycosides preparations under the trade name "TasteRight."

D. Conditions of Intended Use in Food

Qufu, Sunwin, and NuNaturals's TasteRight high purity glucosylated steviol glycosides preparations (≥ 95% total steviol glycosides) are intended for use as a general-purpose sweetener in foods, excluding meat and poultry products and infant formulas, at levels determined by current good manufacturing practices (CGMP).

E. Basis for GRAS Conclusion

Pursuant to 21 CFR 170.30(a) and (b)[2], Qufu, Sunwin, and NuNaturals's TasteRight high purity glucosylated steviol glycosides preparations (≥ 95% total steviol glycosides) have been concluded to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

Purified steviol glycosides are not subject to premarket approval requirements of the FD&C Act based on Qufu, Sunwin, and NuNaturals's conclusion that the substance is GRAS under the conditions of its intended food use.

Qufu, Sunwin, and NuNaturals certify, to the best of our knowledge, that this GRAS notice is a complete, representative, and balanced assessment that includes all relevant information, both favorable and unfavorable, available and pertinent to the evaluation of the safety and GRAS status of high purity glucosylated steviol glycosides.

F. Availability of Information

The data and information that serve as the bases for this GRAS Notice will be maintained at the offices of Sunwin Stevia International, Deerfield, FL, and will be made available during customary business hours.

Qufu, Sunwin, and NuNaturals certify that no data or information contained herein are exempt from disclosure under the Freedom of Information Act (FOIA). No non-public, safety-related data were used by the Expert Panel to reach a GRAS conclusion.

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

A. Chemical Identity of Ingredient

"Enzyme modified steviol glycosides" or "glucosylated steviol glycosides" are the common or usual names of the non-nutritive sweetener derived from the enzymatic glycosylation of a high purity extract of *Stevia rebaudiana* Bertoni. The compositional features of the TasteRight high purity glucosylated steviol glycosides preparations (≥ 95% total steviol glycosides) are described in more detail in this section. "Enzyme Treated Stevia" and "Refined Enzyme Treated Stevia" are the terms

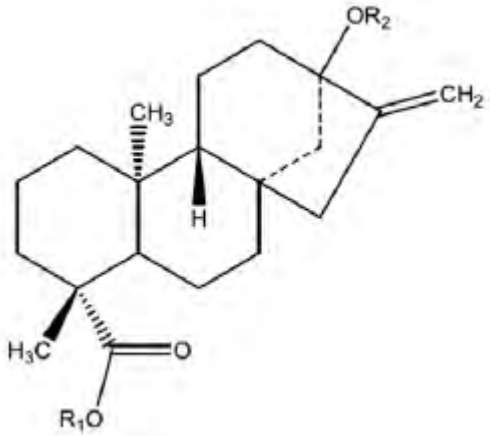
[2] 21 CFR 170.30. Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=170.30 (Accessed 4/18/19)

used by Qufu, Sunwin, and NuNaturals in referring to the notified substance. The preparation is also marketed as TasteRight.

The general chemistry of steviol glycosides and enzyme modified steviol glycosides has previously been reviewed in a number of GRAS Notifications (GRNs) , including GRN 337 (NOW Foods, 2010), GRN 667 (Blue California, 2016), and GRN 715 (Blue California, 2017). Representative chemical structures of steviol glycosides that have been identified to date are presented in Figure 1.

No known toxins have been identified in stevia or stevia-derived products.

Figure 1. Chemical Structures of Various Steviol Glycosides[a]



Compound	R1	R2
Steviol	H-	H-
Stevioside	Glcß1-	Glcß(1-2)Glcß1-
Rebaudioside A	Glcß1-	Glcß(1-2)[Glcß(1-3)]Glcß1-
Rebaudioside B	H-	Glcß(1-2)[Glcß(1-3)]Glcß1-
Rebaudioside C	Glcß1-	Rhaα(1-2)[Glcβ(1-3)]Glcβ-
Rebaudioside D	Glcβ(1-2)Glcβ1-	Glcß(1-2)[Glcß(1-3)]Glcß1-
Rebaudioside E	Glcβ(1-2)Glcβ1-	Glcβ(1-2)Glcβ1-
Rebaudioside F	Glcβ1-	Xylβ(1-2)[Glcβ(1-3)]Glcβ1-
Rebaudioside M	Glcß(1-2)[Glcß(1-3)]Glcß1-	Glcß(1-2)[Glcß(1-3)]Glcß1-
Steviolbioside	H-	Glcβ(1-2)Glcβ1-
Dulcoside A	Glcβ1-	Rhaα(1-2)Glcβ1-
Rubusoside	Glcß1-	Glcß1-
Glc, Rha, and Xyl represent glucose, rhamnose, and xylose sugar moieties, respectively		

[a] From Perrier et al. (2018)

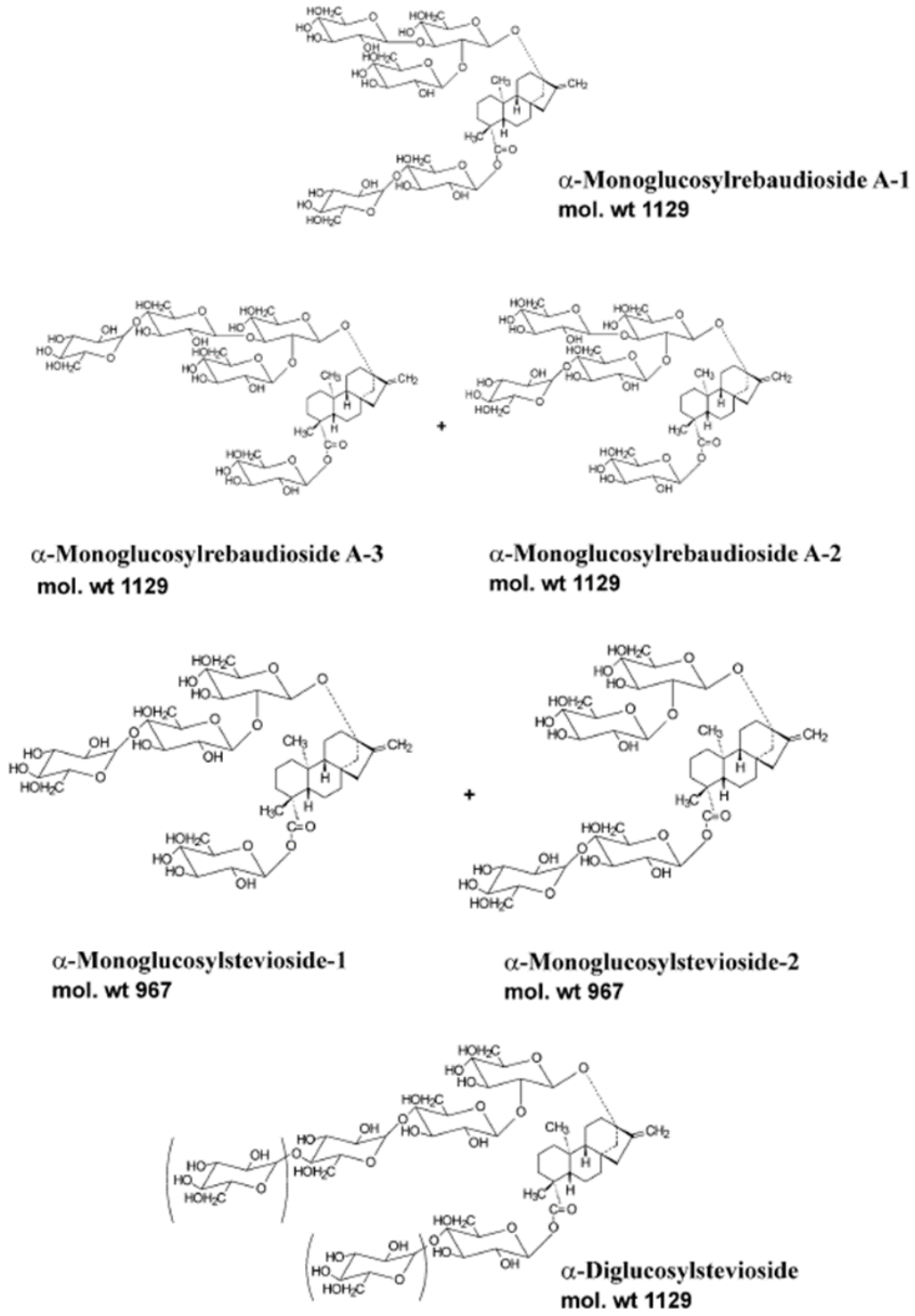
Enzyme modified steviol glycosides are produced when additional glucose moieties are bonded to the original steviol glycoside structure via α(1→4) linkages, resulting in α-glucosylated steviol glycosides. The product α-glucosylated steviol glycosides consists of a mixture of both α-D-glucosylated steviol glycosides and steviol glycosides, including rebaudioside A, rebaudioside C, dulcoside A, steviolbioside, rubusoside, and rebaudioside B. The enzyme attaches the additional glucose residues by sterio- and regio-specific 1,4-α-D-glycosidic bonds, whereas the glucose is attached by β-glycosidic bonds in naturally occurring steviol glycosides. The primary constituents of enzymatically treated stevia have been identified (Koyama et al., 2003a) and are described in Table 1, and the chemical structures are shown in Figure 2.

Table 1. Components Expected to be Present in Glucosylated Steviol Glycosides[a]

Compound	Molecular Weight	Empirical Formula	Level of Enzyme Glycosylation[b]
Steviolbioside	642	C32H50O13	--
Dulcoside A	788	C38H60O17	--
Stevioside	804	C38H60O18	--
Rebaudioside C	950	C44H70O22	--
Rebaudioside A	966	C44H70O23	--
Monoglucosyl Rebaudioside B	966	C44H70O23	+1
Monoglucosyl Stevioside	966	C44H70O23	+1
Monoglucosyl Rebaudioside C	1112	C50H80O27	+1
Monoglucosyl Rebaudioside A	1128	C50H80O28	+1
Diglucosyl Rebaudioside B	1128	C50H80O28	+2
Diglucosylstevioside	1128	C50H80O28	+2
Diglucosyl Rebaudioside C	1274	C56H90O32	+2
Diglucosyl Rebaudioside A	1290	C56H90O33	+2
Triglucosyl Rebaudioside B	1290	C56H90O33	+3
Triglucosyl Rebaudioside A	1452	C62H100O38	+3

[a] Data from Koyama et al. (2003a)
[b] The level of enzymatic glycosylation indicates the number of glucose units that have been added *via* enzyme treatment.

Figure 2. Chemical Structures of Various Glucosylated Steviol Glycosides[a]



[a] From Koyama et al. (2003a)

B. Manufacturing Processes

Qufu, Sunwin, and NuNaturals's TasteRight glucosylated steviol glycosides preparations are manufactured via an enzymatic reaction with *Stevia rebaudiana* Bertoni extract [>95% total steviol glycosides, which meets Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications] using cyclomaltodextrin glucanotransferase (CGTase). The resulting preparation is a high purity enzyme modified steviol glycosides product (≥95% total steviol glycosides and glycosylated steviosides).

For the manufacturing of the starting steviol glycosides, Qufu, Sunwin, and NuNaturals employ a fairly typical process that is used in the industry for the production of stevia extracts. In a proprietary process, dried leaves from *Stevia rebaudiana* Bertoni are extracted in water to obtain a stevia extract powder containing 30-60% stevioside. In order to facilitate the precipitation of steviol glycosides, ferrous sulfate and calcium hydroxide may be used. The stevia extract is further purified via film filtration, absorption resin, and membrane purification to obtain an extract consisting of ≥ 95% total steviol glycosides.

Qufu, Sunwin, and NuNaturals use the purified stevia extract product, corn-derived maltodextrin, α-Amylase G Amano L[3], and purified water to manufacture high purity enzyme modified steviol glycosides. After being heated to 60-70°C at pH 5.5 for 6-8 hours, the mixed starting material is deactivated for three hours at 100°C, absorbed with absorption resin, and eluted with food grade ethanol. The eluted solution is then concentrated and spray dried to obtain TasteRight Enzyme Treated Stevia, which is a mixture of glucosylated steviol glycosides and unmodified steviol glycosides, with residual unreacted maltodextrin. This preparation can be further purified with resin to produce TasteRight Refined Enzyme Treated Stevia, in which the majority of residual unreacted maltodextrin has been removed.

The resin used in the manufacturing process complies with 21 CFR 173.65.[4] Qufu, Sunwin, and NuNaturals's high purity glucosylated steviol glycosides preparations are prepared in accordance with CGMP regulations.

The manufacturing process is summarized in a flow chart provided in Figure 3.

Specifications for the raw materials and processing aids are provided in Appendix 1.

[3] α-Amylase G Amano L, manufactured by Amano, is GRAS as defined in 21 CFR 170.30(a) for use as a processing aid in the manufacture of beta cyclodextrins.
[4] 21 CFR 173.65. Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=173.65 (Accessed 4/18/19)

Figure 3. Flow Chart of Manufacturing Process for Qufu, Sunwin, and NuNaturals's TasteRight High Purity Glucosylated Steviol Glycosides Preparations

C. Product Specifications

1. JECFA Specifications for Steviol Glycosides

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

In the most recent Joint FAO/WHO Expert Committee on Food Additives (JECFA) monograph, published in 2017 (FAO, 2017), steviol glycosides specifications were modified to include a minimum requirement of not less than 95% total steviol glycosides, on a dry basis, "determined as the sum of all compounds containing a steviol backbone conjugated to any number, combination or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose xylose, galactose, arabinose and xylose) occurring in the leaves of *Stevia rebaudiana* Bertoni."

JECFA's 2017 monograph describes steviol glycosides as white-to-yellow powders that are odorless or have a slight characteristic odor and exhibit a sweetness that is 200-300 times greater than that of sucrose. The ingredient must consist of a minimum of 95% total steviol glycosides, as defined above. The steviol glycosides are freely soluble in a 50:50 mixture of ethanol and water, and the 1 in 100 solutions exhibit pH values between 4.5 and 7.0. The product should not have

more than 1% ash, with no more than a 6% loss on drying at 105°C after 2 hours. Any residual methanol levels should not exceed 200 mg per kg, and ethanol residues should not exceed 5,000 mg per kg. Arsenic and lead levels should not exceed 1 mg per kg. Microbiological criteria have also been established, with specifications of no more than 1,000 colony forming units (CFU) per g total plate count, not more than 200 CFU per g yeasts and molds, and *E. coli* and *Salmonella* negative in 1 g and 25 g, respectively.

Qufu, Sunwin, and NuNaturals have adopted specifications for our purified steviol glycosides extract starting material that meet or exceed current JECFA specifications, as demonstrated in Table 2. Typical glycosides content of production batches is provided in Table 3.

Table 2. Specifications for Steviol Glycosides Starting Material

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	Qufu, Sunwin, & NuNaturals's Specifications for Steviol Glycosides Starting Material
Appearance Form	Powder	Powder
Appearance Color	White to light Yellow	White
Solubility	Freely soluble in 50:50 water: ethanol	Freely soluble in 50:50 water: ethanol
Assay	NLT 95% total steviol glycosides[b]	≥ 95% total steviol glycosides
Residual Ethanol	NMT 5,000 mg/kg	≤ 5,000 ppm
Residual Methanol	NMT 200 mg/kg	≤ 200 ppm
Loss on Drying	NMT 6.0%	≤ 6.0%
pH, 1% Solution	4.5-7.0	4.5-7.0
Total Ash	NMT 1%	≤ 0.1%
Arsenic	NMT 1 mg/kg	≤ 0.0001%
Lead	NMT 1 mg/kg	≤ 0.0001%
Total Plate Count	NMT 1,000 cfu/g	< 1,000 cfu/g
Yeast & Mold	NMT 200 cfu/g	< 100 cfu/g
Staphylococcus aureus	NS	Negative
Salmonella	Negative in 25 g	Negative
Escherichia coli	Negative in 1 g	Negative

NLT = not less than; NMT = not more than; NS = not specified; mg = milligram; g = gram; kg = kilogram; cfu = colony forming unit; ppm = part per million

[a] Prepared at 84th JECFA (2017)

[b] Total steviol glycosides as the sum of all compounds containing a steviol backbone conjugated to any number, combination, or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose xylose, galactose, arabinose, and xylose) occurring in the leaves of *Steviana rebaudiana* Bertoni.

Table 3. Typical Levels of Steviol Glycosides in Untreated Stevia Extract Starting Material

Component	Stevia Extract Lot 20160702	Stevia Extract Lot 20161202	Stevia Extract Lot 20161204	Stevia Extract Lot 20170304	Stevia Extract Lot 20170501	Average of 5 lots
Total Steviol Glycosides	95.08%	95.42%	95.42%	95.20%	95.55%	95.33%
Rebaudioside A	7.83%	8.31%	9.52%	9.57%	8.98%	8.84%
Stevioside	82.06%	82.82%	81.11%	80.86%	80.96%	81.56%
Rebaudioside C	1.85%	1.19%	0.36%	0.71%	1.20%	1.06%

2. Specifications for Qufu, Sunwin, and NuNaturals's TasteRight High Purity Glucosylated Steviol Glycosides Preparations and Supporting Methods

Qufu, Sunwin, and NuNaturals have adopted product specifications for our TasteRight high purity glucosylated steviol glycosides preparations that meet or exceed current JECFA recommendations, while also complying with Food Chemicals Codex (FCC, 2010) specifications for rebaudioside A as a consumable human food substance. The compositions of five non-consecutive lots of Qufu, Sunwin, and NuNaturals's TasteRight Enzyme Treated Stevia (≥ 95% total glucosylated steviosides and steviol glycosides) and TasteRight Refined Enzyme Treated Stevia (≥ 95% total glucosylated steviosides and steviol glycosides) preparations are compared to the JECFA and FCC specifications in Table 4 and Table 5, respectively.

Table 4. Specifications for Qufu, Sunwin, and NuNaturals's TasteRight Enzyme Treated Stevia Preparation

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	FCC[b] Specifications Rebaudioside A	Qufu, Sunwin, & NuNaturals's Minimum Specifications for TasteRight Enzyme Treated Stevia	Results of TasteRight Enzyme Treated Stevia Preparations				
				Batch Number 20170302	Batch Number 20170305	Batch Number 20170401	Batch Number 20170502	Batch Number 20170703
Appearance Form	Powder	Crystal, granule or powder	Powder	Pass	Pass	Pass	Pass	Pass
Appearance Color	White to light Yellow	White to off-white	White to off-white	Pass	Pass	Pass	Pass	Pass
Sweetness Intensity[c]	--	--	100-150	110	110	110	110	110
Solubility	Freely soluble in water: ethanol (50:50)	Freely soluble in water: ethanol (50:50)	Freely soluble in water: ethanol (50:50)	Meets specification	Meets specification	Meets specification	Meets specification	Meets specification
Purity (HPLC Area) %	≥95 Steviol Glycosides	≥ 95	≥ 95	99.07	99.74	99.17	99.26	99.74
Dextrin (%)	NA	NA	≤ 20	18.04	19.01	17.21	18.02	17.00
Residual Ethanol	NMT 5,000 mg/kg	NMT 0.5%	≤ 5,000 ppm	249 ppm	272 ppm	270 ppm	266 ppm	199 ppm
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	≤ 200 ppm	32 ppm	26 ppm	53 ppm	50 ppm	30 ppm
Loss on Drying	NMT 6.0%	NMT 6.0%	≤ 6%	2.57%	2.65%	2.66%	2.68%	2.70%
pH, 1% Solution	4.5-7.0	4.5-7.0	4.5-7.0	5.86	5.83	5.80	5.76	5.70
Total Ash	NMT 1%	NMT 1%	≤ 1 %	0.71%	0.71%	0.71%	0.71%	0.71%
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm
Lead	NMT 1 mg/kg	NMT 1 mg/kg	≤ 1 ppm (as total heavy metals)	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm
Total Plate Count (cfu/g, max)	NMT 1,000	NA	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000
Yeast & Mold (cfu/g, max)	NMT 200	NA	< 100	< 100	< 100	< 100	< 100	< 100
E. coli (mpn/g)	Negative in 1 g	NA	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella spp.	Negative in 25 g	NA	Negative in 25 g	Negative	Negative	Negative	Negative	Negative

[a] Prepared at 84th JECFA (2017)
[b] Rebaudioside A monograph. Food Chemicals Codex (7th Ed.) (FCC, 2010)
[c] Compared with a 2% sucrose solution
NA = not applicable; NMT = not more than; mg = milligram; g = gram; kg = kilogram; cfu = colony forming unit; ppm = part per million; mpn = most probable number; HPLC = high-performance liquid chromatography

Table 5. Specifications for Qufu, Sunwin, and NuNaturals's TasteRight Refined Enzyme Treated Stevia Preparation

Physical & Chemical Parameters	JECFA[a] Specifications Steviol Glycosides	FCC[b] Specifications Rebaudioside A	Qufu, Sunwin, & NuNaturals's Minimum Specifications for TasteRight Refined Enzyme Treated Stevia	Results of TasteRight Refined Enzyme Treated Stevia Preparations				
				Batch Number 20170301	Batch Number 20170306	Batch Number 20170402	Batch Number 20170503	Batch Number 20170701
Appearance Form	Powder	Crystal, granule or powder	Powder	Pass	Pass	Pass	Pass	Pass
Appearance Color	White to light Yellow	White to off-white	White to off-white	Pass	Pass	Pass	Pass	Pass
Sweetness Intensity[c]	--	--	≥ 260	Meets specification	Meets specification	Meets specification	Meets specification	Meets specification
Solubility	Freely soluble in water: ethanol (50:50)	Freely soluble in water: ethanol (50:50)	Freely soluble in water: ethanol (50:50)	Meets specification	Meets specification	Meets specification	Meets specification	Meets specification
Purity (HPLC Area) %	≥95% Steviol Glycosides	≥ 95	≥ 95	96.92	96.38	96.78	96.00	97.19
Dextrin (%)	NA	NA	≤ 1	0.1	0.1	0.1	0.1	0.1
Residual Ethanol	NMT 5,000 mg/kg	NMT 0.5%	≤ 5,000 ppm	256 ppm	248 ppm	258 ppm	251 ppm	261 ppm
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	≤ 200 ppm	36 ppm	29 ppm	32 ppm	30 ppm	26 ppm
Loss on Drying	NMT 6.0%	NMT 6.0%	≤ 6%	2.62%	2.68%	2.65%	2.59%	2.58%
pH, 1% Solution	4.5-7.0	4.5-7.0	4.5-7.0	5.52	5.56	5.53	5.53	5.49
Total Ash	NMT 1%	NMT 1%	≤ 1 %	0.09%	0.09%	0.09%	0.09%	0.09%
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm
Lead	NMT 1 mg/kg	NMT 1 mg/kg	≤ 1 ppm (as total heavy metals)	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm	≤ 1 ppm
Total Plate Count (cfu/g, max)	NMT 1,000	NA	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000	< 1,000
Yeast & Mold (cfu/g, max)	NMT 200	NA	< 100	< 100	< 100	< 100	< 100	< 100
E. coli (mpn/g)	Negative in 1 g	NA	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella spp.	Negative in 25 g	NS	Negative in 25 g	Negative	Negative	Negative	Negative	Negative

[a] Prepared at 84th JECFA (2017)
[b] Rebaudioside A monograph. Food Chemicals Codex (7th Ed.) (FCC, 2010)
[c] Compared with a 2% sucrose solution
NA = not applicable; NMT = not more than; mg = milligram; g = gram; kg = kilogram; cfu = colony forming unit; ppm = part per million; mpn = most probable number; HPLC = high-performance liquid chromatography

Qufu, Sunwin, and NuNaturals analyze our TasteRight high purity glucosylated steviol glycosides preparations (≥ 95% total glucosylated steviosides and steviol glycosides) in accordance with the Peoples' Republic of China National Health and Family Planning Commission's monograph for glucosyl steviol glycosides (Appendix 2). In addition to the presentation of key specifications found in Table 2 for comparison with generally accepted purity standards, certificates of analysis for five representative lots of both TasteRight Enzyme Treated Stevia and TasteRight Refined Enzyme Treated Stevia preparations, are provided in Appendix 3 and Appendix 4 respectively. The chromatograms for representative lots of both TasteRight Enzyme Treated Stevia and TasteRight Refined Enzyme Treated Stevia preparations, are provided in Appendix 5 and Appendix 6, respectively. Test reports for analyses of pesticide residues in a representative lot of purified steviol glycosides used as the raw material, as well as in representative lots of TasteRight Enzyme Treated Stevia and TasteRight Refined Enzyme Treated Stevia are located in Appendix 7. The collection of these reports demonstrates that the substance is well characterized and meets the established purity criteria.

D. Physical or Technical Effect

Qufu, Sunwin, and NuNaturals determine the relative sweetness of our TasteRight Enzyme Treated Stevia and TasteRight Refined Enzyme Treated Stevia preparations by organoleptic comparison to a 2% sucrose solution, following the method outlined in Appendix 8. Qufu, Sunwin, and NuNaturals's specifications for relative sweetness of TasteRight Enzyme Treated Stevia is ≥ 100X and for the relative sweetness of TasteRight Refined Enzyme Treated stevia is ≥ 260X, respectively.

E. Stability

1. Stability Data on Steviol Glycosides

The stability of steviol glycosides and enzyme modified steviol glycosides has previously been reviewed in a number of GRAS Notifications, including GRN 337 (NOW Foods, 2010), GRN 667 (Blue California, 2016), and GRN 715 (Blue California, 2017).

Steviol glycosides have been reported to be stable over the pH range 3-9 and can be heated at 100^{o}Capasso et al. (2008) for 1 hour, but, at pH levels greater than 9, it rapidly decomposes (Kinghorn, 2002). In previously submitted GRAS Notifications, GRN 252 (Merisant, 2008), GRN 253 (Cargill, 2008), and GRN 304 (Sunwin/WILD, 2010) reported stability data indicating that Rebaudioside A is stable under the intended conditions of use.

Furthermore, in the over 55 GRAS Notices[5] that have been submitted to FDA to date for steviol glycosides, the presented stability data have supported the position that steviol glycosides are stable and well-suited for the intended uses in foods.

[5] GRN Database. Available at: https://www.fda.gov/food/ingredientspackaginglabeling/gras/noticeinventory/default.htm (Accessed 4/18/19)

2. Stability Data for Qufu, Sunwin, and NuNaturals's TasteRight Enzyme Treated Stevia Preparations

Qufu, Sunwin, and NuNaturals conducted a number of studies on a single batch of TasteRight Enzyme Treated Stevia preparation for stability under high temperature, light, and high humidity conditions. A summary of the stability results is provided in Table 6.

Table 6. TasteRight Enzyme Treated Stevia Stability Under Intense Conditions (Batch 20150401)

Test Condition	Days	Test Parameter			
		Appearance	Moisture-Absorption (Weight Gain %)	Loss on Drying (%)	Assay (%)
	0	White powder, odorless or characteristic	NR	3.02	96.16
High Temperature (60°C)	5	White powder, odorless or characteristic	NR	3.04	96.45
	10	White powder, odorless or characteristic	NR	2.98	96.10
Light (4500 lux)	5	White powder, odorless or characteristic	NR	2.99	96.22
	10	White powder, odorless or characteristic	NR	3.03	96.08
High Humidity (RH 92.5%)	5	White powder, odorless or characteristic	8.2%	NR	95.81
High Humidity (RH 75%)	5	White powder, odorless or characteristic	7.9%	NR	96.09

NR = not reported; RH = relative humidity

For the accelerated stability study, samples of three batches of TasteRight Enzyme Treated Stevia were stored at 40°C ± 2°C at a relative humidity of 75% ± 5% for 0, 1, 2, 3, and 6 months. The stability samples were then tested for loss on drying, ash, and total steviol glycosides parameters. A summary of the accelerated shelf-stability results is presented in Table 7.

Table 7. TasteRight Enzyme Treated Stevia Accelerated Storage Stability Data

Time (months)	Loss on Drying (%)	Ash (%)	Total Steviol Glycosides[a] (%)	Microbial Limits	
				Total Aerobic Bacterial Count (cfu/g)	Yeast & Mold (cfu/g)
BATCH NUMBER 20150401					
0	3.02	0.09	96.16	< 10	< 10
1	3.01	0.09	96.44	< 10	< 10
2	3.02	0.09	96.11	< 10	< 10
3	3.06	0.09	96.05	< 10	< 10
6	3.01	0.09	96.19	< 10	< 10
BATCH NUMBER 20150402					
0	3.16	0.09	96.47	< 10	< 10
1	3.15	0.09	96.56	< 10	< 10
2	3.18	0.09	97.75	< 10	< 10
3	3.14	0.09	96.20	< 10	< 10
6	3.15	0.09	96.68	< 10	< 10
BATCH NUMBER 20150403					
0	3.25	0.09	96.19	< 10	< 10
1	3.25	0.09	96.09	< 10	< 10
2	3.24	0.09	96.43	< 10	< 10
3	3.28	0.09	95.94	< 10	< 10
6	3.19	0.09	96.90	< 10	< 10

[a] Reported as the sum of steviol glycosides and glycosylated steviosides.

Over the course of 24 months, samples of three batches of TasteRight Enzyme Treated Stevia were stored at 25°C ± 2°C at a relative humidity of 60% ± 10% for 0, 3, 6, 9, 12, 18, and 24 months. The stability samples were then tested for loss on drying, ash, total steviol glycosides, and microbial parameters. A summary of the long-term shelf-stability results is presented in Table 8.

Table 8. TasteRight Enzyme Treated Stevia Storage Stability Data

Time (months)	Loss on Drying (%)	Ash (%)	Total Steviol Glycosides[a] (%)	Microbial Limits	
				Total Aerobic Bacterial Count (cfu/g)	Yeast & Mold (cfu/g)
BATCH NUMBER 20150401					
0	3.02	0.09	96.16	< 10	< 10
3	3.06	0.09	96.99	< 10	< 10
6	3.01	0.09	96.40	< 10	< 10
9	3.04	0.09	96.69	< 10	< 10
12	3.02	0.09	96.68	< 10	< 10
18	3.01	0.09	96.94	< 10	< 10
24	3.05	0.09	96.76	< 10	< 10
BATCH NUMBER 20150402					
0	3.16	0.09	96.47	< 10	< 10
3	3.14	0.09	96.66	< 10	< 10
6	3.15	0.09	96.54	< 10	< 10
9	3.18	0.09	97.27	< 10	< 10
12	3.21	0.09	97.73	< 10	< 10
18	3.22	0.09	96.99	< 10	< 10
24	3.23	0.09	96.81	< 10	< 10
BATCH NUMBER 20150403					
0	3.25	0.09	96.19	< 10	< 10
3	3.28	0.09	96.56	< 10	< 10
6	3.19	0.09	96.47	< 10	< 10
9	3.18	0.09	96.58	< 10	< 10
12	3.16	0.09	96.18	< 10	< 10
18	3.15	0.09	96.26	< 10	< 10
24	3.18	0.09	96.33	< 10	< 10

[a] Reported as the sum of steviol glycosides and glycosylated steviosides.

A stability study report for TasteRight Enzyme Treated Stevia is provided in Appendix 9.

The stability data in the scientific literature for stevioside, the JECFA report, and the extensive stability testing for rebaudioside A as presented by Merisant, Cargill, and Sunwin & WILD Flavors, along with Qufu, Sunwin, and NuNaturals's stability testing results, support the position that Qufu, Sunwin, and NuNaturals's Taste Right Enzyme Treated Stevia is well-suited for the intended food uses.

3. Stability Data for Qufu, Sunwin, and NuNaturals's TasteRight Refined Enzyme Treated Stevia Preparations

Qufu, Sunwin, and NuNaturals conducted a number of studies on a single batch of TasteRight Refined Enzyme Treated Stevia preparation for stability under high temperature, light, and high humidity conditions. A summary of the stability results is provided in Table 9.

Table 9. TasteRight Refined Enzyme Treated Stevia Stability Under Intense Conditions (Batch 20150301)

Test Condition	Days	Test Parameter			
		Appearance	Moisture-Absorption (Weight Gain %)	Loss on Drying (%)	Assay (%)
	0	White powder, odorless or characteristic	NR	3.11	96.09
High Temperature (60°C)	5	White powder, odorless or characteristic	NR	3.10	96.80
	10	White powder, odorless or characteristic	NR	3.08	96.24
Light (4500 lux)	5	White powder, odorless or characteristic	NR	3.12	96.57
	10	White powder, odorless or characteristic	NR	3.10	96.36
High Humidity (RH 92.5%)	5	White powder, odorless or characteristic	8.1%	NR	96.55
High Humidity (RH 75%)	5	White powder, odorless or characteristic	7.8%	NR	96.01

NR = not reported; RH = relative humidity

For the accelerated stability study, samples of three batches of TasteRight Refined Enzyme Treated Stevia were stored at 40°C ± 2°C at a relative humidity of 75% ± 5% for 0, 1, 2, 3, and 6 months. The stability samples were then tested for loss on drying, ash, and total steviol glycosides parameters. A summary of the accelerated shelf-stability results is presented in Table 10.

Table 10. TasteRight Refined Enzyme Treated Stevia Accelerated Storage Stability Data

Time (months)	Loss on Drying (%)	Ash (%)	Total Steviol Glycosides[a] (%)	Microbial Limits	
				Total Aerobic Bacterial Count (cfu/g)	Yeast & Mold (cfu/g)
BATCH NUMBER 20150301					
0	3.11	0.10	96.09	< 10	< 10
1	3.12	0.10	96.40	< 10	< 10
2	3.13	0.10	96.22	< 10	< 10
3	3.12	0.10	96.08	< 10	< 10
6	3.10	0.10	95.90	< 10	< 10
BATCH NUMBER 20150302					
0	3.01	0.10	96.85	< 10	< 10
1	3.01	0.10	96.45	< 10	< 10
2	3.02	0.10	96.62	< 10	< 10
3	3.02	0.10	96.66	< 10	< 10
6	3.05	0.10	96.47	< 10	< 10
BATCH NUMBER 20150303					
0	3.06	0.10	96.10	< 10	< 10
1	3.06	0.10	96.00	< 10	< 10
2	3.07	0.10	96.43	< 10	< 10
3	3.05	0.10	96.16	< 10	< 10
6	3.01	0.10	96.92	< 10	< 10

[a] Reported as the sum of steviol glycosides and glycosylated steviosides.

Over the course of 24 months, samples of three batches of TasteRight Refined Enzyme Treated Stevia were stored at 25°C ± 2°C at a relative humidity of 60% ± 10% for 0, 3, 6, 9, 12, 18, and 24 months. The stability samples were then tested for loss on drying, ash, and total steviol glycosides. A summary of the long-term shelf-stability results is presented in Table 11.

Table 11. TasteRight Refined Enzyme Treated Stevia Storage Stability Data

Time (months)	Loss on Drying (%)	Ash (%)	Total Steviol Glycosides[a] (%)	Microbial Limits	
				Total Aerobic Bacterial Count (cfu/g)	Yeast & Mold (cfu/g)
BATCH NUMBER 20150301					
0	3.11	0.10	96.09	< 10	< 10
3	3.12	0.10	96.32	< 10	< 10
6	3.10	0.10	96.60	< 10	< 10
9	3.14	0.10	95.77	< 10	< 10
12	3.16	0.10	96.30	< 10	< 10
18	3.20	0.10	96.56	< 10	< 10
24	3.18	0.10	97.07	< 10	< 10
BATCH NUMBER 20150302					
0	3.01	0.10	96.85	< 10	< 10
3	3.02	0.10	96.42	< 10	< 10
6	3.05	0.10	96.80	< 10	< 10
9	3.07	0.10	97.23	< 10	< 10
12	3.08	0.10	96.88	< 10	< 10
18	3.10	0.10	96.20	< 10	< 10
24	3.12	0.10	96.27	< 10	< 10
BATCH NUMBER 20150303					
0	3.06	0.10	96.10	< 10	< 10
3	3.05	0.10	96.73	< 10	< 10
6	3.01	0.10	96.75	< 10	< 10
9	3.08	0.10	95.92	< 10	< 10
12	3.10	0.10	95.98	< 10	< 10
18	3.12	0.10	96.53	< 10	< 10
24	3.11	0.10	96.16	< 10	< 10

[a] Reported as the sum of steviol glycosides and glycosylated steviosides.

A stability study report for TasteRight Refined Enzyme Treated Stevia is provided in Appendix 10.

The stability data in the scientific literature for stevioside, the JECFA report, and the extensive stability testing for rebaudioside A as presented by Merisant, Cargill, and Sunwin & WILD Flavors, along with Qufu, Sunwin, and NuNaturals's stability testing results, support the position that Qufu, Sunwin, and NuNaturals's TasteRight Refined Enzyme Treated Stevia preparation is well-suited for the intended food uses.

E. Calculation of Steviol Equivalents of TasteRight Glucosylated Steviol Glycosides

For comparative purposes, the content of steviol glycosides is often expressed as steviol or steviol equivalents. Each component steviol glycoside has a steviol equivalence factor that is calculated based upon the ratio of the molecular weights (MW) of steviol and a particular steviol glycoside, as shown in Table 12.

Table 12. Steviol Equivalency Factors for Various Steviol Glycosides

Component Steviol Glycoside	Molecular Weight	Steviol Equivalency Factor[a]
Stevioside	805	0.395
Rebaudioside C	951	0.334
Rebaudioside A	967	0.329

[a] Calculated by dividing the molecular weight of steviol (MW=318) by the molecular weight of each glycoside.

Using these steviol equivalency factors, along with the percent composition of the stevia extract starting material, it is possible to determine the steviol equivalency of the raw material steviol glycosides extract, as presented in Table 13.

Table 13. Steviol Equivalency of TasteRight Preparations

Component Steviol Glycoside	Average Range [a] (%)	Steviol Equivalents[b] (%)
Stevioside	81.56	32.22
Rebaudioside C	1.06	0.35
Rebaudioside A	8.84	2.91
Unspecified steviol glycoside(s)[c,d]	3.87	1.53
Total Steviol Equivalence		37.01

[a] Determined by averaging the representative values for the starting raw material steviol glycosides extract provided in Table 3
[b] Calculated by multiplying the % of the steviol glycoside by the steviol equivalency factor
[c] Percent of unspecified steviol glycosides determined by subtracting the sum of Reb A, stevioside, and Reb C from the total steviol glycosides content in the material based on the average values in Table 3
[d] Steviol equivalent calculated on worst-case basis, using the steviol equivalence factor for stevioside

The stevia extract starting material is enzymatically glycosylated as described in Part 2.B., in a process in which a glucosyltransferase enzyme adds glucose moieties, obtained from a maltodextrin source, to the steviol glycosides present in the raw material. It is reasonable to assume that all steviol glycosides and glucosylated steviol glycosides will maintain the same level

of steviol equivalence described above since no other reactions are known to occur from the known chemistry of the enzyme. Therefore, the steviol equivalency of the TasteRight preparations is expected to be no greater than 37.01 g steviol per 100 g TasteRight.

PART 3. DIETARY EXPOSURE

The subject TasteRight Enzyme Treated Stevia and Refined Enzyme Treated Stevia preparations are intended to be used as table top sweeteners and general purpose non-nutritive sweeteners in various foods other than infant formulas and meat and poultry, as defined in 21 CFR 170.3(o)(19).[6] 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 TasteRight to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation.[7]

A. Estimate of Dietary Exposure to the Substance

There have been many scholarly estimates of potential dietary intake replacement of sweeteners, including steviol glycosides, that have been published (FSANZ, 2008; WHO, 2003; Renwick, 2008) or submitted to FDA (Merisant, 2008). These are summarized in Appendix 11. In GRAS notification 301, a simplified estimate was proposed to, and accepted by, FDA based on the estimates of exposure in "sucrose equivalents" (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90th percentile consumer of a sweetener which is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg per kg (body weight) bw per day for any population subgroup.

The estimated sweetness intensity for TasteRight Enzyme Treated Stevia is approximately 100 times that of sucrose, while the estimated sweetness intensity for TasteRight Refined Enzyme Treated Stevia is approximately 260 times that of sucrose. A weighted sum estimate was used to determine the steviol equivalency factor for the TasteRight material on a worst-case scenario basis, and was determined to be 37.01 g steviol per 100 g TasteRight (as described in Part 2.F.).

The highest 90th percentile consumption by any population subgroup of TasteRight (corresponding to the Enzyme Treated Stevia preparation) would consume approximately 9.90 mg per kg steviol glycosides bw per day. Based on a weighted sum estimate for steviol equivalents provided in Table 13, the consumption would be less than 3.66 mg per kg bw per day on a steviol equivalents basis for any population group, on a worst-case scenario basis, for any of Qufu, Sunwin, and NuNaturals's TasteRight preparations. These calculations are summarized in Table 14 and Table 15 for TasteRight Enzyme Treated Stevia and TasteRight Refined Enzyme Treated Stevia, respectively.

[6] Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.
[7] 21 CFR 182.1(b)(1). Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=182.1

Table 14. Daily Intake of Sweeteners (in Sucrose Equivalents) & Estimated Daily Intakes of TasteRight Enzyme Treated Stevia

Population Group	Intakes of Sweeteners (mg sucrose/kg bw/day)[a]		Calculated Intake of TasteRight Enzyme Treated Stevia (mg/kg bw/day)[b]		Calculated Intake of TasteRight Enzyme Treated Stevia as Steviol Equivalents (mg/kg bw/day)	
	Low	High	Low	High	Low	High
Healthy Population	255	675	2.55	6.75	0.94	2.50
Diabetic Adults	280	897	2.80	8.97	1.04	3.32
Healthy Children	425	990	4.25	9.90	1.57	3.66
Diabetic Children	672	908	6.72	9.08	2.49	3.36

[a] From Renwick (2008).

[b] Calculated by dividing the sucrose intake by the minimum average relative sweetness value of 100 for TasteRight Enzyme Treated Stevia.

Table 15. Daily Intake of Sweeteners (in Sucrose Equivalents) & Estimated Daily Intakes of TasteRight Refined Enzyme Treated Stevia

Population Group	Intakes of Sweeteners (mg sucrose/kg bw/day)[a]		Calculated Intake of TasteRight Refined Enzyme Treated Stevia (mg/kg bw/day)[b]		Calculated Intake of TasteRight Refined Enzyme Treated Stevia as Steviol Equivalents (mg/kg bw/day)	
	Low	High	Low	High	Low	High
Healthy Population	255	675	0.98	2.60	0.36	0.96
Diabetic Adults	280	897	1.08	3.45	0.40	1.28
Healthy Children	425	990	1.63	3.81	0.60	1.41
Diabetic Children	672	908	2.58	3.49	0.96	1.29

[a] From Renwick (2008).

[b] Calculated by dividing the sucrose intake by the minimum average relative sweetness value of 260 for TasteRight Refined Enzyme Treated Stevia.

The values in Table 14 and Table 15 assume that Qufu, Sunwin, and NuNaturals's TasteRight preparations constitute the entire sweetener market, which makes these estimates extremely conservative since the likelihood of that occurrence is minimal. For the general healthy adult population, the estimated maximum intake of purified steviol glycosides is 6.75 mg per kg bw per day (2.50 mg per kg steviol equivalents) for TasteRight Enzyme Treated Stevia and 2.60 mg per kg bw per day (0.96 mg per kg steviol equivalents) for TasteRight Refined Enzyme Treated Stevia. For healthy children, the estimated maximal intake is 9.90 mg per kg bw per day (3.66 mg per kg

as steviol equivalents) for TasteRight Enzyme Treated Stevia and 3.81 mg per kg bw per day (1.41 mg per kg steviol equivalents) for TasteRight Refined Enzyme Treated Stevia. In all population groups, the estimated daily intake of purified steviol glycosides, expressed as steviol equivalents, is well below the JECFA-established acceptable daily intake (ADI) of 4.0 mg per kg bw per day steviol equivalents.

B. Estimated Dietary Exposure to Any Other Substance That is Expected to be Formed In or On Food

This section is not applicable to Qufu, Sunwin, and NuNaturals's TasteRight Enzyme Treated Stevia and Refined Enzyme Treated Stevia products, which would be chemically stable under conditions of use.

C. Dietary Exposure to Contaminants or Byproducts

While a recent publication by Kumari et al. (2016) investigated the Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Antioxidant Capacity (TAC) in *S. rebaudiana* leaf --- and the observed activity has been attributed to naturally-occurring phytochemicals such as phenolics, flavonoids, and pigments in the plant --- the study has minimal relevance with regard to the safety considerations of highly purified stevia extract, of which ≥ 95% consists of the most familiar steviol glycosides and their glucosylated steviosides. These phytochemical contaminants, if present, are in low amounts and were likely similarly present in purified test materials that were used in the toxicology studies summarized in Appendix 12.

Furthermore, no concerns regarding dietary exposure to contaminants or byproducts have been raised by expert regulatory bodies, including the World Health Organization/Joint FAO/WHO Expert Committee on Food Additives (WHO/JECFA), European Food Safety Authority (EFSA), Food Standards Australia New Zealand (FSANZ), and FDA, since JECFA's first steviol glycosides review was performed in 2000 (WHO, 2000).

PART 4. SELF-LIMITING LEVELS OF USE

It has been well-documented in the published literature that the use of steviol glycosides is self-limiting due to organoleptic factors and consumer taste considerations (Kochikyan et al., 2006; Carakostas et al., 2008; Brandle et al., 1998; Prakash et al., 2008; Gupta et al., 2016; Gerwig et al., 2016). These organoleptic factors include bitterness and astringency, as well as a lingering metallic aftertaste (Gerwig et al., 2016).

PART 5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

A. Other Information on Dietary Exposure

1. History of Traditional Medicinal and Human Food Use

Stevia has been used as a traditional medicine and sweetener by native Guarani tribes for centuries (Esen, 2016; Gerwig et al., 2016; Brusick, 2008; Brandle et al., 1998). 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 gram per person per day or more were reported to be necessary (Gregersen et al., 2004).

For about 30 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 (Raintree, 2012). It was previously reported that 40% of the artificial sweetener market in Japan had been stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999). Use of steviol glycosides as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand, and as a natural health product in Canada. It has wide use in China and Japan in food and in dietary supplements. In 2005, it was estimated that sales of stevia in the US reached $45 million (Newsday, 2006).

NewHope360 reported that the global market for stevia in 2014 was $347 million, and that is expected to increase to $565.2 million by 2020. In addition, consumption is expected to increase from 2014 levels of 5,100.6 tons to 8,506.9 tons by 2020 (NewHope360, 2015).

Most recently, Nutritional Outlook reported that Mintel data indicated a 48% increase in stevia-containing products over the last five years (Decker and Prince, 2018).

B. Summary of Regulatory History of Enzyme Modified Steviol Glycosides

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; Watson, 2010; Health Canada, 2012). In the United States, steviol glycosides have been used as a dietary supplement since 1995 (Geuns, 2003).

A brief overview of the most recent regulatory activity regarding steviol glycosides is presented below in Part 5.B. Sections 1-5; a more detailed historical overview is provided in Appendix 13.

1. U.S. Regulatory History

Based on available information from FDA's GRAS Notice Inventory website (FDA, 2019) as of March 25, 2019, FDA has issued 56 "no questions" letters on GRAS notices on rebaudioside A,

rebaudioside D, rebaudioside M, or steviol glycosides, including those undergoing enzyme treatment.

In addition, the Flavor and Extract Manufacturers Association (FEMA) includes 11 steviol glycosides preparations, three of which are for enzymatically modified stevia extracts, on their GRAS lists.

2. Canadian Regulatory History

On November 30, 2012, Health Canada published its final clearance for use of steviol glycosides as a sweetener in foods (Health Canada, 2012). In March 2014, Health Canada updated the List of Permitted Sweeteners (Lists of Permitted Food Additives) to include steviol glycosides in applications as a table-top sweetener and as an ingredient in a variety of foods, beverages, baked goods, meal replacement bars, condiments, and confectionary and gums (Health Canada, 2014). On January 15, 2016, Health Canada approved the use of rebaudioside M for use as a high-intensity sweetener under the same conditions as the previously approved steviol glycosides (Health Canada, 2016).

Most recently, Health Canada's Food Directorate has updated its List of Permitted Sweeteners to allow for the use of steviol glycosides as a sweetener in 'unstandardized snack bars,' including granola bars, cereal bars, fiber bars, and protein isolate-based bars (Health Canada, 2017b). Health Canada (2017a) also modified the List of Permitted Sweeteners to include "all the steviol glycosides in the Stevia rebaudiana Bertoni plant (stevia plant)."

3. European Regulatory History

An amendment to the European Union (EU) food additives regulation 231/2012, which became active on November 3, 2016, removed the previous requirement for stevia blends to contain at least 75% Reb A or stevioside. In addition, the updated regulation ---(EU) 2016/1814---now permits the following steviol glycosides in stevia blends: stevioside, rebaudiosides A, B, C, D, E, F and M, steviolbioside, rubusoside, and dulcoside (Searby, 2016).

The European Food Safety Authority (EFSA) Panel of Food Additives and Nutrient Sources reviewed an application for glucosylated steviol glycoside preparations for use as a new food additive. The Panel concluded that the data supplied by the applicant were "insufficient to assess the safety" of the glucosylated steviol glycosides preparation. It should be noted that no safety concerns were raised by the EFSA Panel, and that their decision was based on the "limited" data provided in the dossier submitted by the applicant (EFSA, 2018).

Recently, JECFA updated the steviol glycosides specifications to include a minimum requirement of not less than 95% total steviol glycosides, on a dry basis, "determined as the sum of all compounds containing a steviol backbone conjugated to any number, combination or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose xylose, galactose, arabinose and xylose) occurring in the leaves of *Stevia rebaudiana* Bertoni." Microbiological criteria were also

established, with specifications of no more than 1,000 CFU per g total plate count, not more than 200 CFU per g yeasts and molds, and *E. coli* and *Salmonella* negative in 1 g and 25 g, respectively (FAO, 2017).

4. Asian Regulatory History

No regulatory updates have been identified in recent years. The Asian regulatory history for steviol glycosides through 2014 is presented in Appendix 13.

5. Other Regulatory History

FSANZ called for submissions on permitting all minor steviol glycosides extracted from stevia leaf to be included in the definition of steviol glycosides in the Food Standards Code, noting that "[no] evidence was found to suggest that the proposed changes pose any public health and safety concerns." The submission period ended on December 19, 2016 (FSANZ, 2016b). Subsequently, on February 8, 2017, FSANZ approved a draft variation of the definition of steviol glycosides to include all steviol glycosides present in the *Stevia rebaudiana* leaf (FSANZ, 2017).

Most recently, FSANZ called for comments on the production of Reb M using enzymes derived from genetically modified yeast. The comment period closed on August 31, 2018 (FSANZ, 2018b). Subsequently, on October 31, 2018, FSANZ approved a draft variation to include a reference to the production method (FSANZ, 2018a).

PART 6. NARRATIVE

The biological, toxicological, and clinical effects of stevia and steviol glycosides have been extensively reviewed (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002). Additionally---and as noted earlier---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; WHO, 2006; WHO, 2007; WHO, 2008), and their findings have been summarized in Part 5.B.3. 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. These studies are summarized in Appendix 14.

Since the JECFA evaluation (WHO, 2008), FDA has received and not objected to over fifty-five GRAS notifications for steviol glycosides or enzyme modified steviol glycosides, many of which were discussed by Perrier et al. (2018). In each case, FDA has agreed with the conclusions that steviol glycosides are GRAS based largely on the 0 – 4 mg per kg bw per day ADI on a steviol equivalence basis that was established by JECFA. A recent publication by Roberts et al. (2016) indicates that the ADI could be higher, as discussed further in Appendix 11. Among the GRAS notifications submitted to FDA, several assessed purified preparations of rebaudioside A, and they were supported by additional toxicology and clinical studies that are summarized in Appendix 12.

Because of their sweetness characteristics, steviol glycosides have viable uses as a non-nutritive sweetener in foods.[8] Periodic reviews by JECFA over the years indicate the progression of knowledge on the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple investigations with *in vivo* and *in vitro* models, which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years, as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic, and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably, the initially reported concerns related to the effects of stevia leaves or crude extracts on fertility were refuted by the well-designed reproductive studies with purified steviol glycosides. All other concerns failed to manifest themselves at the doses employed in the long-term rat studies.

As discussed in Appendix 14 and elsewhere, at its 51st meeting, JECFA determined that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), to establish a temporary ADI of 0 - 2 mg per kg bw per day with an adequate margin of safety (Toyoda et al., 1997). The committee also critically reviewed the lack of carcinogenic response in well-conducted studies. These studies validated the Committee's conclusion that the *in vitro* mutagenic activity of steviol did not present a risk of carcinogenic effects *in vivo* and, therefore, all common steviol glycosides that likely share the same basic metabolic and excretory pathway and that use high purity preparations of various steviol glycosides, are safe as a sugar substitute. Subsequently, the additional clinical data reviewed by JECFA allowed the Committee to establish a permanent ADI of 0 - 4 mg per kg bw per day (based on steviol equivalents).

Recently, JECFA published a safety evaluation of a number of food additives, including steviol glycosides. The JECFA committee reviewed information supporting the safety of a *Yarrowia lipolytica* fermentation-produced rebaudioside A, which included a 90-day rat toxicity study and two *in vitro* genotoxicity studies, as well as *in vitro* colonic microflorae hydrolysis studies in several steviol glycosides, toxicokinetic studies of stevioside in humans and rats, and literature published since the 69th meeting.

The Committee noted that the most recent short-term toxicity studies were consistent with those reviewed at or prior to the 69th meeting, and that the new toxicokinetic study in humans did not

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

have a large enough subject pool to provide reliable toxicokinetic estimates to derive an update ADI for steviol glycosides. The Committee confirmed the current ADI of 0 – 4 mg per kg bw steviol. In addition, the Committee prepared new "tentative" specifications for steviol glycosides, which were expanded to include "any mixture of steviol glycosides compounds derived from *S. rebaudiana* Bertoni" while retaining the requirement that the total percentage of steviol glycosides is ≥ 95% (WHO, 2017).

Qufu, Sunwin, and NuNaturals critically reviewed the JECFA assessments and agree with the calculation of the ADI for steviol glycosides.

Several published and unpublished studies (summarized in Appendix 12) on purified preparations of rebaudioside A showed an absence of toxicological effects in rats (Curry and Roberts, 2008; Nikiforov and Eapen, 2008) and dogs (Eapen, 2008) in subchronic studies, and an absence of reproductive (Curry et al., 2008; Sloter, 2008a) and developmental effects (Sloter, 2008b) in rats. Most notably, pharmacokinetic studies in rats (Roberts and Renwick, 2008) and humans (Wheeler et al., 2008) on purified rebaudioside A follow the same pathway of being degraded to steviol by intestinal bacteria with subsequent rapid glucosylation and elimination in urine and feces.

Qufu, Sunwin, and NuNaturals conclude that these studies on rebaudioside A strengthen the argument that all steviol glycosides that follow the same metabolic pathway are safe at the JECFA established ADI.

Qufu, Sunwin, and NuNaturals have also reviewed the findings from human clinical studies, noting that ---with regard to 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 per kg bw per day in normal individuals, or approximately slightly more than 4 mg per kg bw on the basis of steviol equivalents (Barriocanal et al., 2008). Clinical studies on purified rebaudioside A showed an absence of effects on blood pressure (Maki et al., 2008a) and blood glucose levels (Maki et al., 2008b) at doses slightly higher than the exposures expected in food. Qufu, Sunwin, and NuNaturals conclude that there will be no effects on blood pressure and glucose metabolism in humans at the doses of steviol glycosides expected from its use in food as a non-nutritive sweetener.

Two previously published studies summarized in Appendix 12 raised a potential concern regarding the toxicological effects of steviol glycosides. In one study, DNA damage was seen in a variety of organs as assessed by Comet assay in rats given drinking water containing 4 mg per mL steviol glycosides for up to 45 days (Nunes et al., 2007a). Several experts in the field have since questioned the methodology used in this study (Geuns, 2007; Williams, 2007; Brusick, 2008). Qufu, Sunwin, and NuNaturals have reviewed the cited publications, along with the responses made by the authors (Nunes et al., 2007b; Nunes et al., 2007c), and concur with the challenges to

the methodology utilized by Nunes et al. (2007a), thereby discounting the validity and importance of this study.

In another study with stevioside in rats, tartrate-resistant alkaline phosphatase (TRAP) levels were measured and found to be significantly decreased at doses as low as 15 mg per kg bw (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 toxicology studies on steviol glycosides, nor has it been adequately vetted for application in toxicological studies. Critical reviews of this study 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 it lacked comparison of study findings against laboratory historical control data.

Urban et al. (2013) examined the extensive genotoxicity database on steviol glycosides because some concern has been expressed in two relatively recent publications (Brahmachari et al., 2011; Tandel, 2011) in which the authors concluded that additional testing is necessary to adequately address the genotoxicity profile (Urban et al., 2013). 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 is 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 addition, a recent paper by Shannon et al. (2016) raises a possible concern of endocrine disruption by steviol. Qufu, Sunwin, and NuNaturals reviewed the publication and note that the effects on progesterone production and on the action of progesterone (both antagonistic and agonistic) were observed *in vitro* in sperm cells. Qufu, Sunwin, and NuNaturals conclude that it is difficult to translate *in vitro* concentrations to local concentrations *in vivo* at receptors and that no adverse effects were observed in well-conducted reproductive toxicology studies. Therefore, this study does not alter Qufu, Sunwin, and NuNaturals's opinion that steviol glycosides preparations are generally recognized as safe. A summary of this study is provided in Appendix 12.

Philippaert et al. (2017) demonstrated that stevioside, rebaudioside A, and steviol potentiate the activity of transient receptor potential cation channel subfamily melastatin member 5 (TRPM5), a Ca^{2+}-activated cation channel that is expressed in type II taste receptor cells and pancreatic β-cells. The authors found that the steviol glycosides increased the perception of bitter, sweet, and umami tastes and also enhanced glucose-induced insulin secretion in a TRPM5-dependent

manner. Furthermore, *in vivo* studies indicated that daily consumption of stevioside prevents high-fat-induced diabetic hyperglycemia development in wild-type mice. No adverse events or animal deaths were discussed.

A commercially available steviol glycoside extract (>99%, composition and brand unknown) was used to investigate genotoxicity in human peripheral blood lymphocytes. Uçar et al. (2017) observed no significant differences in chromosomal aberration induction or micronuclei between the control and treatment groups at 24 and 48 h. These data support previous findings that steviol glycosides are not genotoxic.

Panagiotou et al. (2018) observed that steviol and steviol glycosides exert glucocorticoid receptor-mediated effects in human leukemic T-cells (Jurkat cells) but not in normal human peripheral blood mononuclear cells, which they concluded was due to a cell-type specific manner of glucocorticoid receptor-modulation.

Thøgersen et al. (2018) investigated the effect of rebaudioside A, stevioside, and steviol on porcine cytochrome p450 (CYP) expression and activity to assess their potential food-drug interactions in the IPEC-J2 cell line, which is a non-transformed cell line derived from intestinal porcine epithelial cells and in primary hepatocytes. The authors reported that there were no changes in CYP messenger ribonucleic acid (mRNA) expression following treatment of IPEC-J2 cells with rebaudioside A, stevioside, and steviol compared with control. Treatment of primary hepatocytes resulted in increases in CYP329 mRNA at low concentrations of rebaudioside A and steviol, and at all concentrations of stevioside. The authors reported that while treatment with the steviol glycosides tested over 24 hours resulted in minor increases in CYP3A29 mRNA expression (< 2.0 fold), “no direct effect on CYP activity” was observed. The authors concluded that rebaudioside A, stevioside, and steviol are unlikely to cause a food-drug interaction, but noted that the study could not predict long term effects and effects *in vivo*.

A recently published study addressed the genotoxic activity of stevia (Svetia™, purity not reported[9]). Human lymphocytes were treated with 5% and 0.5% Svetia™ for 2 hours. No statistically significant difference in genetic damage was observed in the 0.5% treatment concentration compared to the negative control, while the 5% treatment concentration resulted in a statistically significant difference (P<0.0001) compared to the control, with a decrease in migration average. The authors described the effect as being beneficial. Human lymphocytes treated with 10% Svetia™ demonstrated significant (P<0.0001) genotoxic activity compared to the control; however, at treatment concentrations of 0.05%, 0.5%, and 5% Svetia™, a significant (P<0.0001) decrease in average migration of DNA was observed compared to the control. The authors conclude that these results demonstrate the absence of genotoxicity at concentrations up to 5% Svetia™ (Silva et al., 2018). It should be noted that these observations are inconsistent with data

[9] While the purity of the material used for the study was not reported by Silva et al. (2018), a search of the manufacturer’s website (www.svetia.us) indicates that the trademarked material is a blend of cane sugar and 97% pure Reb A.

reported by Nunes et al. (2007a); however, as discussed above, the validity and importance of the Nunes et al. study has been discounted given the questions surrounding the methodology.

Qufu, Sunwin, and NuNaturals agree with the safety conclusions of the 56 GRAS Expert Panels in the notifications for steviol glycosides previously submitted to FDA that resulted in "no questions" responses from FDA, JECFA (WHO, 2006; WHO, 2008), and Renwick (2008) that a sufficient number of good quality health and safety studies exist to support the determination that purified preparations of steviol glycosides, when added to food at levels up to full replacement of sucrose on a sweetness equivalency basis, meet FDA's definition of safe.

Qufu, Sunwin, and NuNaturals conclude that it is reasonable to apply the JECFA ADI of 4 mg per kg bw per day for steviol glycosides (expressed on a steviol basis) to TasteRight Enzyme Treated Stevia and TasteRight Refined Enzyme Treated Stevia. Therefore, with the steviol equivalence values shown in Table 14, Qufu, Sunwin, and NuNaturals conclude that, for the general population, the estimated maximum daily intake of any TasteRight preparation is 9.90 mg per kg bw or 3.66 mg per kg expressed as steviol equivalents. Based upon these calculations, the intake of all of Qufu, Sunwin, and NuNaturals's TasteRight preparations safely aligns with the 4 mg per kg bw per day ADI expressed as steviol equivalents as determined by JECFA.

Qufu, Sunwin, and NuNaturals's TasteRight Enzyme Treated Stevia and Refined Enzyme Treated Stevia preparations contain not less than 95% total steviol glycosides and glucosylated steviosides. Given the structural similarities with rebaudioside A, stevioside, and other steviol glycosides, and considering analogous metabolic pathways for all these substances, the safety data on stevia and its other components have a direct bearing on the present safety assessment for TasteRight. This is further supported by over a decade and a half of scientific studies on the safety of these substances, along with the fact that the major regulatory bodies view the results of toxicology studies on either stevioside or rebaudioside A as applicable to the safety assessment of all known steviol glycosides, since all are metabolized and excreted by similar pathways, with steviol being the common metabolite for each. The foundational safety of Reb A, other steviol glycosides and steviol has been summarized, with key studies summarized in Appendix 12.

Furthermore, Qufu, Sunwin, and NuNaturals have reviewed this safety information and have concluded that TasteRight Enzyme Treated Stevia and Refined Enzyme Treated Stevia preparations are generally recognized as safe for the proposed uses.

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."[10]

[10] See 21 CFR 170.3 (e)(i) and 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 9/8/18).

Amplification is provided in that the conclusion 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, throughout the expert scientific community knowledgeable about the safety of substances directly or indirectly added to food, that there is reasonable certainty that the substance is not harmful under the conditions of its intended use."
>
> "'Common knowledge' can be based on either "scientific procedures" or on experience based on common use of a substance in food prior to January 1, 1958." [11]

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

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

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

General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive. General recognition of safety through scientific procedures shall be based upon the application of generally available and accepted scientific data, information, or methods, which ordinarily are published, as well as the application of scientific principles, and may be corroborated by the application of unpublished scientific data, information, or methods.

The apparent imprecision of the terms "appreciable," "at the time," and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety in this or any other area (Lu, 1988; Renwick, 1990; Rulis and Levitt, 2009).

[11] See 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 9/8/18).
[12] See Footnote 1.

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. Expert Panel Findings on Safety of TasteRight Preparations

An evaluation of the safety and GRAS status of the intended use of Qufu, Sunwin, and NuNaturals's TasteRight Enzyme Treated Stevia and Refined Enzyme Treated Stevia (≥95% total steviol glycosides and glucosylated steviosides) preparations has been conducted by an Expert Panel convened by GRAS Associates; the Panel consisted of Robert Kapp, Ph.D., Fellow Academy of Toxicological Sciences (ATS), Fellow Royal Society of Biology (FRSB) & European Registered Toxicologist (ERT, UK); Kara Lewis, Ph.D.; and Katrina Emmel, Ph.D., as Panel Chair. The Expert Panel reviewed Qufu, Sunwin, and NuNaturals's dossier as well as other publicly available information available to them. The individuals who served as Expert Panelists are qualified to evaluate the safety of foods and food ingredients by merit of scientific training and experience.

The GRAS Expert Panel report is provided in Appendix 15.

C. Common Knowledge Elements for GRAS Conclusions

The first common knowledge element for a GRAS conclusion requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The second common knowledge element for a GRAS conclusion requires that consensus exists within the broader scientific community.

1. Public Availability of Scientific Information

The majority of the studies reviewed on steviol glycosides and steviol have been published in the scientific literature as summarized in Appendix 12. Most of the literature relied upon by JECFA has also been published---most importantly the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website.

With regard to the safety documentation, the key pharmacokinetic data establish that steviol glycosides are not absorbed through the gastrointestinal (GI) tract, *per se*; they are converted to steviol by bacteria normally present in the large intestine, and the steviol is absorbed but rapidly metabolized and excreted (Gardana et al., 2003; Koyama et al., 2003b). The action of bacteria in the large intestine is directly supported by the published study that showed that steviol glycosides can be converted to steviol in the large intestine by normal anaerobic GI flora as demonstrated by an *in vitro* study in fecal homogenates (Koyama et al., 2003b; Renwick and Tarka, 2008).

The ADI for steviol glycosides has been set largely based on a published chronic study in rats (Toyoda et al., 1997) and several published clinical studies that report no pharmacological effects in humans at doses several fold higher than the ADI (Barriocanal et al., 2006; Barriocanal et al., 2008; Wheeler et al., 2008). As mentioned above, Roberts et al. (2016) noted that the ADI could be higher using a chemical-specific adjustment factor (CSAF), as defined by the WHO in 2005, determined by comparative studies in rats and humans, which they conclude can justify an ADI value of 6-16 mg per kg bw per day for steviol glycosides.

The toxicity of the metabolite steviol has been well reviewed in the published literature (Geuns, 2003; WHO, 2006; Urban et al., 2013).

In addition, there is a large, publicly available, collection of GRNs regarding steviol glycosides on FDA's website.

2. Scientific Consensus

The second common knowledge element for a GRAS conclusion requires that there be a basis to conclude that consensus exists among qualified scientists about the safety of the substance for its intended use.

A number of well-respected regulatory agencies, including JECFA, EFSA, FSANZ, the Switzerland Office of Public Health, and Health Canada, as well as numerous well-respected individual scientists, have indicated that steviol glycosides are safe for human consumption at doses in the range of the JECFA ADI (FAO, 2010; EFSA, 2010; FSANZ, 2008; Switzerland Federal Office of Public Health, 2008; Health Canada, 2012; Xili et al., 1992; Toyoda et al., 1997; Geuns, 2003; Williams, 2007). Since December 2008, over fifty-five GRAS notifications have been submitted to FDA for highly purified stevia-derived sweetener products, and FDA detailed reviews have consistently yielded "no questions" letters.

In summary, a compelling case can be made that scientific consensus exists regarding the safety of steviol glycosides when of sufficiently high purity. The central role of conversion to steviol and subsequent elimination with these naturally occurring steviol glycosides extends to the manner in which the various steviol glycosides molecules are metabolized and eliminated from the body. While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, Qufu, Sunwin, and NuNaturals believe that a wide consensus does exist in the scientific community to support a GRAS conclusion as evidenced by several publications (Carakostas, 2012; Geuns, 2007; Urban et al., 2013; Waddell, 2011; Williams, 2007; Brusick, 2008) that refute safety concerns expressed by a minority of scientists. Roberts et al. (2016) suggests that the ADI could be higher than has been previously accepted by the scientific community.

D. Conclusion

In consideration of the aggregate safety information available on naturally occurring steviol glycosides, Qufu, Sunwin, and NuNaturals conclude that TasteRight Enzyme Treated Stevia and Refined Enzyme Treated Stevia (≥ 95% total steviol glycosides and glucosylated steviosides) preparations defined in the subject notification are safe for use as a general purpose non-nutritive sweetener in foods other than infant formulas and meat and poultry products. The JECFA ADI for steviol glycosides of 4 mg per kg bw per day (as steviol equivalents) can be applied to Qufu, Sunwin, and NuNaturals's TasteRight preparations. Based on published dietary exposure data for other approved sweeteners and adjusting for relative sweetness intensity, intake was estimated for healthy non-diabetic children and adults, and diabetic children and adults with the following findings.

The worst-case estimated intakes of Qufu, Sunwin, and NuNaturals's TasteRight preparations for several population groups summarized in Part 3.A. are no greater than 3.66 mg per kg steviol equivalents per bw per day, which is below the ADI of 4 mg per kg bw expressed as steviol equivalents as established by JECFA. The dietary levels from anticipated food consumption are not likely to exceed the ADI when high purity glucosylated steviol glycosides (≥95% total steviol glycosides and glucosylated steviosides) are used as a general non-nutritive sweetener.

Accordingly, TasteRight Enzyme Treated Stevia and Refined Enzyme Treated Stevia (≥95%total steviol glycosides and glucosylated steviosides) as produced by Qufu, Sunwin, and NuNaturals and declared within the subject notification meet FDA's definition of safety in that there is "reasonable certainty of no harm under the intended conditions of use" as described herein and, therefore, are generally recognized as safe (GRAS).

PART 7. LIST OF SUPPORTING DATA AND INFORMATION IN THE GRAS NOTICE.

A. References

1. List of Acronyms

µg	Microgram
ADI	Acceptable daily intake
ADME	Absorption, Distribution, Metabolism and Excretion
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the plasma-concentration time curve
BP	Blood pressure
bw	Body weight
C	Celsius
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CGMP	Current Good Manufacturing Practice
CGTase	Cyclomaltodextrin glucanotransferase

C_{max}	Maximum (peak) serum concentration of substance is observed
CSAF	Chemical-specific adjustment factor
CYP	Cytochrome P450
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
EDI	Estimated daily intake
EFSA	European Food Safety Authority
EU	European Union
FCC	Food Chemicals Codex
FD&C Act	Federal Food Drug and Cosmetics Act
FDA	Food and Drug Administration
FEMA	Flavor Extract Manufacturers Association
FOIA	Freedom of Information Act
FSANZ	Food Standards Australia New Zealand
FSSAI	Food Safety and Standards Authority of India
g	Gram
GA	GRAS Associates
GEMS	Global Environment Monitoring System
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
gpt	Guanine phosphoribosyltransferase
GRAS	Generally Recognized as Safe
GRN	GRAS Notification
h or hr	Hour
HbA1c	Glycated hemoglobin
HPLC	High-Performance Liquid Chromatography
IADSA	International Alliance of Dietary/Food Supplement Associations
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg	Kilogram
L	Liter
LD_{50}	Median (50%) lethal dose
mg	milligram
mL	Milliliter
MPL	Maximum permitted level
mpn	Most probable number
mRNA	Messenger ribonucleic acid
MW	Molecular weight
n	number
NA	Not applicable
ng	Nanogram
NHANES	National Health and Nutrition Examination Surveys
NHP	Natural Health Products
NLT	Not less than
NMT	Not more than
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NR	Not reported
NS	Not specified
OECD	Organisation for Economic Co-operation and Development

ppm	Parts per million
RH	Relative humidity
SBP	Systolic blood pressure
TAC	Total Antioxidant Capacity
TFC	Total Flavonoid Content
T_{max}	Time at which maximum (peak) plasma concentration (C_{max}) of substance is observed
TPC	Total Phenolic Content
TRAP	Tartrate-resistant alkaline phosphatase
TRPM5	Transient receptor potential cation channel subfamily melastatin member 5
UDS	Unscheduled DNA synthesis
WHO/JECFA	World Health Organizaiton/Joint FAO/WHO Expert Committee on Food Additives

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