ORIGINAL SUBMISSION

PureCircle™

Natural Ingredients

#662

GRN 000662

RECEIVED
AUG 9 2016
OFFICE OF
FOOD ADDITIVE SAFETY

August 5, 2016

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

Dear Dr. Gaynor:

Re: GRAS Exemption Claim for Glucosylated Stevia Leaf Extract

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting one hard copy and one electronic copy (on CD), as the notifier [PureCircle Ltd., 915 Harger Road, Suite 250, Oak Brook, Illinois, 60523], a Notice of the determination, on the basis of scientific procedures, that Glucosylated Stevia Leaf Extract, produced by PureCircle Ltd., as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance and a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of Glucosylated Stevia Leaf Extract under the intended conditions of use, also are enclosed for review by the agency.

The enclosed electronic files for the Notice entitled, "GRAS Assessment of Glucosylated Stevia Leaf Extract" were scanned for viruses prior to submission and is thus certified as being virus-free using McAfee VirusScan 8.8.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Sidd Purkayastha, Ph.D.
VP, Head of Global Scientific & Regulatory Affairs
PureCircle Limited
915 Harger Road, Suite 250
Oak Brook, Illinois 60523
+1 - 630-361-0374x98 (Office)
+1 - 630-480-4365 (Fax)
+1- 217-417-8440 (Mobile)
sidd.purkayastha@purecircle.com

PureCircle

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Oak Brook, Illinois 60523
+1 - 630-361-0374x98 (Office)
+1 - 630-480-4365 (Fax)
+1- 217-417-8440 (Mobile)
sidd.purkayastha@purecircle.com

GRAS Assessment

of

Glucosylated Stevia Leaf Extract

Food Usage Conditions for General Recognition of Safety

For:

PureCircle Ltd.
915 Harger Road, Suite 250
Oak Brook, Illinois
U.S.A. 60523

Evaluation by GRAS Expert Panel:

I. Glenn Sipes, Ph.D.
Stanley M. Tarka, Jr., Ph.D.
John A. Thomas, Ph.D.

August 5, 2016

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I. GRAS EXEMPTION CLAIM

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

PureCircle Ltd.'s (herein "PureCircle") Glucosylated Stevia Leaf Extract has been determined to be Generally Recognized as Safe (GRAS) in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made by an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS evaluation is based on scientific procedures as described in the following sections; the evaluation accurately reflects the conditions of the intended use in foods of Glucosylated Stevia Leaf Extract.

As the notifier, PureCircle accepts responsibility for the GRAS determination that has been made for Glucosylated Stevia Leaf Extract as described in the subject notification. Consequently, Glucosylated Stevia Leaf Extract meeting the conditions described herein is exempt from premarket approval requirements for food ingredients.

Signed,

(b) (6)

August 05, 2016

Sidd Purkayastha, Ph.D
PureCircle Ltd.
915 Harger Road, Suite 250
Oak Brook, Illinois
U.S.A. 60523

Date

B. Name and Address of Notifier

Sidd Purkayastha, Ph.D
PureCircle Ltd.
915 Harger Road, Suite 250
Oak Brook, Illinois
U.S.A. 60523
Telephone: 630-361-0374
Facsimile: 630-480-4365
Email: sidd.purkayastha@purecircle.com

[1] 62 FR 18938 (17 April 1997); Available at http://www.gpo.gov/fdsys/pkg/FR-1997-04-17/pdf/97-9706.pdf (U.S. FDA, 1997).

C. Common Name & Identity of the Notified Substance

Glucosylated Stevia Leaf Extract [glucosylated steviol glycosides + parent steviol glycosides]

D. Conditions of Intended Use in Food

PureCircle intends to market Glucosylated Stevia Leaf Extract as a general purpose sweetening agent in the United States (U.S.), in accordance with current Good Manufacturing Practices (cGMP), as a table-top sweetener and in a variety of traditional food products, excluding infant formulas and meat and poultry products.

Most other high-intensity sweeteners have been approved by the U.S. Food and Drug Administration (FDA) as general purpose sweeteners with no restriction on their use in specific foods or use-levels. Hence, the foods to which high-intensity sweeteners are added to and the use-level is controlled by technological properties (*e.g.,* sweetness potency). As the sweetening potency of Glucosylated Stevia Leaf Extract (167 times sweeter than sucrose) is similar to that of other high-intensity sweeteners, the uses of Glucosylated Stevia Leaf Extract primarily reflect those currently permitted for high-intensity sweeteners in the U.S.

E. Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the *Code of Federal Regulations* (CFR) § 170.30, Glucosylated Stevia Leaf Extract has been determined by PureCircle to be GRAS on the basis of scientific procedures (U.S. FDA, 2015). This GRAS determination is based on data generally available in the public domain pertaining to the safety of glucosylated steviol glycosides and parent steviol glycosides as discussed herein, and on consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of Glucosylated Stevia Leaf Extract as a component of food [see Annex A, entitled, "**Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Determination of Glucosylated Stevia Leaf Extract for Use as a General Purpose Sweetener**"].

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the FDA upon request, or will be available for review and copying at reasonable times at the offices of: PureCircle USA, Inc., 915 Harger Road, Suite 250, Oak Brook, Illinois, U.S.A. 60523.

Should the FDA have any questions or additional information requests regarding this notification, PureCircle will supply these data and information.

II. INTRODUCTION

A. Objective

At the request of PureCircle, an Expert Panel ("the Expert Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended use of Glucosylated Stevia Leaf Extract as a general purpose sweetener is safe and suitable and would be GRAS based on scientific procedures. The Expert Panel consisted of the following qualified scientific experts: Dr. I. Glenn Sipes, Ph.D. (University of Arizona), Dr. Stanley M. Tarka, Jr., Ph.D. (The Tarka Group Inc., and The Pennsylvania State University), and Dr. John A. Thomas, Ph.D. (Indiana University School of Medicine).

B. Foreword

On June 27, 2016, the Expert Panel, independently and collectively, critically evaluated a dossier that included a comprehensive summary of scientific information on Glucosylated Stevia Leaf Extract. The dossier was prepared from information available within the public domain and also included details pertaining to the method of manufacture and product specifications for PureCircle's Glucosylated Stevia Leaf Extract, supportive analytical data, intended use-levels in foods, and consumption estimates for all intended uses. In addition, the Expert Panel evaluated other information deemed appropriate or necessary. Summaries of the data evaluated by the Expert Panel deemed pertinent to the GRAS evaluation of Glucosylated Stevia Leaf Extract are provided in Sections III to V.

C. Summary of Regulatory History of Glucosylated Steviol Glycosides

In the U.S., a number of enzyme-modified steviol glycosides preparations are GRAS for use as flavoring agents and/or general purpose sweeteners in foods. Specifically, a glucosylated steviol glycoside preparation manufactured by PureCircle, similar to the Glucosylated Stevia Leaf Extract that is the subject of this GRAS evaluation, was granted GRAS status by the FEMA GRAS Expert Panel in 2010 for use as a flavoring agent (FEMA # 4728) (Marnett *et al.*, 2013; Leffingwell and Leffingwell, 2014). This preparation recently underwent a self-GRAS determination for use as a flavoring agent with modifying properties, was GRAS Notified to the FDA (GRN 607), and is currently under review by the agency (PureCircle, 2015). Furthermore, 4 GRAS notices (GRN 337, 375, 448, 452) have been submitted to the FDA for review to which the agency raised no objections regarding the petitioners' conclusions that enzyme-modified steviol glycosides are GRAS for use as general purpose sweeteners in foods (U.S. FDA, 2011a,b, 2013a,b).

In several Asian countries, α-glucosylated steviol glycosides are approved for general use as sweeteners in a variety of foods and beverages (Marie, 1991; Das *et al.*, 1992; Ferlow, 2005). α-Glucosylatedtransferase-treated stevia, a substance composed mainly of α-glucosylatedsteviosides obtained from "stevia extract", is approved by The Ministry of Health, Labour and Welfare as a food additive from natural origin (Japan Food Chemical Research Foundation, 2014) and has a safe history of use as a food additive in Japan for over 25 years. Enzymatically modified stevia (glucosyl stevia) is listed in the Korea Food Additives Code as a natural additive (MFDS, 2015), and in Malaysia enzymatically modified stevia is regulated as a sweetening substance (Government of Malaysia, 2014).

III. CHEMISTRY & MANUFACTURING OF GLUCOSYLATED STEVIA LEAF EXTRACT

A. Chemistry of Glucosylated Stevia Leaf Extract

PureCircle's Glucosylated Stevia Leaf Extract is prepared by first extracting steviol glycosides from the leaves of the *Stevia rebaudiana* plant and then purifying this extract so that the total steviol glycoside content is greater than 95%. This purified stevia extract may contain a variety of glycosides, as more than 30 different steviol glycosides have been identified in the extracts obtained from the leaves of the *S. rebaudiana* plant (Purkayastha *et al.*, 2016), and such steviol glycoside preparations have recently been determined to be GRAS with no objection from the FDA (GRN 619) (U.S. FDA, 2016). Next, the purified steviol glycosides (>95%) are enzymatically modified such that additional glucose moieties are conjugated to the parent steviol glycoside structure *via* α-(1-4) linkages. The enzyme treatment generates a mixture of glucosylated steviol glycosides containing from 1 to 20 additional glucose units bound to the parent steviol glycoside; however, based on distribution analyses conducted by PureCircle, the mono-, di-, and tri-glucosylated forms generally predominate. PureCircle's Glucosylated Stevia Leaf Extract contains not less than 95% total steviol glycosides, determined by the sum of the glucosylated steviol glycosides and the parent steviol glycosides. Typical components of Glucosylated Stevia Leaf Extract preparations are outlined in Table 1.

PureCircle's Glucosylated Stevia Leaf Extract is a white to off-white powder that has a clean taste with a mild odor, is freely soluble in water, and is approximately 167 times sweeter than sucrose. Figure 1 depicts the chemical structure of some representative glucosylated steviol glycosides found in PureCircle's Glucosylated Stevia Leaf Extract, including glucosylated stevioside and glucosylated rebaudioside A (Koyama *et al.*, 2003a). The final Glucosylated Stevia Leaf Extract consists of not less than 95% total steviol glycosides, made up of a mixture of glucosylated steviol glycosides of different molecular weights as well as any parent steviol glycosides.

Table 1 Typical Components of Glucosylated Stevia Leaf Extract Preparations		
Molecule Name	**Molecular Formula**	**Molecular Weight (Da)**
Example Glucosylated Steviol Glycosides (~ 80 – 92% final steviol glycoside content)		
n-Glucosylated stevioside	$C_{(38+n*6)}H_{(60+n*10)}O_{(18+n*5)}$	804.87 + n*162.15
n-Glucosylated rebaudioside C	$C_{(44+n*6)}H_{(70+n*10)}O_{(22+n*5)}$	951.01 + n*162.15
n-Glucosylated rebaudioside A	$C_{(44+n*6)}H_{(70+n*10)}O_{(23+n*5)}$	967.01 + n*162.15
n-Glucosylated rebaudioside D	$C_{(50+n*6)}H_{(80+n*10)}O_{(28+n*5)}$	1129.2 + n*162.15
n-Glucosylated rebaudioside N	$C_{(56+n*6)}H_{(90+n*10)}O_{(32+n*5)}$	1275.3 + n*162.15
n-Glucosylated rebaudioside M	$C_{(56+n*6)}H_{(90+n*10)}O_{(33+n*5)}$	1291.3 + n*162.15
Example Parent Steviol Glycosides (~ 5 – 15% final steviol glycoside content)		
Rubusoside	$C_{32}H_{50}O_{13}$	642.73
Steviolbioside	$C_{32}H_{50}O_{13}$	642.73
Dulcoside A	$C_{38}H_{60}O_{17}$	788.87
Stevioside	$C_{38}H_{60}O_{18}$	804.87
Rebaudioside B	$C_{38}H_{60}O_{18}$	804.87
Rebaudioside F	$C_{43}H_{68}O_{22}$	936.99
Rebaudioside C	$C_{44}H_{70}O_{22}$	951.01
Rebaudioside A	$C_{44}H_{70}O_{23}$	967.01
Rebaudioside E	$C_{44}H_{70}O_{23}$	967.01
Rebaudioside D	$C_{50}H_{80}O_{28}$	1129.2
Rebaudioside N	$C_{56}H_{90}O_{32}$	1275.3
Rebaudioside M	$C_{56}H_{90}O_{33}$	1291.3
Rebaudioside O	$C_{62}H_{100}O_{37}$	1437.4

n = the number of glucose units enzymatically added to the parent steviol glycoside; n* = n multiplied by

Chemical Name: Not applicable [a mixture of glucosylated steviol glycosides and parent steviol glycosides]

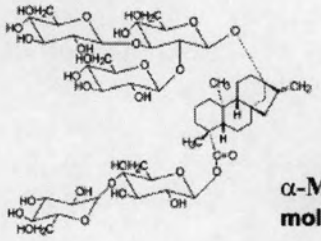
Chemical Formula: Not applicable

Formula Weight: Example estimated weighted average molecular weight: 1287.37 g/mol [see Table 4]

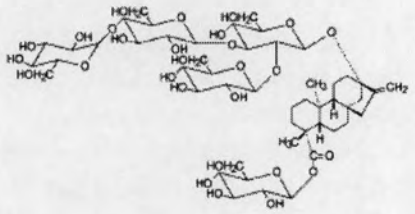
CAS Number: Not applicable

Structural Formula: See Figure 1

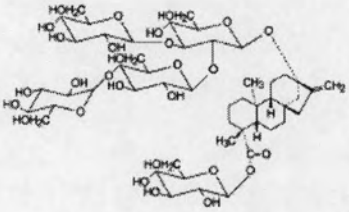
Figure 1 Representative Structures of Glucosylated Steviol Glycosides Found in Glucosylated Stevia Leaf Extract



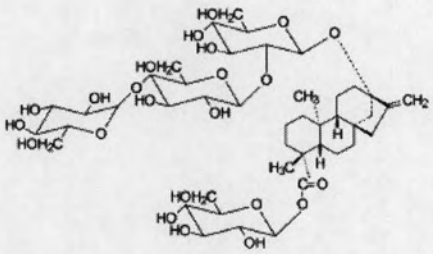
α-Monoglucosylrebaudioside A-1
mol. wt 1129



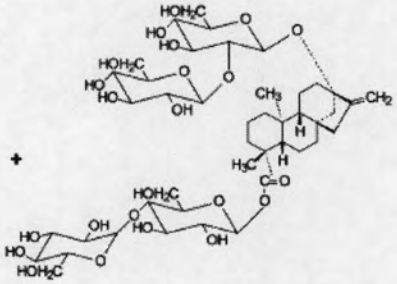
α-Monoglucosylrebaudioside A-3
mol. wt 1129



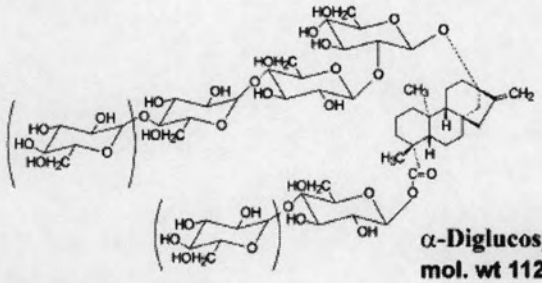
α-Monoglucosylrebaudioside A-2
mol. wt 1129



α-Monoglucosylstevioside-1
mol. wt 967



α-Monoglucosylstevioside-2
mol. wt 967



α-Diglucosylstevioside
mol. wt 1129

Source: adapted from Koyama *et al.*, 2003a

B. Manufacturing Process

1. Production Process

A schematic overview of an example production process for Glucosylated Stevia Leaf Extract, utilizing purified rebaudioside A (>95%) as the steviol glycoside starting material to be glucosylated, is illustrated below in Figure 2. The production process is consistent with the methodologies for the manufacture of steviol glycosides as described in the respective Chemical and Technical Assessment (CTA) published by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2007a). Glucosylated Stevia Leaf Extract is manufactured in a facility certified under Food Safety System Certification 22000:2010.

Stage 1

The production of a refined rebaudioside A crystal powder follows the procedure discussed below. Alternative example stevia extract powders containing other major steviol glycoside molecules, such as stevioside, rebaudiosides B, C, D, M, or N can be produced in the same manner.

S. rebaudiana leaves are placed in hot water at 50 to 60°C for 1 to 2 hours in continuous countercurrent extractors. The filtrate is separated using mesh screens, collected in a holding tank, and subsequently treated with a flocculant (*e.g.*, calcium hydroxide) to remove the mechanical particles, proteins, polysaccharides, and coloring agents. A plate-and-frame filter press is used to separate the resulting precipitate from the filtrate, and the filtrate is deionized by ion-exchange resins in (H^+) and (OH^-) form.

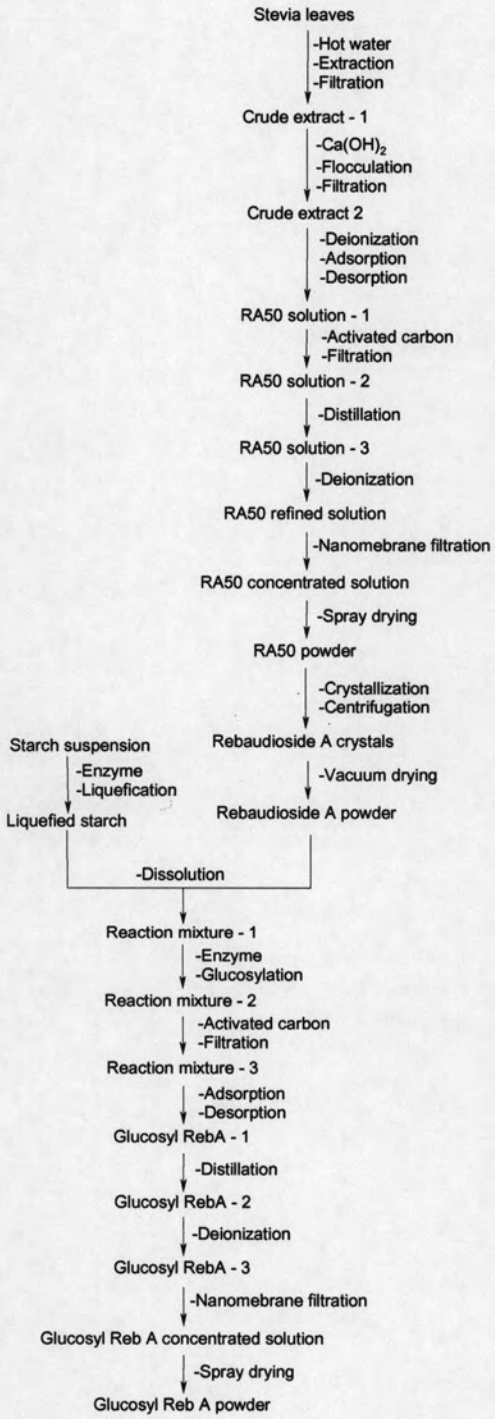
The deionized filtrate is fed to a column system packed with macroporous adsorption resin that retains the glycosides. The column is washed with deionized water to remove impurities that did not adsorb to the resin and then the glycosides are desorbed using aqueous ethanol. The obtained glycoside solution is treated with activated carbon and the carbon is separated from the solution by plate-and-frame filter press. A standard evaporator is used to remove the ethanol and the resulting aqueous solution is deionized again by ion-exchange resins in (H^+) and (OH^-) forms. The refined solution is concentrated using a nanofiltration membrane and the concentrated solution is spray dried to yield stevia extract powder containing >50% rebaudioside A (RA50). The RA50 stevia extract powder may be further purified by dissolving in aqueous ethanol and incubating at low temperature for several hours to allow for rebaudioside A to crystalize. The rebaudioside A crystals containing >95% rebaudioside A can be separated by conventional centrifugation and dried in a rotary drum vacuum dryer at 110°C and 10 mbar. Stevia extract powders are sifted through US 80 mesh stainless steel screens and passed through metal detectors to be packed in aluminum foil bags. All stevia extract powders (*i.e.*, stevioside, rebaudiosides A, B, C, D, M, or N) contain >95% total steviol glycosides.

Stage 2

Tapioca starch, a source of glucose units, is dissolved in reverse osmosis water and liquefied by the addition of cyclomaltodextrin glucanotransferase (CGTase) and/or α-amylase. Purified stevia extract powder (*i.e.,* RA50, >95% rebaudioside A) is added to the liquefied tapioca starch along with an additional portion of CGTase, and the reaction mixture is incubated at 60°C for 48 hours to generate mixtures of glucosylated steviol glycosides. If >95% rebaudioside A steviol glycoside powder is used as the starting material, the resulting glucosylated product can be referred to as glucosylated rebaudioside A. Likewise, utilization of other major steviol glycoside molecules as the starting material generates glucosylated stevioside and other glucosylated rebaudiosides (*i.e.,* B, C, D, M, and N). The enzymes are inactivated at the end of the reaction by heating for 15 minutes at 100°C. The reaction mixture is treated with activated carbon to remove the inactivated enzymes and the carbon is separated from the solution by plate-and-frame filter press. The filtrate is fed to a column system packed with macroporous adsorption resin and impurities (*i.e.,* dextrins) are removed by washing the column with deionized water. Note that an example glucosylated preparation utilizing RA50 as the source of steviol glycoside starting material is described in GRN 607, in which dextrins (<20%) are not removed from the final glucosylated steviol glycoside product (>80% steviol glycosides; ~7% parent steviol glycosides, ~75% glucosylated steviol glycosides) (PureCircle, 2015).

All steviol glycosides, both glucosylated and parent, are desorbed from the macroporous adsorption resin using aqueous ethanol. A standard evaporator is used to remove the ethanol and the resulting aqueous solution containing the Glucosylated Stevia Leaf Extract is deionized by ion-exchange resin in (H^+) and (OH^-) forms. The refined solution is concentrated using a nanofiltration membrane and then spray dried to yield Glucosylated Stevia Leaf Extract powder. The powder is sifted through US 80 mesh stainless steel screens and passed through metal detectors to be packed in aluminum foil bags. The bags are placed in high-density polyethylene drums sealed with tamper evident seals.

Figure 2 Example Manufacturing Process for Glucosylated Stevia Leaf Extract



Abbreviations: RA50, stevia extract powder containing >50% rebaudioside A; Reb, rebaudioside. [Note: This flow chart, as an example, shows Rebaudioside A (>95%) being used in the glucosylation reaction; however, it is also possible to use other 95% steviol glycoside preparations, such as RA50, stevioside, rebaudiosides B, C, D, M, or N.]

2. Raw Materials, Processing Aids and Equipment Specifications

All raw materials, processing aids, and purification equipment used to manufacture steviol glycosides (Stage 1) are food-grade ingredients[2] permitted by U.S. regulation or have been previously determined to be GRAS for their respective uses (Table 2).

Table 2 Raw Materials, Processing Aids, and Purification Aids Used in the Production of Steviol Glycosides

Material	Purpose	Regulatory Status
Stevia rebaudiana leaves	Source of steviol glycosides	N/A
High-purity calcium hydroxide	Flocculant	Permitted for use in food as a direct food additive with no limitations apart from cGMP, 21 CFR §184.1205
Ethanol, food-grade	Crystallization and desorption solvent	GRAS when used in accordance with cGMP
Activated carbon, food-grade	Decolorizing agent	GRAS
Ion-exchange resin	Purification	Used in accordance with 21 CFR §173.25
Divinyl benzene adsorption resin	Purification	Used in accordance with 21 CFR §173.25

CFR = Code of Federal Regulations (U.S. FDA, 2015); cGMP = current Good Manufacturing Practices; GRAS = Generally Recognized as Safe; N/A = not applicable

The manufacture of Glucosylated Stevia Leaf Extract, involving the enzymatic addition of glucose moieties to purified steviol glycosides (Stage 2), requires the use of the following additional raw materials and processing aids that are suitable food-grade materials and are used in accordance with applicable U.S. federal regulations.

i. Enzymes

CGTase derived from a non-genetically modified and non-pathogenic strain of *Bacillus stearothermophilus* (EC 2.4.1.19; CAS No. 9030-09-5) is used as a processing aid in the manufacture of Glucosylated Stevia Leaf Extract. CGTase is GRAS when used in accordance with cGMP [21 CFR §184.1012 (U.S. FDA, 2015)].

α-Amylase derived from a non-genetically modified and non-pathogenic strain of *Bacillus licheniformis* (Termamyl® Classic; EC 3.2.1.1) is used as a processing aid in the manufacture of Glucosylated Stevia Leaf Extract. α-Amylase is GRAS when used in accordance with cGMP [21 CFR §184.1148 (U.S. FDA, 2015)] and is widely used in the starch industry for liquefaction.

In the manufacturing process, several steps are undertaken to inactivate and remove the enzyme system used to catalyze the production of Glucosylated Stevia Leaf Extract. These

[2] Compliant with the specifications set forth in the Food Chemicals Codex (FCC) or equivalent international food or pharmacopeia standard (*e.g.*, JECFA, CODEX, EP).

processes include heating, treatment with activated carbon, resin purification, resin deionization, and nanofiltration.

ii. Carbohydrate Source

Tapioca starch (CAS No. 9005-25-8), derived from Cassava roots (*Manihot esculenta* Crantz Cassava), acts as a donor of glucose units when manufacturing Glucosylated Stevia Leaf Extract. Tapioca starch is commonly used in the food industry.

C. Product Specifications & Supporting Methods

The specifications outlined by PureCircle for Glucosylated Stevia Leaf Extract are based on those established for steviol glycosides by the JECFA following their 73rd meeting (JECFA, 2010), the specifications for rebaudioside A published in the Food Chemicals Codex (FCC, 2014a), as well as the proposed FCC specification for steviol glycosides (FCC, 2014b). Since Glucosylated Stevia Leaf Extract primarily consists of steviol glycosides, the purity specification of not less than 95% total steviol glycosides is consistent with the intent of the purity definition established by JECFA. A summary of the specifications for Glucosylated Stevia Leaf Extract established by PureCircle is provided in Table 3 along with the analyses of 5 non-consecutive lots of final product. The methods of analysis used are based on internationally recognized standards.

Table 3 Chemical and Microbial Specifications for PureCircle's Glucosylated Stevia Leaf Extract & Batch Analyses for 5 Nonconsecutive Lots

Specification Parameter	Limit	Manufacturing Lot				
		PT051015	PT071015	PT091015	PT121015	PT141015
Chemical Specifications						
Appearance	White to off-white powder	Conforms	Conforms	Conforms	Conforms	Conforms
Total Steviol Glycosides, % (anhydrous basis)	> 95.0	95.60	95.81	95.45	95.07	96.28
Loss on Drying, %	≤ 6.0	1.79	2.27	1.70	1.73	1.88
pH (1% solution)	4.5 to 7.0	6.50	6.24	6.19	6.29	6.65
Residual Ethanol, %	< 0.30	0.016	0.017	0.015	0.016	0.018
Residual Methanol, %	< 0.02	0.002	0.001	0.001	0.001	0.001
Total Ash, %	< 1.0	0.35	0.24	0.22	0.40	0.28
Lead (as Pb), ppm	< 1.0	0.025	0.021	0.019	0.032	0.054
Arsenic (as As), ppm	< 1.0	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Cadmium (as Cd), ppm	< 1.0	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Mercury (as Hg), ppm	< 1.0	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Microbial Specifications						
Total Plate Count, (CFU/g)	< 1,000	ND	ND	ND	ND	ND

Table 3 Chemical and Microbial Specifications for PureCircle's Glucosylated Stevia Leaf Extract & Batch Analyses for 5 Nonconsecutive Lots

Specification Parameter	Limit	Manufacturing Lot				
		PT051015	PT071015	PT091015	PT121015	PT141015
Yeast & Mold, (CFU/g)	< 200	ND	ND	ND	ND	ND
Total Coliforms, (MPN/g)	ND	ND	ND	ND	ND	ND
Escherichia coli count, (MPN/g)	ND	ND	ND	ND	ND	ND
Salmonella sp. (in 25 g)	Absent	Absent	Absent	Absent	Absent	Absent

CFU = colony forming units; MPN = most probable number; ND = not detected; ppm = parts per million.

The total steviol glycoside content of the final Glucosylated Stevia Leaf Extract product, including both glucosylated and parent steviol glycosides, was measured in accordance with the Japanese Ministry of Health, Labour and Welfare method for determining the purity of α-glucosylatedtransferase treated stevia (MHLW, 2009) and the JECFA method for measuring steviol glycosides (JECFA, 2008). PureCircle assessed the steviol glycoside content of the same 5 non-consecutive lots of Glucosylated Stevia Leaf Extract (Table 4) and the data demonstrate that the finished ingredient consistently meets the purity specification of greater than 95% total steviol glycosides (glucosylated + parent glycosides).

Table 4 Steviol Glycoside Content Present in 5 Nonconsecutive Lots of Glucosylated Stevia Leaf Extract

	Manufacturing Lot				
	PT051015	PT071015	PT091015	PT121015	PT141015
Glucosylated steviol glycosides (GSG)[a]					
Mono & di-GSG	38.27	39.01	38.68	37.67	38.44
Tri & tetra-GSG	25.29	26.25	28.94	25.73	26.95
n-GSG (n=5-20)	24.77	23.31	20.61	24.41	23.46
Total GSG (%)	88.33	88.57	88.23	87.82	88.85
Parent glycosides[b]					
Rebaudioside A (%)	6.81	6.82	6.79	6.77	7.00
Stevioside (%)	0.26	0.24	0.24	0.29	0.23
Rebaudioside C (%)	0.15	0.14	0.15	0.14	0.16
Rebaudioside F (%)	0.05	0.04	0.04	0.05	0.04
Total parent glycosides (%)	7.27	7.24	7.22	7.25	7.43
Total steviol glycosides (GSG + parent glycosides)	95.60	95.81	95.45	95.07	96.28
Other components including dextrin (estimated)	4.40	4.19	4.55	4.93	3.72

GSG = glucosylated steviol glycosides.
[a] GSG analyzed by MHLW method (MHLW, 2009).
[b] Steviol glycosides measured as per FAO JECFA Monograph 10 (JECFA, 2010), p17-21.

E. Additional Chemical Characterization

1. Pesticide Analysis

Pesticide analyses are conducted on all final Glucosylated Stevia Leaf Extract products. The same 5 non-consecutive lots of Glucosylated Stevia Leaf Extract tested above were subjected to a multi-residue pesticide screen that covered a range of commonly applied pesticides. No pesticide residues were detected in any of the finished products.

2. Sweetness Potency

The sweetness potency of PureCircle's Glucosylated Stevia Leaf Extract relative to sucrose was evaluated by an expert sensory panel. Glucosylated Stevia Leaf Extract (glucosylated rebaudioside A; Lot # GSG0915704) powder was dissolved in acidified water (pH 3.2) and test samples were prepared at the following concentrations: 100, 200, 250, 300, 350, and 400 ppm. Sucrose samples were also prepared in acidified water (pH 3.2) at 0, 2.5, 5, and 7.5%. Expert panelists (N=5) were instructed to first taste the sucrose reference samples, rinse with salt water, and then evaluate the test samples. The panel consensus was that the 300 ppm Glucosylated Stevia Leaf Extract test sample provided a sweetness equivalent to the 5% sucrose reference sample, indicating that PureCircle's Glucosylated Stevia Leaf Extract is around 167 times sweeter than sucrose.

3. Stability of Glucosylated Stevia Leaf Extract

The stability of steviol glycosides has been previously reviewed by a number of scientific advisory bodies involved in the evaluation of steviol glycoside safety [JECFA, the European Food Safety Authority (EFSA), and the Food Standards Australia/New Zealand (FSANZ)] and is also discussed in several published studies (Chang and Cook, 1983; Kroyer, 1999). Specifically, JECFA evaluated data on the stability of steviol glycosides under conditions mimicking their use in foods at their 68th meeting (JECFA, 2007b). The Committee noted that steviol glycosides do not undergo browning or caramelization when heated, and are reasonably stable under elevated temperatures used in food processing. Under acidic conditions (pH 2 to 4), steviol glycosides (approximately 90 to 94% purity), are stable for at least 180 days when stored at temperatures up to 24°C. When exposed to elevated temperatures (80°C, in water, 8 hours), however, 4 and 8% decomposition was observed in solutions of steviol glycosides at pH 4.0 and 3.0, respectively, indicating that the stability of steviol glycosides is pH and temperature dependent. When the temperature was increased to 100°C, expectedly higher rates of steviol glycoside decomposition (10 and 40% at pH 4.0 and 3.0, respectively) were observed. Based on the above findings, as well as additional publicly available stability studies, JECFA concluded that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions.

It is expected that the stability of Glucosylated Stevia Leaf Extract would be similar to individual steviol glycosides given the similarities in structure. Additional stability studies of Glucosylated Stevia Leaf Extract powders under normal and/or accelerated storage conditions as well as in solution at various pH levels and temperatures were conducted for confirmation. These studies are summarized in the sections below and demonstrate that the stability of Glucosylated Stevia Leaf Extract is similar to parent steviol glycosides.

i. Storage Stability of Glucosylated Stevia Leaf Extract

The storage stability of Glucosylated Stevia Leaf Extract Lot PT141015 (a glucosylated rebaudioside A preparation) was assessed. Powder samples were packed in aluminum bags and stored for up to 4 weeks at 40°C and 75% relative humidity (RH). To assess storage stability, samples were tested at baseline, 2 weeks, and 4 weeks, and the percent total steviol glycosides was compared between baseline and the time points thereafter. Total steviol glycoside content was found to remain stable over 4 weeks at 40°C/75% RH.

ii. pH Stability of Glucosylated Stevia Leaf Extract

The stability of Glucosylated Stevia Leaf Extract (Lot PT141015, a glucosylated rebaudioside A preparation) was assessed over a pH range of 2.0 to 8.0 for a total of 7 weeks at 4 different temperatures: 5, 25, 37, and 56°C. Samples were prepared at concentrations of approximately 50,000 mg/L in 500 mL of buffer solution and stored in amber glass vials. Buffer was prepared by mixing different ratios of 0.1 M sodium dihydrogen phosphate, 0.1 M phosphoric acid, or 0.1 M sodium hydrogen phosphate to obtain the target pH. Total steviol glycosides present in the stability samples, measured as the sum of rebaudioside A and glucosylated rebaudioside A, were evaluated at baseline as well as at weeks 2, 4, and 7.

The extent and rate of degradation of Glucosylated Stevia Leaf Extract, based on measured total steviol glycosides, was shown to be dependent on pH, temperature, and time. The samples were generally stable over 7 weeks at pH 4.0 to 8.0 at all 4 temperatures tested, as the majority of samples were within 5% of the starting baseline value. The least amount of degradation over 7 weeks (less than 1%) was reported in the following samples: pH 4.0, 5.0, and 8.0 at 5°C, pH 7.0 and 8.0 at 25°C, and pH 8.0 at 37°C. Some samples showed slightly higher levels of degradation, for example, after 7 weeks at 5°C, the pH 6.0 and pH 7.0 samples experienced losses of 10.5 and 13.9% respectively, and after 7 weeks at 56°C, the pH 6.0 and pH 8.0 samples degraded by 10.7 and 11.3%, respectively. Glucosylated Stevia Leaf Extract was also found to be stable over 7 weeks at pH 2.0 and 3.0, but only at the three lowest temperatures tested (the majority of samples were within 5% of baseline). Significant degradation (up to 45.2%) was reported when the low pH samples were stored at 56°C for 7 weeks.

Similar to individual steviol glycosides, the stability of Glucosylated Stevia Leaf Extract followed the same degradation pathway and was pH-, temperature-, and time-dependent. Therefore, the conclusions regarding the stability of steviol glycosides made by JECFA and other scientific bodies (that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions) can be extended to include the Glucosylated Stevia Leaf Extract that is the subject of this safety assessment.

IV. SELF-LIMITING LEVELS OF USE

The use of Glucosylated Stevia Leaf Extract in food is largely limited by the desired sweetness intended for a particular food or beverage product; therefore, the use of Glucosylated Stevia Leaf Extract as a general purpose sweetener in foods is self-limiting based on its organoleptic properties.

V. BASIS FOR GRAS DETERMINATION

The information summarized below demonstrates that Glucosylated Stevia Leaf Extract as a general purpose sweetener is GRAS based on scientific procedures. The Expert Panel reviewed the publically available safety data on glucosylated steviol glycosides (*i.e.,* enzyme-modified steviol glycosides; α-glucosylated steviol glycosides) during their GRAS assessment, including safe history of use information, *in vitro* metabolic studies, and available toxicological studies. In addition, given that glucosylated steviol glycosides are subject to the same metabolic fate as steviol glycosides, the results of the safety and toxicology studies conducted with parent steviol glycosides were considered applicable to the safety of glucosylated steviol glycosides and specifically Glucosylated Stevia Leaf Extract. Therefore, the Expert Panel also reviewed the data and information deemed pivotal in determining parent steviol glycoside safety during their GRAS assessment, including a detailed summary of the conclusions made by global scientific and regulatory authorities regarding the safety of steviol glycosides as well as data pertaining to their metabolic fate in rats and humans. Based on these data, the Expert Panel concluded that Glucosylated Stevia Leaf Extract was GRAS under the aforementioned intended conditions of use in food based on scientific procedures. A summary of the data reviewed by the Expert Panel is presented herein.

A. Intended Dietary Uses

Glucosylated Stevia Leaf Extract, with a relative sweetening potency of 167 times that of sucrose, is intended for use as a general purpose sweetening agent, in accordance with cGMP. A number of high-intensity sweeteners have been approved by the FDA as general purpose sweeteners without their uses being restricted to specific foods or use-levels (*e.g.,* acesulfame-potassium, aspartame, sucralose). Hence, the foods to which high-intensity sweeteners are added and the use-levels are controlled by technological properties (*e.g.,* sweetness potency).

The sweetness potency of PureCircle's Glucosylated Stevia Leaf Extract, specifically a glucosylated rebaudioside A preparation, was determined to be 167 times sweeter than sucrose. Considering that Glucosylated Stevia Leaf Extract is characterized by a sweetness profile that is, for the most part, comparable to that of other glucosylated steviol glycoside preparations (GRN 337, 375, 448, 452), the uses and use-levels of PureCircle's Glucosylated Stevia Leaf Extract are likely to reflect those currently permitted for glucosylated steviol glycoside sweeteners produced in accordance with cGMP in the U.S. (U.S. FDA, 2011a,b, 2013a,b).

B. Estimated Daily Intake

Numerous surveys have been completed in various global jurisdictions (U.S., Canada, Brazil, Australia/New Zealand, and countries in the European Union) to assess daily consumption estimates of other well-established high-intensity sweeteners in the marketplace (*e.g.,* aspartame, cyclamate, saccharin, sucralose). Although intake modeling is commonly used to estimate the consumption of a particular ingredient, a more realistic, but conservative approach is to estimate the intake of Glucosylated Stevia Leaf Extract based on the intake figures reported in these published studies.

For example, Renwick (2008) used the available post-market surveillance data for other high-intensity sweeteners as the basis for determining the estimated dietary exposure for rebaudioside A by assuming full replacement of the currently approved intense sweeteners with the new sweetener. In order to estimate rebaudioside A intakes, Renwick (2008) first expressed the post-market surveillance intake estimates for intense sweeteners presently used in the global marketplace as sucrose equivalents in various population groups (average and high-end non-diabetic and diabetic adult and child consumers) (see Table 5). The data used in these analyses were primarily derived from studies that used specifically designed food diaries combined with actual use-levels or approved levels in different foods and beverages (Renwick, 2008). In order to predict dietary exposure to rebaudioside A, the intake estimates for the high-intensity sweeteners (expressed as sucrose equivalents) were adjusted for the sweetness intensity of rebaudioside A relative to sucrose (approximately 200). This intake assessment methodology yields intake estimates that while conservative, as it is unlikely that the new sweetener would entirely replace all other sweeteners in the marketplace, are realistic in that they reflect actual post-market intakes of high-intensity sweeteners.

In the case of PureCircle's Glucosylated Stevia Leaf Extract, the same methodology utilized by Renwick (2008) was applied to estimate intake values. Since PureCircle's glucosylated rebaudioside A, was determined to be 167 times sweeter than sucrose, the intake values for intense sweeteners presented in Table 5 were adjusted accordingly to derive an estimated intake for Glucosylated Stevia Leaf Extract. The estimated intakes were then converted to steviol equivalents based upon an estimated weighted average molecular weight of

1,287.37 g/mol for glucosylated rebaudioside A that was generated based upon the composition data provided by PureCircle in Table 4. The predicted intakes of Glucosylated Stevia Leaf Extract presented in Table 5, expressed as steviol equivalents, are all below the current acceptable daily intake (ADI) defined by the JECFA for steviol glycosides of 0 to 4 mg/kg body weight/day expressed as steviol equivalents (JECFA, 2007b).

Table 5 Estimated Consumption of Glucosylated Stevia Leaf Extract Using Renwick's (2008) Methodology of Intense Sweetener Intake Assessment Based on Post-Market Surveillance Intake Data for Currently Used Sweeteners

Population Group	Intakes of intense sweeteners [expressed as sucrose equivalents] (mg/kg bw/day)		Consumption estimates for:			
			Glucosylated Stevia Leaf Extract[a] (mg/kg bw/day)		Glucosylated Stevia Leaf Extract as steviol equivalents[b] (mg/kg bw/day)	
	Average Consumer	High Consumer	Average Consumer	High Consumer	Average Consumer	High Consumer
Non-diabetic Adults	255	675	1.53	4.04	0.38	1.00
Diabetic Adults	280	897	1.68	5.37	0.42	1.33
Non-diabetic Children	425	990	2.54	5.93	0.63	1.47
Diabetic Children	672	908	4.02	5.44	1.00	1.35

[a] Glucosylated Stevia Leaf Extract is 167 times as sweet as sucrose.
[b] Calculated based on an estimated weighted average molecular weight of 1287.37 g/mol for Glucosylated Stevia Leaf Extract described in Table 4 [conversion factor of 4.04, based on a molecular weight of 318.45 g/mol for steviol].

As part of their evaluation of the safety of steviol glycosides in 2008, JECFA considered various intake models for the estimation of dietary exposure to steviol glycosides, including the intake analysis conducted by Renwick (2008). Although higher intake estimates than those presented by Renwick (2008) were identified using other methodologies, including ones considering replacement of all sweeteners used in food (up to approximately 6 mg/kg body weight/day, expressed as steviol equivalents), it was noted by JECFA that such replacement estimates were highly conservative and that actual exposures to steviol glycosides (expressed as steviol equivalents) would be 20 to 30% of these values (1 to 2 mg/kg body weight/day, expressed as steviol equivalents). Furthermore, JECFA noted that the intake estimates based on post-market surveillance further confirmed the lower range.

C. History of Use

Glucosylated steviol glycosides, also described as enzyme-modified steviol glycosides, were developed to improve the sweetness qualities of steviol glycosides. α-Glucosylatedtransferase-treated stevia, a substance composed mainly of α-glucosylatedsteviosides obtained from "stevia extract", is approved by The Ministry of Health, Labour and Welfare as a food additive from

natural origin (Japan Food Chemical Research Foundation, 2014) and has a safe history of use as a food additive in Japan for over 25 years. Likewise, in several other Asian countries, α-glucosylated steviol glycosides are approved for general use as sweeteners in a variety of foods and beverages (Marie, 1991; Das *et al.*, 1992; Ferlow, 2005). Enzymatically modified stevia (glucosyl stevia), for example, is listed in the Korea Food Additives Code as a natural additive (MFDS, 2015), and in Malaysia enzymatically modified stevia is regulated as a sweetening substance (Government of Malaysia, 2014). In the U.S., a number of enzyme-modified steviol glycosides preparations are GRAS for use as flavoring agents and/or general purpose sweeteners in foods. Specifically, a glucosylated steviol glycoside preparation manufactured by PureCircle, similar to the Glucosylated Stevia Leaf Extract that is the subject of this GRAS evaluation, was granted GRAS status by the FEMA GRAS Expert Panel in 2010 for use as a flavoring agent (FEMA # 4728) (Marnett *et al.*, 2013; Leffingwell and Leffingwell, 2014). This preparation was also recently determined to be self-GRAS for use as a flavoring agent with modifying properties, has been notified to the FDA (GRN 607), and is currently under review by the agency (PureCircle, 2015). Furthermore, 4 GRAS notices (GRN 337, 375, 448, 452) were submitted to the FDA for review and the agency raised no objections regarding the petitioners' conclusions that enzyme-modified steviol glycosides are GRAS for use as general purpose sweeteners in foods (U.S. FDA, 2011a,b, 2013a,b).

D. Safety Data for Glucosylated Stevia Leaf Extract

The scientific conclusions regarding the safety of Glucosylated Stevia Leaf Extract are primarily based on the fact that glucosylated steviol glycosides are metabolized to steviol in a similar manner as non-modified steviol glycosides. Therefore, the safety conclusions for steviol glycosides in general can be extended to include Glucosylated Stevia Leaf Extract. As such, presented below is a detailed summary of the data deemed pivotal in determining the safety of steviol glycosides, in addition to the publically available safety data obtained specifically with glucosylated steviol glycoside preparations (*i.e.*, enzyme-modified steviol glycosides; α-glucosylated steviol glycosides). To identify scientific publications specifically relevant to the safety of Glucosylated Stevia Leaf Extract, a comprehensive and detailed search of the published scientific literature was conducted through May 2016 and the following databases were accessed: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®.

1. Metabolic Fate of Glucosylated Steviol Glycosides

The microbial metabolism of enzymatically-modified steviol glycosides was investigated *in vitro* using human fecal homogenates (Koyama *et al.*, 2003a). Two glucosylated steviol glycosides, α-monoglucosylated rebaudioside A and α-monoglucosylated stevioside, were incubated with

human fecal homogenates for up to 24 hours at 37°C under anaerobic conditions and metabolites were analyzed and identified by liquid chromatography-mass spectrometry (LC-MS). Both glucosylated compounds were reported to be completely metabolized to steviol after 24 hours. Similarly, a mixture of enzymatically-modified stevia containing the primary components α-glucosylated rebaudioside A, α-glucosylated stevioside, α-glucosylated rebaudioside C, and α-glucosylated dulcoside A, was also reported to hydrolyze to steviol following a 24-hour *in vitro* incubation with human fecal homogenates. The metabolic pathway for α-glucosylated steviol glycosides was shown to start with α-deglucosylation to the parent steviol glycoside, followed by hydrolysis to steviol, similar to that established for parent steviol glycosides.

As part of the safety assessment provided in GRAS Notification GRN 337 for glucosylated enzyme treated stevia, gastrointestinal degradation was evaluated in 2 *in vitro* studies (U.S. FDA, 2011a). In the first study, glucosylated stevia was incubated for 4 hours at 37°C in simulated gastric juices (sodium chloride, hydrochloric acid, pepsin, pH 1.2) followed by LC-MS analysis to detect any enzymatic or non-enzymatic degradation. No cleavage of the added glucose units was reported and the structure was determined to remain intact in simulated gastric juices. In the second study, glucosylated stevia was incubated with human fecal homogenates under anaerobic conditions for 24 hours at 37°C. Glucosylated stevia was shown to hydrolyze to steviol in a concentration- and time-dependent manner and exhibited similar hydrolysis rates over 24 hours to those measured for non-modified steviol glycosides.

Metabolic studies with steviol glycosides report that human digestive enzymes are not capable of hydrolyzing β-glycosidic bonds (Hutapea *et al.,* 1997; Geuns *et al.,* 2007), and, thus, steviol glycosides are not digested in the upper gastrointestinal tract. Since glucosylated steviol glycosides can contain α-oriented glycosidic bonds, it is possible that these bonds could be hydrolyzed by digestive enzymes (*i.e.,* salivary and/or pancreatic α-amylase). Enzymatically treated stevia has in fact been experimentally exposed to amylase *in vitro*, and based on the limited details reported in the abstract of this study, enzymatically treated stevia did convert to parent steviol glycosides when incubated with amylase (Shibasato, 1995). Although the details of this study were reported in Japanese, it appears that amylase can generate parent steviol glycosides in the upper gastrointestinal tract. This would allow for the parent steviol glycosides to be degraded by the established metabolic pathway to steviol, and would release the α-glycosidic bonded sugar moieties to be absorbed in the intestine and metabolized *via* normal carbohydrate metabolism pathways.

2. Toxicological Studies Conducted with Glucosylated Steviol Glycosides

The subchronic toxicity of an α-glucosylated steviol glycoside preparation was evaluated by Toyo Sugar and the results of the study were reported in GRAS Notification GRN 375 (U.S. FDA, 2011b). Rats [strain not specified] (10/sex/group) consumed α-glucosylated steviol glycosides mixed in the diet at concentrations of 0, 1.25, 2.5, or 5.0% (equivalent to 0, 253, 519,

or 1,059 or 0, 289, 601, or 1,153 mg steviol equivalents/kg body weight/day, for males or females, respectively) for 13 weeks. No deaths, clinical signs of toxicity, or abnormalities in ophthalmoscopic examinations were reported throughout the study. Some measures of hematology, clinical chemistry, urinalysis, and organ weights were reported to be statistically different between some of the groups, but were determined to be of no toxicological significance due to the facts that findings were not consistent between sexes, lacked dose-dependency, and could not be confirmed by related macroscopic or microscopic findings during organ examinations. Overall, Toyo Sugar concluded that their α-glucosylated steviol glycosides were safe and well-tolerated and that the no-observed-adverse-effect level (NOAEL) in rats for 13 weeks was the highest concentration tested (5%), equivalent to 1,059 and 1,153 mg steviol equivalents/kg body weight/day for males and females, respectively.

Toyo Sugar also evaluated the genotoxic potential of their α-glucosylated steviol glycoside product both *in vitro* and *in vivo* and reported in GRN 375 that the outcomes of all genotoxic assays were negative, indicating a lack of genotoxicity (U.S. FDA, 2011b). Further details of the experiments were not provided and these studies have not been published.

A number of studies with enzymatically treated stevia were summarized in a Japanese article by Shibasato (1995). Enzymatically treated stevia studied *in vitro* was reported to be non-mutagenic in a bacterial assay and the acute oral median lethal dose in mice was reported to be greater than 60 g/kg body weight. Lastly, when administered to rats chronically (22 to 24 months) at a dose of 550 mg/kg body weight/day the study results were described as "negative".

3. Summary of Safety Conclusions for Steviol Glycosides

The safety of steviol glycosides has been considered by several scientific bodies and regulatory agencies, including the FDA, JECFA, European Commission's Scientific Committee on Food (SCF), EFSA, FSANZ, and Health Canada. Interest in the use of steviol glycosides as sweeteners has encouraged extensive testing of the compounds and as such a large safety database exists. This database includes a thorough examination of the comparative metabolism and pharmacokinetics of steviol glycosides in experimental animals and humans, acute toxicity studies, short- and long-term toxicity and carcinogenicity studies, reproductive and developmental toxicology studies, *in vitro* and *in vivo* mutagenicity/genotoxicity studies, and human studies. Although many earlier studies examining the safety of steviol glycosides were conducted with stevioside due to the predominance of stevioside in *S. rebaudiana* leaves (Aze *et al.*, 1991; Toyoda *et al.*, 1997), the database pertaining to the safety of steviol glycosides was expanded following the completion of additional short-term toxicity, reproductive toxicity, *in vitro* and *in vivo* mutagenicity/genotoxicity studies, and human studies on rebaudioside A (Curry and Roberts, 2008; Curry *et al.*, 2008; Nikiforov and Eapen, 2008; Williams and Burdock, 2009). Although the majority of toxicity studies have been conducted with either purified stevioside or rebaudioside A, the extensive database on the common metabolic fate of steviol glycosides has

permitted the scientific bodies and regulatory agencies to extend their safety opinion to all steviol glycosides, rather than just individual glycosides.

i. Metabolic Fate of Steviol Glycosides

In vitro and *ex vivo* studies have demonstrated that steviol glycosides are not hydrolyzed by digestive enzymes of the upper gastrointestinal tract and are not absorbed through the upper portion of the gastrointestinal tract (Hutapea *et al.*, 1997; Geuns *et al.*, 2003, 2007; Koyama *et al.*, 2003b). Therefore, steviol glycosides enter the colon intact, where they are subject to microbial degradation by members of the *Bacteroidaceae* family, resulting in the release of the aglycone steviol (Gardana *et al.*, 2003; Renwick and Tarka, 2008). Several *in vitro* studies mimicking the anaerobic conditions of the colon, reviewed extensively by Renwick and Tarka (2008), have confirmed the ability of gut microflora from mice, rats, hamsters, and humans to hydrolyze steviol glycosides completely to steviol (Wingard *et al.*, 1980; Hutapea *et al.*, 1997; Gardana *et al.*, 2003; Koyama *et al.*, 2003a). Numerous studies have been conducted that demonstrate the similarities in the microbial metabolism among the different steviol glycosides. For instance, the degradation of a stevia mixture containing rebaudioside A, stevioside, rebaudioside C, and dulcoside A (percent composition not reported) was investigated in the presence of human fecal homogenates under anaerobic conditions and was found to degrade completely to steviol within 24 hours (Koyama *et al.*, 2003a). Rebaudioside E incubated *in vitro* with crude pectinase, an enzyme associated with the resident pectinolyic bacteria of the human intestine, hydrolyzed to steviol (Jensen and Canale-Parola, 1985). Rebaudioside D incubated with rat cecal contents for 90 minutes was reported to hydrolyze to stevioside and steviol, and was comparable to that of rebaudioside A (Nikiforov *et al.*, 2013). These similarities in microbial metabolism have been confirmed in several parallel *in vitro* comparisons with rebaudioside A, and a remarkable similarity with respect to the rate of hydrolysis of the individual steviol glycosides to steviol, particularly during the first 24 hours of incubation, indicates that the number and location of sugar units attached to the steviol backbone does not significantly impact the rate of hydrolysis (Purkayastha *et al.*, 2014, 2015, 2016).

Steviol is absorbed systemically into the portal vein and distributed to a number of organs and tissues, including the liver, spleen, adrenal glands, fat, and blood (Nakayama *et al.*, 1986; Sung, 2002 [unpublished]; Koyama *et al.*, 2003b; Wang *et al.*, 2004; Roberts and Renwick, 2008). In the liver, steviol primarily undergoes conjugation with glucuronic acid to form steviol glucuronide. In rats, free steviol (82 to 86% of chromatographed radioactivity), steviol glucuronide (10 to 12% of chromatographed radioactivity), and 2 unidentified metabolites (5 to 6% of chromatographed radioactivity) were detected in the plasma 8 hours after oral administration with either rebaudioside A or stevioside (Roberts and Renwick, 2008). Similarly, steviol glucuronide was identified in the plasma following ingestion of stevioside or rebaudioside A in humans, with maximal concentrations detected 8 and 12 hours after administration,

respectively (Geuns and Pietta, 2004 [unpublished]; Simonetti *et al.*, 2004; Geuns *et al.*, 2007; Wheeler *et al.*, 2008).

In rats, free and conjugated steviol, as well as any unhydrolyzed fraction of the administered glycosides, are excreted primarily in the feces *via* the bile (generally within 48 hours), with smaller amounts appearing in the urine (less than 3%) (Wingard *et al.*, 1980; Nakayama *et al.*, 1986; Sung, 2002 [unpublished]; Roberts and Renwick, 2008). Conversely, in humans elimination of steviol glycosides, primarily as steviol glucuronide with very small amounts of the unchanged glycoside or steviol, occurs *via* the urine. Relative to amounts recovered in urine, larger amounts of steviol (unabsorbed steviol released from steviol glycosides in the colon or from small amounts of steviol glucuronide secreted back into the gut *via* the bile) were also eliminated in the feces (Kraemer and Maurer, 1994; Geuns and Pietta, 2004 [unpublished]; Simonetti *et al.*, 2004; Geuns *et al.*, 2006, 2007; Wheeler *et al.*, 2008). The inter-species difference in the route of elimination of systemically absorbed steviol as steviol glucuronide (*via* the bile in rats and in the urine in humans) occurs as a result of the lower molecular weight threshold for biliary excretion in rats (325 Da) as compared to humans (500 to 600 Da; molecular weight of steviol glucuronide is 495 Da) (Renwick, 2007). The difference in the route of elimination is considered to be of no toxicological significance due to the fact that the water soluble phase II metabolites are rapidly cleared in both species. Therefore, toxicology data generated in rats are considered applicable to the assessment of the safety of steviol glycosides in humans given the similarities in metabolic fate.

ii. Safety Opinions Issued by Scientific & Regulatory Authorities

a) The Joint FAO/WHO Expert Committee on Food Additives (JECFA)

The safety of steviol glycosides was reviewed by JECFA at 4 separate meetings (51st, 63rd, 68th, and 69th) in 1998, 2004, 2007, 2008 (JECFA, 1998, 2004, 2007a,b, 2008). The committee has reviewed data demonstrating that stevioside and rebaudioside A are not genotoxic as well as data pertaining to the metabolic fate of steviol glycosides in rats and humans (Roberts and Renwick, 2008; Wheeler *et al.*, 2008), subchronic and reproductive/developmental toxicity of rebaudioside A (Curry and Roberts, 2008; Curry *et al.*, 2008; Nikiforov and Eapen, 2008), and the potential pharmacological effects of steviol glycosides in diabetic populations and individuals with normal or low-normal blood pressure (Maki *et al.*, 2008a,b). An ADI of 0 to 4 mg/kg body weight/day (expressed as steviol equivalents) for steviol glycosides was allocated by the committee, based on a NOAEL of 970 mg/kg body weight/day (383 mg/kg body weight/day as steviol) from a 2-year study in rats (Toyoda *et al.*, 1997) and application of a safety factor of 100. The current specifications for steviol glycosides require not less than 95% of the 9 named steviol glycosides to be present in the final product on a dry weight basis, including stevioside, rebaudioside A, B, C, D, F, dulcoside A, rubusoside, and/or steviolbioside. Specific studies have not been conducted with each of these individual steviol glycosides, and therefore, their

inclusion within JECFA's purity specification confirms that the safety demonstrated for one glycoside is relevant to all glycosides in general based on the general recognition that all steviol glycosides are metabolized to steviol.

b) Food Standards Australia/New Zealand (FSANZ)

In its safety assessment of steviol glycosides, FSANZ considered the data previously reviewed by JECFA, as well as supplementary data consisting of published and unpublished studies (FSANZ, 2008). FSANZ considered the toxicological database for stevioside to cover a range of toxicological endpoints, and concluded that the ADI for steviol glycosides was 0 to 4 mg/kg body weight/day steviol equivalents. Recently, FSANZ has published a draft notification (FSANZ, 2015) to amend the steviol glycosides specification to include rebaudioside M along with the 9 other glycosides as approved by JECFA (2010).

c) European Food Safety Authority (EFSA)

In an independent review of the safety data previously reviewed by JECFA at its 69th meeting, EFSA corroborated JECFA's conclusion regarding the safety of steviol glycosides and concurred with the ADI previously established by JECFA of 0 to 4 mg/kg body weight/day, expressed as steviol equivalents (EFSA, 2010). Moreover, EFSA recently confirmed JECFA's previous determination that safety studies conducted with an individual steviol glycoside can extend to other steviol glycosides due to the shared metabolic fate (EFSA, 2015).

d) Health Canada

Health Canada also conducted its own independent review of the available safety data for steviol glycosides (Health Canada, 2012). Further corroborating the conclusions by JECFA, FSANZ, and EFSA, Health Canada also established an ADI of 0 to 4 mg/kg body weight/day for steviol glycosides, expressed as steviol equivalents. Furthermore, Health Canada recently amended the steviol glycoside purity definition to include to include rebaudioside M (Health Canada, 2016).

E. Summary and Basis for GRAS Conclusion

The GRAS determination for the use of Glucosylated Stevia Leaf Extract as a general purpose sweetener is based on scientific procedures. Glucosylated Stevia Leaf Extract is produced using a multi-step process, beginning with the hot-water extraction of *S. rebaudiana* leaves, followed by various purification and concentration steps to produce purified steviol glycoside extracts (>95%). The purified steviol glycosides (>95%) are then enzymatically modified such that additional glucose moieties are conjugated to the parent steviol glycoside structure *via* α-(1-4) linkages. The enzymes are inactivated by heat and the reaction mixture is purified and concentrated to yield Glucosylated Stevia Leaf Extract. PureCircle's Glucosylated Stevia Leaf

Extract contains not less than 95% total steviol glycosides, determined by the sum of the glucosylated steviol glycosides and the parent steviol glycosides.

Chemical specifications, based on those established by JECFA and FCC for steviol glycosides, and microbial specifications were established for Glucosylated Stevia Leaf Extract. Batch samples of Glucosylated Stevia Leaf Extract are routinely tested to verify compliance with the established product specifications. Additionally, since Glucosylated Stevia Leaf Extract is obtained from a plant source that may be exposed to various pesticides during cultivation, the final preparations were subjected to a multi-residue pesticide screen. Results of the analyses showed the absence of any pesticide residues. PureCircle undertook a series of studies to confirm the stability of Glucosylated Stevia Leaf Extract as a powder under normal and accelerated conditions and in solution at various pH levels and temperatures. Similar to the conclusions by JECFA for parent steviol glycosides, that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions, Glucosylated Stevia Leaf Extract was shown to be stable at pH values between 4.0 to 8.0 under normal processing and storage conditions.

PureCircle intends to market Glucosylated Stevia Leaf Extract as a general purpose sweetener, containing a mixture of glucosylated steviol glycosides and parent steviol glycosides, consisting of not less than 95% total steviol glycosides. Based on its intended use as a general purpose sweetening agent, Glucosylated Stevia Leaf Extract will be added to a variety of food products, excluding infant formulas and meat and poultry products, consistent with the current uses of other related high-intensity sweeteners that are already in the market.

Intakes of Glucosylated Stevia Leaf Extract were estimated based on post-market surveillance data for other high-intensity sweeteners and by adjusting these intake values for a relative sweetness intensity of 167 times that of sucrose. The estimated intakes of Glucosylated Stevia Leaf Extract in average consumers were predicted to range from 1.53 mg/kg body weight/day for non-diabetic adults to 4.02 mg/kg body weight/day for diabetic children, equivalent to 0.38 and 1.00 mg steviol equivalents/kg body weight/day, respectively. Predicted intakes for heavy consumers ranged from 4.04 mg/kg body weight/day for non-diabetic adults to 5.93 mg/kg body weight/day for non-diabetic children, equivalent to 1.00 and 1.47 mg steviol equivalents/kg body weight/day, respectively. Accordingly, the highest intake estimate for Glucosylated Stevia Leaf Extract of 1.47 mg/kg body weight/day steviol equivalents for non-diabetic children, under the proposed conditions of use, is below the current ADI for steviol glycosides of 0 to 4 mg/kg body weight, expressed as steviol, as set by JECFA.

Glucosylated steviol glycosides have a history of safe consumption by humans in the U.S. as well as globally. PureCircle's Glucosylated Stevia Leaf Extract is similar to several enzyme-modified steviol glycoside preparations that have been determined to be GRAS for use as general purpose sweeteners with no objection from the FDA (GRN 337, 375, 448, 452; U.S. FDA, 2011a,b, 2013a,b). A similar glucosylated steviol glycoside preparation manufactured by

PureCircle has obtained FEMA GRAS status for use as a flavoring agent, and recently this same preparation underwent a self-GRAS determination, has been notified to the FDA (GRN 607), and is currently under review by the agency (PureCircle, 2015). In several Asian countries, α-glucosylated steviol glycosides are approved for general use as sweeteners in a variety of foods and beverages and specifically has a safe history of use as a food additive in Japan for over 25 years (Marie, 1991; Das *et al.*, 1992; Ferlow, 2005; Government of Malaysia, 2014; Japan Food Chemical Research Foundation, 2014; MFDS, 2015).

The scientific conclusions regarding the safety of Glucosylated Stevia Leaf Extract are primarily based on the fact that glucosylated steviol glycosides are subject to microbial metabolism in a similar manner as parent steviol glycosides, ultimately generating the common primary metabolite steviol. Given this shared metabolic fate with parent steviol glycosides, the extensive safety database that has been established for steviol glycosides can be extrapolated to support the safe use of Glucosylated Stevia Leaf Extract. Furthermore, none of the publicly available safety studies conducted with similar glucosylated steviol glycoside preparations was found to be associated with any toxicological concerns.

The Expert Panel convened on behalf of PureCircle, independently and collectively, critically evaluated the data and information summarized above, and concluded that the intended uses of Glucosylated Stevia Leaf Extract meeting appropriate food-grade specifications and manufactured according to cGMP, are safe and suitable and are GRAS based on scientific procedures. It is also PureCircle's opinion that other qualified scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, PureCircle has concluded that Glucosylated Stevia Leaf Extract is GRAS under the intended conditions of use on the basis of scientific procedures; therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

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Part	**Section §**	**Section Title**
173—Secondary direct food additives permitted in food for human consumption	173.25	Ion-exchange resins
184—Direct food substances affirmed as generally recognized as safe	184.1012	α-Amylase enzyme preparation from Bacillus stearothermophilus
	184.1148	Bacterially-derived carbohydrase enzyme preparation
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Annex A

Expert Panel Consensus Statement

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Determination of Glucosylated Stevia Leaf Extract for Use as a General Purpose Sweetener

June 27, 2016

PureCircle Limited (hereafter PureCircle) intends to market Glucosylated Stevia Leaf Extract as a general purpose sweetener. Glucosylated Stevia Leaf Extract is produced by first extracting and purifying steviol glycosides (>95%) from the dried leaves of the *Stevia rebaudiana* Bertoni (*S. rebaudiana*) plant. Steviol glycosides are natural constituents of *S. rebaudiana* and more than 30 different steviol glycosides have been identified in the extracts obtained from the leaves of this plant (Purkayastha *et al.,* 2016). The purified steviol glycosides (>95%) are reacted with glucotransferase enzymes in the presence of glucose, which results in the addition of glucose to the parent steviol glycosides. PureCircle's Glucosylated Stevia Leaf Extract (glucosylated steviol glycosides + parent steviol glycosides) contains not less than 95% total steviol glycosides, determined by the sum of the glucosylated steviol glycosides and any parent steviol glycosides. Based on its intended use as a general purpose sweetening agent, Glucosylated Stevia Leaf Extract will be added to a variety of food products, consistent with the current uses of similar glucosylated steviol glycoside sweeteners that are already in the market.

At the request of PureCircle, a panel (the "Expert Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended uses of Glucosylated Stevia Leaf Extract as a sweetening agent is safe, suitable, and would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Expert Panel consisted of Dr. I. Glenn Sipes, Ph.D. (University of Arizona), Dr. Stanley M. Tarka Jr., Ph.D. (The Tarka Group Inc., and The Pennsylvania State University), and Dr. John A. Thomas, Ph.D. (Indiana University School of Medicine).

The Expert Panel, independently and collectively, evaluated a dossier that included a comprehensive summary of scientific information on glucosylated steviol glycosides prepared from information available within the public domain and also included details pertaining to the method of manufacture and product specifications for PureCircle's Glucosylated Stevia Leaf Extract, supportive analytical data, intended use-levels in foods, and consumption estimates for all intended uses. In addition, the Expert Panel evaluated other information deemed appropriate or necessary. Following its independent, critical evaluation of such data and information, the Expert Panel convened on June 27, 2016 *via* teleconference and unanimously concluded that the intended uses described herein for Glucosylated Stevia Leaf Extract, meeting appropriate food-grade specifications as described in the supporting dossier [Documentation Supporting the

Evaluation of Glucosylated Stevia Leaf Extract as Generally Recognized as Safe (GRAS) for Use as a General Purpose Sweetener] and manufactured according to current Good Manufacturing Practice (cGMP), are safe, suitable, and GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided below.

SUMMARY AND BASIS FOR GRAS DETERMINATION

Chemistry and Manufacturing

PureCircle's Glucosylated Stevia Leaf Extract is prepared by first extracting steviol glycosides from the leaves of the *S. rebaudiana* plant and then purifying this extract so that the total steviol glycoside content is greater than 95%. This stevia extract may contain a variety of glycosides, as more than 30 different steviol glycosides have been identified in the extracts obtained from the *S. rebaudiana* leaves (Purkayastha *et al.,* 2016), and such steviol glycoside preparations have recently been determined to be GRAS with no objection from the U.S. Food and Drug Administration (FDA) (GRN 619) (U.S. FDA, 2016). Next, the purified steviol glycosides (>95%) are enzymatically modified such that additional glucose moieties are conjugated to the parent steviol glycoside structure *via* α-(1-4) linkages. The enzyme treatment generates a mixture of glucosylated steviol glycosides containing from 1 to 20 additional glucose units bound to the parent steviol glycoside; however, based on the distribution analyses conducted by PureCircle, the mono-, di-, and tri-glucosylated forms generally predominate. PureCircle's Glucosylated Stevia Leaf Extract contains not less than 95% total steviol glycosides, determined by the sum of the glucosylated steviol glycosides and the parent steviol glycosides. The final product is a white to off-white powder that has a clean taste with a mild odor and is freely soluble in water. Typical components of Glucosylated Stevia Leaf Extract preparations are outlined in Table 1.

Table 1 Typical Components of Glucosylated Stevia Leaf Extract Preparations		
Molecules	**Molecular Formula**	**Molecular Weight (Da)**
Example Glucosylated Steviol Glycosides (~ 80 – 92% final steviol glycoside content)		
n-Glucosylated stevioside	$C_{(38+n*6)}H_{(60+n*10)}O_{(18+n*5)}$	804.87 + n*162.15
n-Glucosylated rebaudioside C	$C_{(44+n*6)}H_{(70+n*10)}O_{(22+n*5)}$	951.01 + n*162.15
n-Glucosylated rebaudioside A	$C_{(44+n*6)}H_{(70+n*10)}O_{(23+n*5)}$	967.01 + n*162.15
n-Glucosylated rebaudioside D	$C_{(50+n*6)}H_{(80+n*10)}O_{(28+n*5)}$	1129.2 + n*162.15
n-Glucosylated rebaudioside N	$C_{(56+n*6)}H_{(90+n*10)}O_{(32+n*5)}$	1275.3 + n*162.15
n-Glucosylated rebaudioside M	$C_{(56+n*6)}H_{(90+n*10)}O_{(33+n*5)}$	1291.3 + n*162.15
Example Parent Steviol Glycosides (~ 5 – 15% final steviol glycoside content)		
Rubusoside	$C_{32}H_{50}O_{13}$	642.73
Steviolbioside	$C_{32}H_{50}O_{13}$	642.73
Dulcoside A	$C_{38}H_{60}O_{17}$	788.87
Stevioside	$C_{38}H_{60}O_{18}$	804.87
Rebaudioside B	$C_{38}H_{60}O_{18}$	804.87
Rebaudioside F	$C_{43}H_{68}O_{22}$	936.99
Rebaudioside C	$C_{44}H_{70}O_{22}$	951.01
Rebaudioside A	$C_{44}H_{70}O_{23}$	967.01
Rebaudioside E	$C_{44}H_{70}O_{23}$	967.01
Rebaudioside D	$C_{50}H_{80}O_{28}$	1129.2
Rebaudioside N	$C_{56}H_{90}O_{32}$	1275.3
Rebaudioside M	$C_{56}H_{90}O_{33}$	1291.3
Rebaudioside O	$C_{62}H_{100}O_{37}$	1437.4

n = the number of glucose units enzymatically added to the parent steviol glycoside; n* = n multiplied by

The production of Glucosylated Stevia Leaf Extract begins with the hot-water extraction of steviol glycosides from the leaves of the *S. rebaudiana* plant, followed by initial purification, filtration, and deionization steps. The filtrate is fed through a column packed with macroporous adsorption resin that retains the steviol glycosides, the column is washed to remove any impurities, and the purified glycosides are desorbed using aqueous ethanol. The glycoside solution is treated with activated carbon, filtered, evaporated, deionized, and concentrated by nanofiltration. The concentrated solution is spray dried to yield stevia extract powder containing >50% rebaudioside A (RA50). This powder may be further processed to obtain more highly purified steviol glycosides. For instance, crystallization in ethanol at low temperature for several hours will yield rebaudioside A crystals of >95% purity. The rebaudioside A crystals can be separated by conventional centrifugation and dried in a rotary drum vacuum dryer. Overall, all stevia extract powders contain >95% total steviol glycosides and this first stage of the production process is consistent with the methodologies for the manufacture of steviol glycosides as described in the respective Chemical and Technical Assessment (CTA) published by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2007a).

Tapioca starch dissolved in water and liquefied by the addition of CGTase and/or α-amylase is then reacted with the steviol glycoside powder (>95% purity) (*e.g.,* RA50, stevioside, rebaudiosides A, B, C, D, M, or N) and additional CGTase for 48 hours at 60°C to generate glucosylated steviol glycosides. At the end of the reaction, the enzymes are inactivated by heating for 15 minutes at 100°C, the reaction mixture is treated with activated carbon to remove the inactivated enzymes and then filtered. The filtrate is again fed through a column packed with macroporous adsorption resin, impurities are washed away (*i.e.,* dextrins), and the steviol glycosides (both glucosylated and parent) are desorbed from the resin using aqueous ethanol. The ethanol is evaporated and the resulting aqueous solution is deionized and concentrated by nanofiltration. The concentrated solution is spray dried to yield Glucosylated Stevia Leaf Extract. Note that an example glucosylated preparation utilizing RA50 as the source of steviol glycosides is described in GRN 607, in which dextrins (<20%) are not removed from the final glucosylated steviol glycoside product (>80% steviol glycosides; ~7% parent steviol glycosides, ~75% glucosylated steviol glycosides) (PureCircle, 2015).

Physical and chemical specifications, based on those determined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for steviol glycosides (JECFA, 2010), were established for Glucosylated Stevia Leaf Extract. Since the ingredient is obtained from a natural source (*S. rebaudiana* leaves), the potential presence of microbial contaminants was limited by establishing rigorous microbiological specification parameters. Batch samples of Glucosylated Stevia Leaf Extract are routinely tested to verify compliance with the established chemical and microbiological parameters. Additionally, since Glucosylated Stevia Leaf Extract is obtained from a plant source that may be subjected to various pesticides during cultivation, the final ingredient is also subjected to a multi-residue pesticide screen. Results of the batch analyses showed the absence of any pesticide residues. PureCircle undertook a series of studies to confirm that the storage stability and pH and temperature stability of Glucosylated Stevia Leaf Extract is similar to individual steviol glycosides. As per the conclusions drawn by JECFA, that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions, Glucosylated Stevia Leaf Extract was shown to be stable under normal processing and storage conditions at pH values between 4.0 to 8.0.

Intended Food Uses and Estimated Intake

Glucosylated Stevia Leaf Extract is proposed for use as a general purpose sweetener that will be added to a variety of food products, consistent with the current uses of other related glucosylated steviol glycoside products that are already in the market (GRN 337, 375, 448, 452) (U.S. FDA, 2011a,b, 2013a,b). Based on post-market surveillance data for other high-intensity sweeteners and adjusting for relative sweetness intensity of Glucosylated Stevia Leaf Extract (167 times sweeter than sucrose), the estimated intakes of Glucosylated Stevia Leaf Extract were calculated for adults (diabetic and non-diabetic) and children (diabetic and non-diabetic) (Table 2). The estimated intakes were then converted to steviol equivalents based upon an

estimated weighted average molecular weight for Glucosylated Stevia Leaf Extract (1287.37 g/mol) that was generated based upon composition data provided by PureCircle.

The mean intake of Glucosylated Stevia Leaf Extract for average consumers was predicted to range across all groups from 1.53 mg/kg body weight/day for non-diabetic adults to 4.02 mg/kg body weight/day for diabetic children, equivalent to 0.38 and 1.00 mg steviol equivalents/kg body weight/day, respectively. Predicted intakes for heavy consumers ranged across all groups from 4.04 mg/kg body weight/day for non-diabetic adults to 5.93 mg/kg body weight/day for non-diabetic children, equivalent to 1.00 and 1.47 mg steviol equivalents/kg body weight/day, respectively. Accordingly, the predicted intakes of Glucosylated Stevia Leaf Extract are all below the current acceptable daily intake (ADI) defined by the JECFA for steviol glycosides of 0 to 4 mg/kg body weight/day expressed as steviol equivalents (JECFA, 2007b).

Table 2 Estimated Consumption of Glucosylated Stevia Leaf Extract Using Renwick's (2008) Methodology of Intense Sweetener Intake Assessment Based on Post-Market Surveillance Intake Data for Currently Used Sweeteners

Population Group	Intakes of intense sweeteners [expressed as sucrose equivalents] (mg/kg bw/day)		Consumption estimates for:			
			Glucosylated Stevia Leaf Extract[a] (mg/kg bw/day)		Glucosylated Stevia Leaf Extract as steviol equivalents[b] (mg/kg bw/day)	
	Average Consumer	High Consumer	Average Consumer	High Consumer	Average Consumer	High Consumer
Non-diabetic Adults	255	675	1.53	4.04	0.38	1.00
Diabetic Adults	280	897	1.68	5.37	0.42	1.33
Non-diabetic Children	425	990	2.54	5.93	0.63	1.47
Diabetic Children	672	908	4.02	5.44	1.00	1.35

bw = body weight
[a] Glucosylated Stevia Leaf Extract is 167 times as sweet as sucrose.
[b] Calculated based on an estimated weighted average molecular weight of 1287.37 g/mol for Glucosylated Stevia Leaf Extract [conversion factor of 4.04, based on a molecular weight of 318.45 g/mol for steviol].

Information to Establish Safety

The Expert Panel reviewed the publically available safety data on glucosylated steviol glycosides (*i.e.,* enzyme-modified steviol glycosides; α-glucosylated steviol glycosides) during their GRAS assessment, including safe history of use information, *in vitro* metabolic studies, and available toxicological studies. In addition, given that glucosylated steviol glycosides are subject to the same metabolic fate as steviol glycosides, the results of the safety and toxicology studies conducted with parent steviol glycosides were considered applicable to the safety of glucosylated steviol glycosides and specifically Glucosylated Stevia Leaf Extract. Therefore, the

Expert Panel also reviewed the data and information deemed pivotal in determining parent steviol glycoside safety during their GRAS assessment, including a detailed summary of the conclusions made by global scientific and regulatory authorities regarding the safety of steviol glycosides as well as data pertaining to their metabolic fate in rats and humans.

Metabolic Fate of Glucosylated Steviol Glycosides and Steviol

In vitro studies have demonstrated that glucosylated steviol glycosides are hydrolyzed to steviol by microbes present in the human gut and therefore are subject to the same metabolic fate as parent steviol glycosides. The metabolic pathway for α-glucosylated steviol glycosides has been shown to start with α-deglucosylation to the parent steviol glycoside, followed by hydrolysis to steviol (Koyama *et al.,* 2003a). Specifically, 2 glucosylated steviol glycosides, α-monoglucosylrebaudioside A and α-monoglucosylstevioside, were incubated with human fecal homogenates for up to 24 hours at 37°C under anaerobic conditions and after 24 hours both compounds were reported to be completely metabolized to steviol. Furthermore, a mixture of enzymatically-modified stevia containing the primary components α-glucosylrebaudioside A, α-glucosylstevioside, α-glucosylrebaudioside C, and α-glucosyldulcoside A, was also reported to hydrolyze to steviol following a 24-hour *in vitro* incubation with human fecal homogenates (Koyama *et al.,* 2003a). A commercial preparation referred to as glucosylated enzyme treated stevia was incubated with human fecal homogenates under anaerobic conditions for 24 hours at 37°C, and similarly, was shown to hydrolyze to steviol in a concentration- and time-dependent manner and exhibited similar hydrolysis rates over 24 hours to those measured for non-modified steviol glycosides (U.S. FDA, 2011a).

Steviol is absorbed systemically from the colon into the portal vein and distributed to a number of organs and tissues, including the liver, where steviol primarily undergoes conjugation with glucuronic acid to form steviol glucuronide. In humans, steviol glucuronide and very small amounts of unchanged glycoside or steviol are eliminated *via* the urine. Relative to amounts eliminated in urine, larger amounts of steviol (unabsorbed steviol released from steviol glycosides in the colon or from small amounts of steviol glucuronide secreted back into the gut *via* the bile) are also eliminated in the feces. Circulating steviol glycosides have not been detected in the plasma of humans, nor in the majority of animal studies conducted, indicating that the parent compound is not absorbed systemically (Koyama *et al.,* 2003b; Geuns *et al*., 2006, 2007; Roberts and Renwick, 2008; Wheeler *et al*., 2008).

Metabolic studies with steviol glycosides report that human digestive enzymes are not capable of hydrolyzing β-glycosidic bonds, and thus, steviol glycosides are not digested in the upper gastrointestinal tract (Hutapea *et al.,* 1997; Koyama *et al.,* 2003b; Geuns *et al.,* 2007). In addition to these same β-oriented glycosidic bonds, glucosylated steviol glycosides do, however, also contain α-glycosidic bonds. Therefore, it is possible that the α-glycosidic bonds could be hydrolyzed by digestive enzymes present in the upper gastrointestinal tract (*i.e.,* salivary α-amylase). Enzymatically treated stevia has in fact been experimentally exposed to

amylase *in vitro*, and based on the limited details reported in the abstract for this study, enzymatically treated stevia did convert to parent steviol glycosides when incubated with amylase (Shibasato, 1995). Although the details of this study were reported in Japanese, it appears that amylase can generate the parent steviol glycosides in the upper gastrointestinal tract. This would allow for the parent steviol glycosides to be degraded by the established metabolic pathway to steviol, and would release the α-glycosidic bonded sugar moieties to be absorbed in the intestine and metabolized *via* normal carbohydrate metabolism pathways.

Overall, the Expert Panel concurred that the available data demonstrate that glucosylated steviol glycosides are subject to the same metabolic fate as steviol glycosides, and therefore the safety conclusions that have been drawn for steviol glycosides can be extended to PureCircle's Glucosylated Stevia Leaf Extract.

Summary of Safety Conclusions for Steviol Glycosides

Interest in the use of steviol glycosides as sweeteners has encouraged extensive testing of the compounds and as such a large safety database exists. This database includes a thorough examination of the comparative metabolism and pharmacokinetics of steviol glycosides in experimental animals and humans, acute toxicity studies, short- and long-term toxicity and carcinogenicity studies, reproductive and developmental toxicology studies, *in vitro* and *in vivo* mutagenicity/genotoxicity studies, and human studies. The safety of steviol glycosides in general is based on the general recognition that all steviol glycosides are metabolized to the aglycone steviol and that the safety demonstrated for one glycoside, therefore, is relevant to all glycosides.

The safety of steviol glycosides was reviewed by JECFA at 4 separate meetings (51st, 63rd, 68th, and 69th) in 1998, 2004, 2007a,b, and 2008 and following extensive evaluations, JECFA established an ADI for steviol glycosides of 0 to 4 mg/kg body weight expressed as steviol equivalents (JECFA, 1998, 2004, 2007a,b, 2008). Several other scientific bodies and regulatory agencies including Food Standards Australia/New Zealand (FSANZ), the European Food Safety Authority (EFSA), and Health Canada, have conducted independent safety reviews and all have concurred with the ADI established by JECFA (FSANZ, 2008, 2015; EFSA, 2010, 2015; Health Canada, 2012). Given that glucosylated steviol glycosides are metabolized to steviol in a similar manner as parent steviol glycosides, the Expert Panel concluded that JECFA's ADI for steviol glycosides would also extend to Glucosylated Stevia Leaf Extract.

Toxicological Studies with Glucosylated Steviol Glycosides

A few toxicological studies have been conducted with glucosylated steviol glycoside preparations and findings from these studies consistently support their safety. For example, the subchronic toxicity of an α-glucosylated steviol glycoside preparation was evaluated and reported in GRAS Notification GRN 375 (U.S. FDA, 2011b). Rats [strain not specified]

(10/sex/group) consumed glucosylated stevia mixed in the diet at concentrations of 0, 1.25, 2.5, or 5.0% (equivalent to 0, 253, 519, or 1,059 or 0, 289, 601, or 1,153 mg steviol equivalents/kg body weight/day, for males or females, respectively) for 13 weeks. No deaths, clinical signs of toxicity, or abnormalities in ophthalmoscopic examinations were reported throughout the study. Some measures of hematology, clinical chemistry, urinalysis, and organ weights were reported to be statistically different between some of the groups, but were determined to be of no toxicological significance due to the fact that findings were not consistent between sexes, lacked dose-dependency and were not confirmed by related macroscopic or microscopic findings during organ examinations. Overall, glucosylated stevia was concluded to be safe and well-tolerated and the no-observed-adverse-effect level in rats for 13 weeks was determined to be the highest concentration tested (5%), equivalent to 1,059 and 1,153 mg steviol equivalents/kg body weight/day for males and females, respectively. This same product was also evaluated in some *in vitro* and *in vivo* genotoxicity assays and GRN 375 reports that the outcomes of all assays were negative, indicating a lack of genotoxicity (U.S. FDA, 2011b). Further details of these experiments are not publically available.

A number of studies with enzymatically treated stevia were summarized in a Japanese article by Shibasato (1995). Enzymatically treated stevia studied *in vitro* was reported to be non-mutagenic in a bacterial assay and the acute oral median lethal dose (LD_{50}) of enzymatically treated stevia in mice was reported to be greater than 60 g/kg body weight. When administered to rats chronically for 22 to 24 months at a dose of 550 mg/kg body weight/day, the results were described as “negative”.

History of Use and Current Regulatory Status of Glucosylated Steviol Glycosides

Glucosylated steviol glycosides, also described as enzyme-modified steviol glycosides, were developed to improve the sweetness qualities of steviol glycosides. α-Glucosyltransferase-treated stevia, a substance composed mainly of α-glucosylsteviosides obtained from “stevia extract”, is approved by The Ministry of Health, Labour and Welfare (MHLW) as a food additive from natural origin (Japan Food Chemical Research Foundation, 2014) and has a safe history of use as a food additive in Japan for over 25 years. Likewise, in several other Asian countries, α-glucosylated steviol glycosides are approved for general use as sweeteners in a variety of foods and beverages (Marie, 1991; Das *et al.*, 1992; Ferlow, 2005). Enzymatically modified stevia (glucosyl stevia), for example, is listed in the Korea Food Additives Code as a natural additive (MFDS, 2015), and in Malaysia, enzymatically modified stevia is regulated as a sweetening substance (Government of Malaysia, 2014). In the U.S., a number of enzyme-modified steviol glycosides preparations are GRAS for use as flavoring agents and/or general purpose sweeteners in foods. Specifically, a glucosylated steviol glycoside preparation manufactured by PureCircle, similar to the Glucosylated Stevia Leaf Extract that is the subject of this GRAS evaluation, was granted GRAS status by the FEMA GRAS Expert Panel in 2010 for use as a flavoring agent (FEMA # 4728) (Marnett *et al.*, 2013; Leffingwell and Leffingwell, 2014).

This same preparation has also recently undergone a self-GRAS determination by PureCircle for use as a flavoring agent with modifying properties, has been notified to the FDA (GRN 607), and is currently under review by the agency (PureCircle, 2015). Furthermore, four (4) GRAS notices (GRN 337, 375, 448, 452) have been submitted to the FDA for review and the agency raised no questions regarding the petitioners' conclusions that enzyme-modified steviol glycosides are GRAS for use as general purpose sweeteners in foods (U.S. FDA, 2011a,b, 2013a,b).

The scientific evidence examined by the Expert Panel demonstrates that under the conditions of intended use Glucosylated Stevia Leaf Extract would not produce any adverse health effects.

CONCLUSIONS

We, the Expert Panel, have independently and collectively, critically evaluated the data and information summarized above as well as other information that we deemed pertinent to the safety of the proposed uses of PureCircle's Glucosylated Stevia Leaf Extract (glucosylated steviol glycosides + parent steviol glycosides). We unanimously conclude that the intended use of Glucosylated Stevia Leaf Extract as a general purpose sweetener, meeting appropriate food-grade specifications as stated in the supporting dossier entitled [Documentation Supporting the Evaluation of Glucosylated Stevia Leaf Extract as Generally Recognized as Safe (GRAS) for Use as a General Purpose Sweetener] and manufactured in accordance with cGMP, is safe and suitable and GRAS based on scientific procedures.

It is our opinion that other qualified experts, critically evaluating the same information, would concur with our conclusion.

(b) (6)

Dr. I. Glenn Sipes, Ph.D.
Fellow AAAS and ATS and The University of Arizona

05 July 2016
Date

(b) (6)

Dr. Stanley M. Tarka, Ph.D.
The Tarka Group Inc. and The Pennsylvania State University

01 July 2016
Date

(b) (6)

Dr. John A. Thomas, Ph.D.
Fellow A.C.T. and ATS
University of Indiana School of Medicine

30 June 2016
Date

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