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March 14, 2018

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740-3835

Dear Dr. Gaynor

RE: GRAS Exemption Claim for Stevia Leaf Extracts

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 [Cargill, Incorporated, 15407 McGinty Road West, M.S. 163, Wayzata, Minnesota, 55391], a Notice of the determination, on the basis of scientific procedures, that stevia leaf extracts, as defined in the enclosed documents and manufactured according to current Good Manufacturing Practices, is GRAS under specific conditions of use as an ingredient in food and beverages, 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 stevia leaf extracts under the intended conditions of use, also are enclosed for review by the agency.

The enclosed electronic files for the Notice entitled, "GRAS Notice for Stevia Leaf Extracts" were scanned for viruses prior to submission and is thus certified as being virus-free using Symantec Endpoint Protection 12.1.5.

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.

Yours sincerely

(b) (6)

Nicole Cuellar-Kingston
Principal Scientist, Scientific & Regulatory Affairs
Cargill, Incorporated

Encl. (2)

GRAS NOTICE FOR STEVIA LEAF EXTRACTS

PREPARED FOR:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740

DATE:
14 March 2018

GRAS Notice for Stevia Leaf Extracts

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GRAS Notice for Stevia Leaf Extracts

Part 1 §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through §170.285, Cargill Incorporated (Cargill) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that stevia leaf extracts, manufactured according to current Good Manufacturing Practices (cGMP) is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Cargill's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below. In addition, as a responsible official of Cargill, Nicole Cuellar-Kingston hereby certifies that all data and information presented in this notice represents a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to Cargill and pertinent to the evaluation of the safety and GRAS status of stevia leaf extracts as an ingredient for addition to food.

Signed,

(b)(6)

3/14/18

Nicole Cuellar-Kingston, M.S.
Principal Scientist, Scientific & Regulatory Affairs
Cargill Incorporated

Date

1.1 Name and Address of Notifier

Nicole Cuellar-Kingston
Cargill Incorporated
15407 McGinty Road West, M.S. 163
Wayzata, MN
U.S.A. 55391

Telephone: 952-742-2113
Email: Nicole_Cuellar-Kingston@cargill.com

1.2 Common Name of Notified Substance

Stevia leaf extract; stevia leaf extracts; steviol glycosides

1.3 Conditions of Use

Cargill intends to market stevia leaf extracts as an ingredient in the U.S. for use as general-purpose sweetening agents in foods and beverages in accordance with the principles of cGMP, under the same conditions of use as defined for steviol glycoside extract preparations in GRAS notification GRN 000619 (U.S. FDA, 2016).

Stevia leaf extracts are hot water-extracts from the leaves of the *Stevia rebaudiana* (*S. rebaudiana*) Bertoni plant containing various individual steviol glycosides. The sweetness intensity of stevia leaf extracts depends on the ratio of individual steviol glycosides present in a preparation, and may range from 200 to 350 times sweeter than sucrose. The use levels of other high-intensity sweeteners (HIS) which have been approved by the FDA as general-purpose sweeteners (or otherwise received 'no questions' letters from the FDA upon submission of a GRAS notification) are not restricted to specific foods or use-levels. Instead, the use levels of HIS are self-limiting based on their organoleptic properties (*i.e.*, sweetness potency).

Steviol glycosides have a sweetness intensity comparable to aspartame which is 200 times as sweet as sucrose and has been used as a basis for determining the use-levels described in several previous GRAS notifications. As such, the uses and use-levels of stevia leaf extracts are expected to be similar to those currently permitted for other HIS which are permitted for use in the U.S.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a) and (b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2017a), stevia leaf extracts manufactured by Cargill's suppliers under the intended uses described in Section 1.3, have been concluded to have GRAS status on the basis of scientific procedures. This GRAS determination has used data pertaining to the safety of stevia leaf extracts which are generally available in the public domain. A panel of experts who are qualified by scientific training and experience to evaluate the safety of stevia leaf extracts as a component of food was convened and the panel of experts concurred with Cargill's GRAS determination.

The scientific data pertaining to the safety of stevia leaf extracts is presented herein. All information presented within this notification was reviewed by a panel of experts qualified in their field by scientific training to evaluate the safety of stevia leaf extracts. The consensus statement of the panel of experts is provided in Annex A entitled "**Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of Stevia Leaf Extracts for Use in Foods as General Purpose Sweeteners**".

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the FDA for review and copying upon request during business hours at the offices of:

Cargill Incorporated
15407 McGinty Road West, M.S. 163
Wayzata, MN
U.S.A. 55391

In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency`s review of the notice, Cargill will supply these data and information.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Cargill's view that all data and information presented in Parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. 552.

Part 2 §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Description

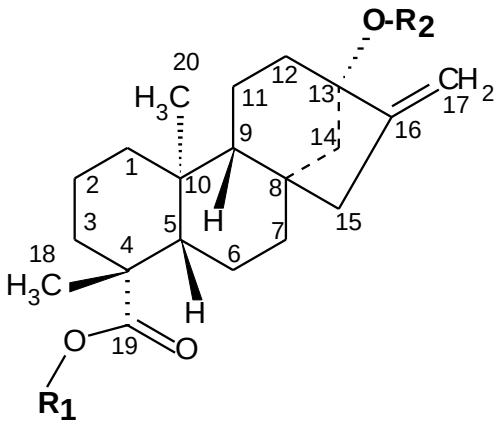
The ingredients that are the subject of this GRAS determination are the water-extracts from the leaves of the *Stevia rebaudiana* (*S. rebaudiana*) Bertoni plant containing various individual steviol glycosides (steviol glycosides represent a group of over 40 natural constituents) in any combination or ratio with a total steviol glycoside content of ≥95%. Additional description of the ingredient and information characterizing the identity of the source organism is presented below.

2.1.1 Chemical and Physical Characteristics

Steviol glycosides that have been identified from the leaves of *S. rebaudiana* are generally white to off-white powders with a characteristic sweet taste.

All steviol glycosides within stevia leaf extracts contain a steviol backbone that is conjugated with different numbers and combinations of sugar moieties, including glucose, xylose, rhamnose, fructose, deoxyglucose, galactose, and/or arabinose (Ibrahim *et al.,* 2016; Purkayastha *et al.,* 2016; JECFA, 2017). The structural formula for steviol glycosides is presented in Figure 2.1.1-1.

Figure 2.1.1-1 Backbone Structure for Steviol Glycosides



The molecular structure of steviol glycosides consists of a steviol backbone that is linked to mono-, di-, or oligosaccharide groups at the R_1 and R_2 positions on carbons 19 and 13, respectively. As previously established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the purity criteria for steviol glycosides includes any sugar moiety (*e.g.*, glucose, rhamnose, xylose, fructose, deoxyglucose, galactose, and/or arabinose) in any combination or orientation, such that the total steviol glycoside content of the product is not less than 95% total steviol glycosides (JECFA, 2016a, 2017). The Chemical Abstract Service (CAS) numbers, empirical formulae, molecular weights, and the R_1 and R_2 groups for steviol glycosides, as well as the aglycone steviol are summarized in Table 2.1.1-1. Some minor steviol glycosides which contain a more diverse selection of sugar moieties have also been recently identified and their information is also included in Table 2.1.1-1.

Table 2.1.1-1 Molecular Weight and Formula, and R-Groups in Backbone Structure (See Figure 2.1.1-1)

#	Common Name	CAS Number	Molecular Weight	Trivial Formula	R_1	R_2
-	Steviol	471-80-7	318.46	C20H30O3	H	H
1) Steviol + Glucose						
1.1	Steviolmonoside	-	480.59	$C_{25}H_{40}O_8$	H	Glcβ1-
1.2	Steviol-19-O-β-D-glucoside	60129-60-4	480.59	$C_{25}H_{40}O_8$	Glcβ1-	H
1.3	Rubusoside	64849-39-4	642.73	$C_{32}H_{50}O_{13}$	β-Glc	β-Glc
1.4	Steviolbioside	41093-60-1	642.73	$C_{32}H_{50}O_{13}$	H	β-Glc-β-Glc(2-1)
1.5	Stevioside	57817-89-7	804.88	$C_{38}H_{60}O_{18}$	β-Glc	β-Glc-β-Glc(2-1)
1.6	Stevioside A	-	804.88	$C_{38}H_{60}O_{18}$	β-Glc-β-Glc(2-1)	β-Glc
1.7	Rebaudioside B	58543-17-2	804.88	$C_{38}H_{60}O_{18}$	H	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
1.8	Rebaudioside G	127345-21-5	804.88	$C_{38}H_{60}O_{18}$	Glcβ1-	Glcβ(1-3)Glcβ1-
1.9	Stevioside B	-	804.88	$C_{38}H_{60}O_{18}$	Glcβ(1-3)Glcβ1-	Glcβ1-
1.10	Rebaudioside E	63279-14-1	967.01	$C_{44}H_{70}O_{23}$	Glcβ(1-2)Glcβ1-	Glcβ(1-2)Glcβ1-
1.11	Rebaudioside A	58543-16-1	967.01	$C_{44}H_{70}O_{23}$	β-Glc	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
1.12	Rebaudioside A2	-	967.01	$C_{44}H_{70}O_{23}$	Glcβ1-	Glcβ(1-6)Glcβ(1-2)Glcβ1-
1.13	Rebaudioside D	63279-13-0	1,129.15	$C_{50}H_{80}O_{28}$	B-Glc-β-Glc(2-1)	Glcβ(1-2)[Glcβ(1-3)]Glcβ1
1.14	Rebaudioside I	-	1,129.15	$C_{50}H_{80}O_{28}$	Glcβ(1-3)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
1.15	Rebaudioside L	-	1,129.15	$C_{50}H_{80}O_{28}$	Glcβ1-	Glcβ(1-6)Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
1.16	Rebaudioside Q2	-	1,129.15	$C_{50}H_{80}O_{28}$	Glcα(1-2)Glcα(1-4)Glcβ1-	Glcβ(1-2)Glcβ1-
1.17	Rebaudioside Q	-	1,129.15	$C_{50}H_{80}O_{28}$	Glcβ1-	Glcα(1-4)Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
1.18	Rebaudioside I2	-	1,129.15	$C_{50}H_{80}O_{28}$	Glcβ1-	Glcα(1-3)Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
1.19	Rebaudioside Q3	-	1,129.15	$C_{50}H_{80}O_{28}$	Glcβ1-	Glcα(1-4)Glcβ(1-3)[Glcβ(1-2)]Glcβ1-
1.20	Rebaudioside I3	-	1,129.15	$C_{50}H_{80}O_{28}$	Glcβ(1-2)[Glcβ(1-6)]Glcβ1-	Glcβ(1-2)Glcβ1-
1.21	Rebaudioside M	1220616-44-3	1,291.3	$C_{56}H_{90}O_{33}$	Glcβ(1-2)[Glcβ (1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
2) Steviol + Rhamnose + Glucose						
2.1	Dulcoside A	64432-06-0	788.88	$C_{38}H_{60}O_{17}$	β-Glc	β-Glc-α-Rha(2-1)
2.2	Dulcoside B	63550-99-2	788.88	$C_{38}H_{60}O_{17}$	H	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-
2.3	Rebaudioside C	63550-99-2	951.02	$C_{44}H_{70}O_{22}$	β-Glc	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-
2.4	Rebaudioside C (isomer)	-	951.02	$C_{44}H_{70}O_{22}$	Rhaα(1-2)Glcβ1-	Glcβ(1-3)Glcβ1-
2.5	Rebaudioside H	-	1,113.16	$C_{50}H_{80}O_{27}$	Glcβ1-	Glcβ(1-3)Rhaα(1-2)[Glcβ(1-3)]Glcβ1-
2.6	Rebaudioside K	-	1,113.16	$C_{50}H_{80}O_{27}$	Glcβ(1-2)Glcβ1-	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-
2.7	Rebaudioside J	-	1,113.16	$C_{50}H_{80}O_{27}$	Rhaα(1-2)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
2.8	Rebaudioside N	1220616-46-5	1,275.30	$C_{56}H_{90}O_{32}$	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-
2.9	Rebaudioside O	-	1,437.44	$C_{62}H_{100}O_{37}$	Glcβ(1-3)Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-

Table 2.1.1-1 Molecular Weight and Formula, and R-Groups in Backbone Structure (See Figure 2.1.1-1)

#	Common Name	CAS Number	Molecular Weight	Trivial Formula	R_1	R_2
3) Steviol + Xylose + glucose						
3.1	Stevioside F	-	774.85	$C_{37}H_{58}O_{17}$	Glcβ1-	Xylβ(1-2)Glcβ1-
3.2	Rebaudioside F	438045-89-7	936.99	$C_{43}H_{68}O_{22}$	β-Glc	β-Glc-β-Xly(2-1)
3.3	Rebaudioside F2	-	936.99	$C_{43}H_{68}O_{22}$	Glcβ1-	Glcβ(1-2)[Xylβ(1-3)]Glcβ1-
3.4	Rebaudioside F3	-	936.99	$C_{43}H_{68}O_{22}$	Xylβ(1-6)Glcβ1-	Glcβ(1-2)Glcβ1-
4) Steviol + Fructose + Glucose						
4.1	Rebaudioside A3	-	967.01	$C_{44}H_{70}O_{23}$	Glcβ1-	Glcβ(1-2)[Fruβ(1-3)]Glcβ1-
5) Steviol + Deoxyglucose + Glucose						
5.1	Stevioside D	-	788.88	$C_{38}H_{60}O_{17}$	Glcβ1-	6-deoxyGlcβ(1-2)Glcβ1-
5.2	Stevioside E	-	951.02	$C_{44}H_{70}O_{22}$	Glcβ1-	6-deoxyGlcβ(1-2)[Glcβ(1-3)]Glcβ1-
5.3	Stevioside E2	-	951.02	$C_{44}H_{70}O_{22}$	6-deoxyGlcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-

deoxyGlc = deoxyglucose; Fru = fructose; Glc = glucose; H = hydrogen; Rha = rhamnose; Xyl = xylose.
Adapted from Purkayastha *et al*. (2016)

Adding to the well-established steviol glycosides described in Table 2.1.1-1, as-yet not fully characterized steviol glycosides have been reported to have been identified in stevia leaf extracts (Kohda *et al.*, 1976; Kinghorn *et al.*, 1999; Kennelly, 2002; Wölwer-Rieck, 2012). These glycosides may be produced under certain extraction and/or storage conditions which could permit hydrolysis of sugar moieties or from steviol glycosides with fewer sugar moieties (Kobayashi *et al*., 1977; Chang and Cook, 1983; Woelwer-Rieck *et al.*, 2010). One example of this is the case of rebaudioside A which can be hydrolyzed under alkaline conditions to form rebaudioside B for commercial use (Bridges *et al.*, 2012; Furlano *et al.*, 2012; Markosyan, 2013).

2.2 Source Organism

2.2.1 Phenotypic Identity

The source organism, *Stevia rebaudiana* Bertoni, is a plant officially discovered in 1887 by Antonio Bertoni, although *S. rebaudiana* Bertoni and steviol glycosides have been consumed as sweeteners in foods and beverages for hundreds of years by humans in various countries with no reports of adverse effects following use (Lee *et al.,* 1979; Geuns, 2003; Ferlow, 2005).

2.2.2 Part(s) of Source Organism Used

Stevia leaf extracts are produced from the leaves of *S. rebaudiana* Bertoni which have traditionally been used in the production of other steviol glycosides which have received “no questions” letters from the FDA (U.S. FDA, 2013, 2015, 2016).

In a recent study, Molina-Calle *et al.* (2017) obtained crude polar and non-polar extracts of stevia leaves using either a 35:65 (volume/volume) ethanol-water mixture or n-hexane and analyzed the extracts using liquid chromatography quadrupole-time of flight tandem mass spectrometry. The authors identified at least 6 steviol glycosides which had not been identified previously in the literature. Other components which were identified in the crude polar and non-polar extracts of the stevia leaf included other terpenoids, phenolic compounds, amino acids, glycerolipids, fatty acids, and derivatives; however, due to the processes

used in the production of stevia leaf extracts which results in products that comply with established purity specifications, these other components are not expected to be present at meaningful concentrations.

2.3 Manufacturing

Cargill intends to market stevia leaf extracts manufactured by Cargill and their suppliers that are manufactured according to practices which are commonly used and previously described in previous GRAS notices [GRN 000456 (rebaudioside D), GRN 000548 (rebaudioside D), and GRN 000619 (purified steviol glycosides with rebaudioside A and stevioside as the principal components)] as well as methods described in the most recent Chemical and Technical Assessment published by JECFA (JECFA, 2016b; U.S. FDA, 2013, 2015, 2016).

Different pH and temperature conditions can be used during the production of stevia leaf extracts which can result in stevia leaf extracts with different profiles of individual steviol glycosides. A description of the manufacturing method is provided below.

Steviol glycosides are first obtained by water-extraction of leaves from the *S. rebaudiana* Bertoni plant. The extract is then centrifuged or filtered using a filter press. Optionally, a flocculant that permitted for use in food in the U.S. may be added prior to the filtration. The filtrate is passed through cation and anion exchange columns to remove mineral impurities and colored substances before it is passed through an adsorption resin. The adsorption resin is washed with deionized water to remove impurities that did not adsorb on the resin, and is subsequently washed with an organic solvent to elute the steviol glycosides. Another optional filtration step may be employed to remove any particulate material remaining in the solution.

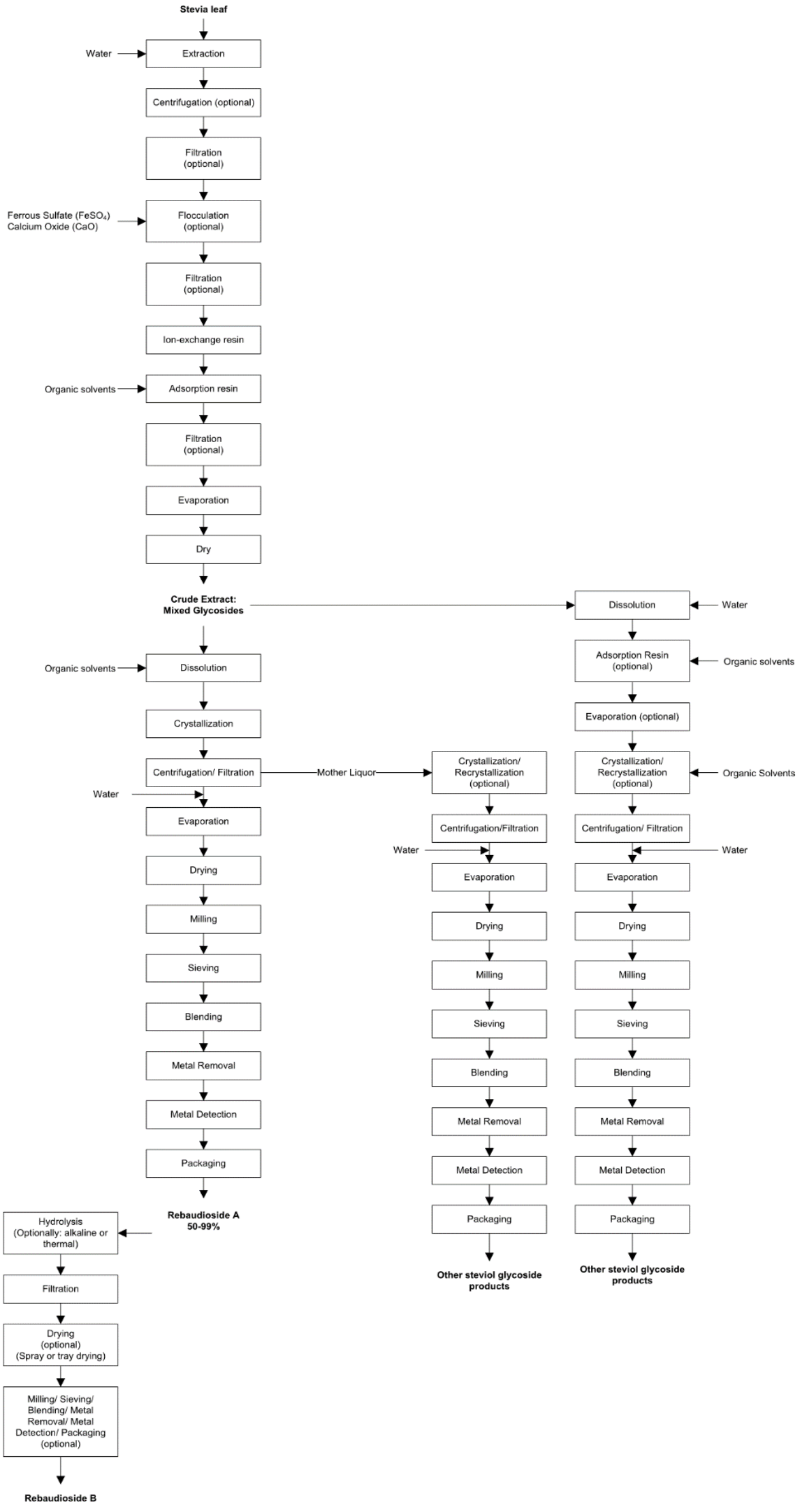
The obtained glycoside eluent is concentrated by evaporation to remove the solvent from solution. The glycoside concentrate is dried to yield the crude extract that contains mixed steviol glycosides. The steviol glycoside crude extract is further refined in 2 different ways:

In one way, the steviol glycoside crude extract is dissolved in either food grade organic solvents like ethanol, or in a mixture of food-grade organic solvents and water. The temperature is lowered to form crystals. Subsequently, the crystals are filtered from the mother liquor by centrifuge or filtration. The crystals are subjected to sequential rinsing with water, and then through evaporation, drying, milling, sieving, blending, metal removing, and packing processes to produce steviol glycoside products with a rebaudioside A content of 50 to 99%. These rebaudioside A products can be further hydrolyzed (thermal or alkaline) to produce rebaudioside B products. The mother liquor from the crystallization step can be further treated by crystallization or re-crystallization to produce other steviol glycoside products.

In the other way, the steviol glycoside crude extract is dissolved in water and can be passed through an adsorption resin as an optional step. Optional evaporation and crystallization/re-crystallization steps in an organic solvent like ethanol can be applied before it is subjected to centrifugation and filtration to produce crystals. The crystals undergo sequential rinsing with water, and then concentrated through evaporation, drying, milling, sieving, blending, metal removing (using a magnet), and packing processes to yield other steviol glycoside products.

A schematic diagram of the production process of stevia leaf extracts is provided in Figure 2.3-1.

Figure 2.3-1 Schematic of the Production Process of Stevia Leaf Extracts



The raw materials, processing aids, and equipment used in the manufacture of stevia leaf extracts are suitable food-grade materials and are used in accordance with applicable U.S. federal regulations. The regulatory status of example raw materials and processing aids used during the manufacture of stevia leaf extracts is provided in Table 2.3-1

Table 2.3-1 Regulatory Status of Example Raw Materials, Processing Aids, and Equipment Used in the Manufacture of Stevia Leaf Extracts

Raw Material	Use	Regulatory Status	
		21 CFR	Approved Uses
Stevia rebaudiana Bertoni leaves	Starting material		GRAS for use in the manufacture of purified steviol glycosides
Ferrous sulfate	Flocculant	§184.1315	Affirmed as GRAS for use in food as a nutrient supplement and processing aid with no limitation other than cGMP
Calcium oxide	Flocculant	§184.1210	Affirmed as GRAS for use in food with no limitation other than cGMP
Ethanol[a]	Elution solvent Crystallization	§182.1	GRAS when used in accordance with cGMP
Methanol[b]	Elution solvent	§182.1	GRAS when used in accordance with cGMP
Ion-exchange resin	Purification		Used in accordance with §173.25
Adsorption resin	Purification		Used in accordance with §173.25

CFR = Code of Federal Regulations (U.S. FDA, 2017a); cGMP = current Good Manufacturing Practices; GRAS = Generally Recognized as Safe.

[a] JECFA specifications for steviol glycosides specify a level of not more than 5,000 ppm for ethanol residues.

[b] JECFA specifications for steviol glycosides specify a level of not more than 200 ppm for methanol residues.

2.4 Product Specifications and Batch Analyses

2.4.1 Proposed Product Specifications

The proposed product specifications for the stevia leaf extracts are provided in Table 2.4.1-1.

Table 2.4.1-1 Product Specifications – Stevia Leaf Extracts

Parameter	Specification	Method of Analysis Used by Cargill
Identity		
Assay (steviol glycosides)	NLT 95%	STV-002-06
Appearance	Loose powder or crystals, white to off-white	STV-003-01
Purity		
Ash	NMT 1.0%	AOAC 945.46
Loss on drying	NMT 6.0%	STV-006-02
Residual solvents	NMT 0.02 methanol NMT 0.5 ethanol	STV-009-01
Heavy Metals		
Arsenic	NMT 1 mg/kg	USP 730 ICP-MS
Cadmium	NMT 1 mg/kg	USP 730 ICP-MS
Lead	NMT 1 mg/kg	USP 730 ICP-MS
Mercury	NMT 1 mg/kg	USP 730 ICP-MS

Table 2.4.1-1 Product Specifications – Stevia Leaf Extracts

Parameter	Specification	Method of Analysis Used by Cargill
Microorganisms		
Aerobic plate count	LT 1,000 CFU/g	AOAC 966.23
Yeast	NMT 50 CFU/g	FDA BAM, 7th edition
Mold	NMT 50 CFU/g	FDA BAM, 7th edition
Salmonella spp.	Negative/25 g	AOAC-R1 100201

AOAC = Association of Official Analytical Chemists; BAM = bacteriological annual manual; CFU = colony forming unit; FDA = United States Food and Drug Administration; ICP = inductively coupled plasma; MS = mass spectrometry; NLT = not less than; NMT = not more than; USP = United States Pharmacopeia

[a] The assay value for steviol glycosides is defined by JECFA as not less than 95% of "*a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties in any of the orientations occurring in the leaves of Stevia rebaudiana Bertoni including, glucose, rhamnose, xylose, fructose, and deoxyglucose*"; additional saccharides (galactose and arabinose) have also been included in this definition (JECFA, 2016a, 2017). Cargill reports the assay value as the sum of rebaudioside A, B, C, D, F, M, stevioside, dulcoside, rubusoside, and steviolbioside.

2.4.2 Batch Analyses

The results of 3 non-consecutive lots from 2 example stevia leaf extracts (*i.e.,* a total of 6 lots of final product) shows that the ingredient is manufactured consistent with the proposed product specifications (Table 2.4.2-1). Complete certificates of analysis for these 6 lots are provided in Annex B.

Table 2.4.2-1 Results of 3 Batch Analyses of Stevia Leaf Extracts

Parameter	Specification	Truvia® Stevia RA50 Batch Numbers			ViaTech™ Batch Numbers		
		20170604	170614-02	20170702	170816-B1	170816-B3	170816-B5
Identity							
Assay (steviol glycosides)	NLT 95%	99	99	98	97	97	97
Appearance	Loose powder or crystals, white to off-white	Pass	Pass	Pass	Pass	Pass	Pass
Purity							
Ash	NMT 1.0%	0.5	0.1	< 0.04	0.2	0.1	0.2
Loss on drying	NMT 6.0%	2	3	1	1	1	1
Residual methanol	NMT 0.02%	0.00	0.00	0.00	0.0	0.01	0.00
Residual ethanol	NMT 0.5%	0.1	0.0	0.0	0.1	0.1	0.1
Heavy Metals							
Arsenic	NMT 1 mg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cadmium	NMT 1 mg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Lead	NMT 1 mg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Mercury	NMT 1 mg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Microorganisms							
Aerobic plate count	LT 1,000 CFU/g	< 10	< 10	< 10	< 10	< 10	< 10
Yeast	NMT 50 CFU/g	< 10	< 10	< 10	< 10	< 10	< 10

Table 2.4.2-1 Results of 3 Batch Analyses of Stevia Leaf Extracts

Parameter	Specification	Truvia® Stevia RA50 Batch Numbers			ViaTech™ Batch Numbers		
		20170604	170614-02	20170702	170816-B1	170816-B3	170816-B5
Mold	NMT 50 CFU/g	10	< 10	< 10	< 10	< 10	< 10
Salmonella spp.	Negative/25 g	Negative	Negative	Negative	Negative	Negative	Negative

CFU = colony forming units; FDA = United States Food and Drug Administration; ICP = inductively coupled plasma; MS = mass spectrometry; NLT = not less than; NMT = not more than.

2.4.3 Additional Analytical Information

In addition to the above analytical data for example batches of stevia leaf extracts, the steviol glycoside distribution for 3 non-consecutive lots of 2 example stevia leaf extracts were analyzed and presented in Table 2.4.3-1. The distribution of specific steviol glycosides within stevia leaf extracts are dependent upon the extraction conditions (time, temperature, pressure, and solvents used), the adsorption and desorption of the steviol glycosides to and from the separation columns, and crystallization steps used during the production process. Thus, while all stevia leaf extracts have a total steviol glycoside content of no less than 95%, each stevia leaf extract preparation has a different and specific distribution of individual steviol glycosides.

Table 2.4.3-1 Steviol Glycoside Distribution for 6 Non-Consecutive Lots of 2 Stevia Leaf Extracts (Truvia® Stevia RA50, ViaTech™ Batch Numbers)

Steviol Glycoside (% wt/wt)	Truvia® Stevia RA50 Batch Numbers			ViaTech™ Batch Numbers		
	20170604	170614-02	20170702	170816-B1	170816-B3	170816-B5
Rubusoside	0.41	0.28	0.58	< 0.10	< 0.10	< 0.10
Steviolbioside	0.51	0.65	0.28	0.21	0.21	0.20
Stevioside	37.44	40.29	36.45	< 0.10	< 0.10	< 0.10
Rebaudioside B	0.86	1.00	0.56	34.86	36.94	34.21
Rebaudioside A	57.01	52.43	51.18	30.38	31.62	29.82
Rebaudioside D	< 0.10	0.23	0.16	23.03	18.74	23.56
Rebaudioside M	< 0.10	< 0.10	< 0.10	8.80	9.18	9.00
Dulcoside A	0.21	< 0.10	0.50	< 0.10	< 0.10	< 0.10
Rebaudioside C	2.12	3.08	7.01	< 0.10	< 0.10	< 0.10
Rebaudioside F	0.65	0.66	1.13	< 0.10	< 0.10	< 0.10
TOTAL	**99.20**	**98.62**	**97.86**	**97.27**	**96.70**	**96.78**

% wt/wt = percentage by weight.

Multi-residue pesticide screens were conducted on 2 non-consecutive lots of example stevia leaf extracts. The pesticide analyses included detection and quantification of commonly applied pesticides. No pesticides were detected above the limits of detection in either of the lots of stevia leaf extracts which were examined.

2.5 Stability

At its 68th meeting, JECFA considered the stability of steviol glycosides under conditions mimicking their use in foods (JECFA, 2007). According to this evaluation, steviol glycosides do not undergo browning or caramelization when heated, and are reasonably stable under elevated temperatures used in food

processing. JECFA concluded that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverage under normal processing and storage conditions. In particular, high-purity steviol glycosides (90 to 94%) are stable for at least 180 days when stored at temperatures up to 24°C in acidic solutions (pH 2 to 4). However, when solutions of steviol glycosides were exposed to elevated temperatures (80°C in water, 8 hours) at pH 4.0 and 3.0, 4 and 8% decomposition, respectively, was observed, indicating that the stability is pH and temperature dependent. When the temperature was increased to 100°C, higher rates of steviol glycoside decomposition (10 and 40% at pH 4.0 and 3.0, respectively) were observed.

2.5.1 Storage Stability

The stability of stevia leaf extracts under typical storage conditions are expected to be similar to that of steviol glycosides which were the subjects of previous GRAS notifications, in particular GRN 000456, GRN 000548, and GRN 000619 (U.S. FDA, 2013, 2015, 2016). Steviol glycoside preparations were reported to minimally degrade (<4%) over a period of 122 weeks at 40°C and 75% relative humidity (U.S. FDA, 2016).

2.5.2 pH Stability

The pH stability of the steviol glycoside rebaudioside D was described in a GRN 000456 and GRN 000548 and are applicable to the stability of stevia leaf extracts on the basis of the structural similarity between steviol glycosides (U.S. FDA, 2013, 2015). When stored for up to 24 weeks at pH values between 2.0 and 8.0 and at 4 different temperatures (5, 20, 37, and 56°C), rebaudioside D was stable at low temperatures between pH 3.0 and 8.0 with between 3 and 5% of the sample degrading over the storage period. However, when stored at a pH of 2.0 at 5°C, approximately 14% of the original rebaudioside D sample degraded. At high temperatures (56°C), complete degradation was observed after 4 weeks of storage only when the pH was 2.0. Overall, rebaudioside D stability decreased when stored at increasing temperatures. Sample chromatograms indicated that as the concentration of rebaudioside D decreased, the concentrations of rebaudioside A and B increased, suggesting that rebaudioside D is degraded to rebaudioside A and B by the cleavage of 1 glucose unit at a time, occurring at the C19 position of the steviol backbone.

Part 3 §170.235 Dietary Exposure

3.1 History of Use in Food

The history of use of the *S. rebaudiana* plant as a source of steviol glycosides and as a sweetener have been previously discussed in Section 2.2.

The steviol glycoside stevioside has been used in Asia since at least 1995 when use levels were reported to be approximately 160,000 metric tons as sucrose equivalents (SE) and have since increased to approximately 200,000 metric tons (SE) in 1999 (International Sugar Organization, 2001). In addition to these usage rates, stevioside has been used in Japan for more than 30 years with no occurrence of adverse effects (Ferlow, 2005). Stevioside and *S. rebaudiana* have been used in China, Brazil, South Korea, and Paraguay for at least 25 to over 100 years (Geuns, 2003). According to the Global Stevia Institute (2017), stevia glycosides are used as food additives in dozens of countries in North America, South America, Asia, Africa, and Europe.

Steviol glycosides were first used in the United States as dietary supplements in 1995 (Geuns, 2003). There have been at least 47 GRAS notices for steviol glycosides, including purified steviol glycosides (≥95% purity) and enzyme-modified steviol glycosides for a variety of food and beverage use which have been submitted to the U.S. FDA; most of these notices have received a "no questions" letter with the remainder currently undergoing review. Steviol glycosides have also been approved for use in other jurisdictions by regulatory bodies including Food Standards Australia and New Zealand (FSANZ), Health Canada, the European Food Safety Authority (EFSA), and JECFA (FSANZ, 2008, 2015, 2017; Health Canada 2012a,b, 2016, 2017; EFSA, 2010, 2015; JECFA, 1999, 2006, 2007, 2009, 2016b, 2017). The opinions of these authorities are discussed in Section 6.3.

A limited number of hypersensitivity and/or allergic reactions in response to stevia sweeteners have been reported in individuals in Japan (2 cases) and the U.S. (1 case); however, these cases were suspected by the study authors to be a result of stevia sweeteners which did not meet the JECFA purity specification of ≥95% steviol glycosides (Kimata, 2007; Esmail and Kabadi, 2012). Thus, it is hypothesized that the few causes of adverse reactions are from impurities present in the final product.

3.2 Estimated Consumption of Stevia Leaf Extracts from Proposed Food Uses

Estimation of steviol glycoside consumption based on the *per capita* consumption of caloric sweeteners in the U.S. is possible; however, assuming that purified steviol glycosides would replace all sugar consumption, this would correspond to an estimated stevia leaf extract intake of 1.5 to 7.8 mg/kg body weight/day as steviol equivalents[1]. These estimated intakes are highly conservative since it is unlikely that stevia leaf extracts would completely replace sugar consumption. In their re-assessment of steviol glycosides at their 82nd meeting, the JECFA Committee considered dietary exposure estimates for mixtures of steviol glycosides and noted that "*sugar substitution methods were generally overestimates of dietary exposure, as not all sugar in food products would be replaced by intense sweeteners, and a number of intense sweeteners are used in the marketplace"* (JECFA, 2016b).

[1] This estimate is based on production data for caloric sweeteners in the U.S. which result in a *per capita* consumption of up to 141 g caloric sweeteners/day (USDA/ARS, 2016) and assumes purified steviol glycosides would replace all sugar consumption and have sweetness equivalency values of between 200 and 350, relative to sugar.

Thus, the estimated consumption of stevia leaf extracts is based on the approach by Renwick (2008). Renwick (2008) estimated the intake of rebaudioside A using published data on dietary exposure to approved intense sweeteners, such as aspartame, from post-market surveillance studies (*i.e.*, studies that used specifically designed food diaries combined with actual use levels or approved levels in different foods and beverages). The exposure from these sweeteners was adjusted based on their relative sweetness intensities to sucrose, assuming a relative sweetness for rebaudioside A of 200 times that of sucrose, and data were pooled to provide realistic, but conservative, estimates of potential consumption of rebaudioside A as steviol equivalents.

In the GRAS notification GRN 000619, PureCircle generated consumption estimates for its steviol glycoside extracts containing different mixtures of individual steviol glycosides utilizing the Renwick (2008) paradigm and using a range of molecular weights (480.62 to 1,437.6 g/mol) and sweetness intensities 200 to 350 times sweeter than sucrose) thereby encompassing all steviol glycosides (U.S. FDA, 2016).

Cargill's stevia leaf extracts are similar to PureCircle's steviol glycoside extracts in that they are also comprised of mixtures of individual steviol glycosides. Thus, the consumption estimates reported in GRAS notification GRN 000619 have been adapted and are presented in Table 3.2-1. These estimations were calculated using steviol glycosides listed in Table 2.1.1-1 with the lowest and highest molecular weights. The predicted intakes of stevia leaf extracts containing mixtures of individual steviol glycosides, expressed as steviol equivalents are similar to those reported in GRN 000619 and are below the current acceptable daily intake (ADI) of 0 to 4 mg/kg body weight as steviol which was defined by the JECFA for steviol glycosides (JECFA, 2009, 2016a).

Table 3.2-1 Estimated Consumption of Steviol Glycosides from Preparations Containing Mixtures of Individual Steviol Glycosides (adapted from GRN 000619, U.S. FDA, 2016)

Population Group	Intakes of High-Intensity Sweeteners (expressed as sucrose equivalents)[a] (mg/kg bw/day)		Consumption Estimates for Mixtures of Steviol Glycosides[b] (mg/kg bw/day)		Consumption Estimates for Mixtures of Steviol Glycosides (as steviol equivalents)[c] (mg/kg bw/day)	
	Average Consumer	High Consumer	Average Consumer	High Consumer	Average Consumer	High Consumer
Non-diabetic Adults	255	675	0.73 to 1.28	1.93 to 3.38	0.16 to 0.85[d]	0.43 to 2.24
Diabetic Adults	280	897	0.80 to 1.40	2.56 to 4.49	0.18 to 0.93	0.57 to 2.97
Non-diabetic Children	425	990	1.21 to 2.13	2.83 to 4.95	0.27 to 1.41	0.63 to 3.28
Diabetic Children	672	908	1.92 to 3.36	2.59 to 4.54	0.43 to 2.23	0.57 to 3.01

bw = body weight.

[a] Source: Renwick AG (2008). The use of a sweetener substitution method to predict dietary exposures for the intense sweetener rebaudioside A. Food Chem Toxicol 46(Suppl. 7):S61-S69. DOI:10.1016/j.fct.2008.05.009.

[b] Mixtures of steviol glycosides are approximately 200 to 350 times as sweet as sucrose.

[c] Calculated based on a range of molecular weights for steviol glycosides, from the lowest possible molecular weight of 480.62 g/mol to the highest possible molecular weight of 1,437.6 g/mol [conversion factors of 1.51 and 4.51, based on a molecular weight of 318.45 g/mol for steviol.].

[d] Example calculations of range: 0.73 mg/kg bw/day / 4.51 = 0.16 mg/kg bw/day; 1.28 mg/kg bw/day / 1.51 = 0.85 mg/kg bw/day.

Part 4 §170.240 Self-Limiting Levels of Use

Stevia leaf extracts have a sweetness potency approximately 200 to 350 times greater than sucrose, depending upon the desired combination of steviol glycosides present. Under its intended use as a general-purpose sweetener, the use of stevia leaf extracts in food and beverages is limited by the desired amount of sweetness. Thus, the use of stevia leaf extracts is self-limiting based on its organoleptic properties when used as a general-purpose sweetener.

Part 5 §170.245 Experience Based on Common Use in Food Before 1958

Not applicable

Part 6 §170.250 Narrative and Safety Information

6.1 Introduction

The safety of Cargill's stevia leaf extracts under the conditions of its intended uses are based on scientific procedures. In particular, the safety of stevia leaf extracts as general-purpose sweeteners in food and beverages is based on the shared metabolic fate of all steviol glycosides; available safety information for steviol glycosides in the public domain, conclusions made by several scientific and regulatory authoritative bodies on the safety of steviol glycosides, and, the estimated intake of stevia leaf extracts as steviol equivalents described in Part 3 of this notification.

Information on the shared metabolic fate of steviol glycosides provides the basis for comparison between different steviol glycosides and is included in Section 6.2. A summary of safety opinions pertaining to steviol glycosides made by scientific and regulatory authorities is presented in Section 6.3. Safety data for steviol glycoside preparations were previously summarized and reviewed by the U.S. FDA in GRAS notification GRN 000619 in 2015, therefore these data are incorporated by reference while new safety data pertaining to the safety of steviol glycosides published between January 2015 and July 2017 are summarized in Section 6.4. In addition, the allergenicity potential of stevia leaf extracts was considered and is discussed in Section 6.5.

All information used to establish the safety in use of stevia leaf extracts is available in the public domain and, as such, there are no data which are exempt from disclosure under the Freedom of Information Act.

6.2 Absorption, Distribution, Metabolism, and Excretion and Common Metabolic Fate of Steviol Glycosides

Data pertaining to the pharmacokinetics, metabolism, and elimination of several steviol glycosides has been reviewed by multiple scientific bodies and regulatory authorities including the U.S. FDA, thus a brief discussion on the metabolic fate of steviol glycosides, which is relevant for all steviol glycosides including stevia leaf extracts, is provided rather than detailed study-by-study summaries.

The metabolism of steviol glycosides has also been extensively studied *in vivo* in both rodents and humans. A number of metabolic studies have demonstrated that steviol glycosides (stevioside and rebaudioside A) are not readily absorbed from the upper gastrointestinal tract, and as reported *in vitro*, are hydrolyzed by the colonic flora to steviol (Wingard *et al.*, 1980; Nakayama *et al.*, 1986; Gardana *et al.*, 2003; Koyama *et al.*, 2003a; Wang *et al.*, 2004; Geuns *et al.,* 2006*,* 2007; Wheeler *et al.,* 2008; Roberts *et al.,* 2016). This is because digestive enzymes of the upper gastrointestinal tract cannot hydrolyze steviol glycosides according to findings from *in vitro* and *ex vivo* studies (Hutapea *et al*., 1997; Geuns *et al*., 2003, 2007; Koyama *et al*., 2003b). Therefore, in the colon, degradation of steviol glycosides by members of the *Bacteroidaceae* family occurs resulting in sugars and the aglycone steviol (Renwick and Tarka, 2008). The hydrolysis of steviol glycosides to produce steviol has been confirmed to occur in several rodent models (rats, mice, hamsters) and humans in several *in vitro* studies which have mimicked the anaerobic conditions of the colon (Wingard *et al*., 1980; Hutapea *et al*., 1997; Gardana *et al*., 2003; Koyama *et al*., 2003b; Nikiforov *et al*., 2013; Purkayastha *et al.,* 2014*,* 2015, 2016). *In vitro* metabolism studies have also been conducted with crude pectinase from *Aspergillus niger,* as pectinolytic bacteria are known to reside in the human intestine (Jensen and Canale-Parola, 1985), and likewise, steviol was detected following incubation of rebaudioside E with pectinase (Chaturvedula and Prakash, 2013).

A recent re-assessment of the existing *in vitro* metabolism data was carried out by Purkayastha *et al*. (2016). The re-assessment focused on steviol glycoside metabolism studies which used human fecal homogenates. The study author collected and compared studies that compared the metabolism of individual steviol glycosides (rebaudiosides B, C, D, E, F, M, dulcoside A, and steviolbioside) to rebaudioside A under similar test concentrations (0.2 to 2.0 mg/mL, depending on solubility) and incubation times (up to 48 hours). This assessment demonstrated that steviol glycosides are metabolized at generally similar hydrolysis rates regardless of the type of sugar moiety (*e.g.,* glucose, rhamnose, xylose) or the number of sugar moieties attached to the steviol backbone.

Once hydrolyzed, steviol is readily absorbed from the colon to the portal vein and distributed to a number of organs and tissues, including the liver, spleen, adrenal glands, and fat. After it is absorbed from the colon, steviol is primarily conjugated with glucuronic acid to steviol glucuronide in the liver. Pharmacokinetic studies demonstrated that steviol glucuronide is excreted in rats primarily *via* the bile (Wingard *et al*., 1980; Nakayama *et al*., 1986; Sung, 2002; Roberts and Renwick, 2008), whereas in humans steviol glucuronide is cleared primarily *via* the urine (Kraemer and Maurer, 1994; Geuns and Pietta, 2004; Simonetti *et al*., 2004; Geuns *et al*., 2006, 2007; Wheeler *et al*., 2008). It is 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) which causes the difference in the route of elimination between these 2 species (Renwick, 2007). Regardless, water soluble glucuronide conjugates are rapidly cleared in both species, thus, these differences in the route of elimination are considered to be of no toxicological significance.

To more accurately characterize the pharmacokinetic/toxicokinetic differences in the production of steviol/steviol glucuronide following oral consumption of steviol glycosides between rats and humans, Roberts *et al.* (2016) recently conducted comparative studies in rats and humans. Male Sprague-Dawley rats and healthy male human volunteers were orally administered a single dose of stevioside (40 mg/kg body weight; equivalent to 16 mg steviol equivalents/kg body weight) and plasma samples collected over the following 72 hours were analyzed for steviol and steviol glucuronide by liquid chromatography-tandem mass spectrometry. Although peak plasma concentrations (C_{max}) of steviol and steviol glucuronide occurred slightly later in humans in comparison to rats, C_{max} values of plasma steviol were similar between rats and humans (~72 to 77 ng/mL). C_{max} values for steviol glucuronide, however, were approximately 25-fold higher in humans than rats (~4,400 ng/mL *vs.* 180 ng/mL). Systemic exposure was determined based on the area-under-the-curve (AUC) of the concentration *vs.* time data, and steviol and steviol glucuronide exposure were measured to be 2.8-fold higher (~1,650 ng*h/mL *vs.* 590 ng*h/mL) and 57-fold higher (~136,000 ng*h/mL *vs.* 2,400 ng*h/mL), respectively, in humans compared to rats.

Collectively, the available degradation and pharmacokinetic studies on steviol glycosides confirm the common metabolic pathway for all steviol glycosides: steviol glycosides are rapidly hydrolyzed to steviol, steviol is absorbed and conjugated with glucuronic acid, and steviol glucuronide is excreted *via* the urine in humans. This is consistent with the fact that with the exception of having different numbers and types of sugar moieties, steviol glycosides share the same structural backbone, steviol. Due to this shared metabolic fate of steviol glycosides the safety data and conclusions drawn for individual steviol glycosides, therefore, can be extended to include all steviol glycosides including those within stevia leaf extracts which is the subject of this application.

6.3 Summary of Safety Opinions by Scientific and Regulatory Authorities

As previously noted, steviol glycosides have been previously evaluated by several scientific and regulatory authorities. JECFA, in particular, has extensively reviewed the safety of steviol glycosides at several of the Committee meetings (JECFA, 1999, 2006, 2007, 2009, 2016b, 2017). JECFA concluded that the metabolic fate of steviol glycosides is similar in humans and rats, in that they are converted to steviol through hydrolysis of sugar moieties by intestinal bacteria and steviol is absorbed from the colon. Steviol is rapidly metabolized and excrete *via* the urine in humans. The Committee also concluded that steviol is not mutagenic *in vivo*. When provided to human subjects for with type 2 diabetes mellitus for 16 weeks or individuals with normal or low-normal blood pressure for 4 weeks, steviol glycosides which meet the established purity criteria do not cause adverse effects when consumed at doses of up to 4 mg/kg body weight/day, as steviol equivalents. At their 51st meeting the Committee evaluated a carcinogenicity study in rats conducted with stevioside in which a no-observed-adverse-effect level (NOAEL) of 970 mg stevioside/kg body weight/day (equivalent to 383 mg/kg body weight/day as steviol) was determined (Toyoda *et al.*, 1997). Based on this study, JECFA applied a 100-fold safety factor for inter- and intra-species differences to the NOAEL to derive an ADI for steviol glycosides of 0 to 4 mg/kg body weight, expressed as steviol equivalents. The initial ADI and specifications for steviol glycosides established by JECFA (2010) were limited to 9 named steviol glycosides (stevioside, rebaudioside A, B, C, D, and F, dulcoside A, rubusoside, and steviolbioside) and stipulated that the purity of steviol glycoside preparations was to be not less than 95% (JECFA, 2010). JECFA recently re-assessed the safety of steviol glycosides at the 82nd meeting by reviewing all new data which had become available since the previous evaluation, and the ADI for steviol glycosides was confirmed. Based on the new data, a tentative specification was established for "Steviol glycosides from *Stevia rebaudiana* Bertoni" which defined steviol glycosides as "all compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni, including glucose, rhamnose, xylose, fructose, and deoxyglucose[2]" (JECFA, 2016a). The inclusion of all steviol glycosides within JECFA's purity specification further confirms that the safety of steviol glycosides is based on the general recognition that all glycosides are hydrolyzed to the aglycone steviol and that the safety demonstrated for one glycoside is relevant to all glycosides in general.

Similarly, an ADI of 4 mg/kg body weight, expressed as steviol equivalents has been established by other authoritative bodies including EFSA, FSANZ, and Health Canada following their safety evaluations of steviol glycosides (EFSA, 2010; FSANZ, 2008, 2015, 2017; Health Canada, 2012a, 2017).

EFSA (2010) evaluated the safety of steviol glycosides[3] for use in food in the European Union at the request of the European Commission as part of the authorization process for food additives. Following this safety opinion and the setting of the ADI of 4 mg/kg body weight, as steviol equivalents which was established therein, the European Commission permitted the use of steviol glycoside as a sweetening agent under Commission Regulation (EU) No 1131/2011 (EU, 2011). Subsequently, EFSA expanded the definition of steviol glycosides to include rebaudiosides D and M and concluded that "extending the current specifications to include [two additional steviol glycosides], rebaudiosides D and M, as alternatives to rebaudioside A in the predominant components of steviol glycosides would not be of safety concern" and

[2] The Committee reviewed a validated HPLC-ultraviolet method for the assay at the 84th meeting and based on these data the 2 additional saccharides (galactose and arabinose) were included in the definition and the tentative status was removed from the specification (JECFA, 2017).

[3] Consisting of stevioside, rebaudioside A, B, C, D, and F, dulcoside A, rubusoside, and steviol bioside (EFSA, 2010)

that “the ADI of 4 mg/kg body weight can also be applied where total steviol glycosides comprise more than 95% of the material” (EFSA, 2015).

FSANZ has recently approved a request to amend the definition of steviol glycosides in the Food Standards Code to include “all minor steviol glycosides” extracted from the *S. rebaudiana* Bertoni leaf in addition to the 10 steviol glycosides (stevioside, rebaudioside A, B, C, D, F, and M, dulcoside A, rubusoside, and steviolbioside) which were approved previously (FSANZ, 2008, 2015, 2017). As part of the approval process, FSANZ performed a risk assessment in which it concluded that steviosides, rebaudiosides, and dulcosides are all biotransformed to steviol and are consistent with steviol glycosides previously approved in Australia and New Zealand. FSANZ concluded that the ADI for steviol glycosides from *S. rebaudiana* Bertoni leaf of 0 to 4 mg/kg body weight (as steviol) is “applicable to all steviol glycosides in stevia leaf” which FSANZ recognizes includes at least 40 different steviol glycosides (FSANZ, 2017). FSANZ also prepared new specifications for the expanded steviol glycoside definition with the intent that these new specifications will be removed some time after the specifications recently established by JECFA (2016a) are finalized.

Steviol glycosides as initially defined by JECFA were approved by Health Canada for use as sweetening agents at levels of up to 0.35% calculated as steviol equivalents (Health Canada, 2012b). Health Canada has, after receiving a request, decided to expand the steviol glycoside food additive description ‘steviol glycosides’ to include all steviol glycosides in the *S. rebaudiana* Bertoni plant (Health Canada, 2017).

6.4 New Safety Data

Cargill’s stevia leaf extracts are similar to steviol glycoside preparations which were reviewed by the U.S. FDA in GRAS notification GRN 000619 (U.S. FDA, 2016). It has been previously established by regulatory bodies that the safety data for any steviol glycoside is applicable to any other steviol glycoside. Thus, the publicly-available safety data contained within GRN 000619 which is current up to January 2015 is incorporated by reference into the safety discussion of this GRAS dossier. New safety data published after January 2015 were obtained through a comprehensive and detailed search of the published scientific literature published between January 2015 and July 2017. The literature search was completed using ProQuest and included searches of the following databases for pertinent literature on the safety of steviol glycosides: AdisInsight: Trials, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, ToxFile®. Due to the purity criteria laid down in several specifications, studies were excluded if the test article investigated had a purity of less than 95% steviol glycosides.

New safety data identified included a 90-day repeat-dose study in rats provided diets containing rebaudioside A (>95% purity), a 90-day repeat-dose study in rats provided diets containing an ethanolic extract from the leaves of *S. rebaudiana*, a 7-month repeat dose study in mice provided diets containing rebaudioside A (>95% purity), *in vitro* genotoxicity studies evaluating rebaudioside A (>95% purity), and *in vitro* and *in vivo* genotoxicity studies evaluating an ethanolic extract obtained from *S. rebaudiana* leaves. Repeat-dose studies and genotoxicity studies are summarized in Sections 6.4.1 and 6.4.2, respectively.

While not directly related to safety, a study was performed to evaluate the effect of steviol glycoside consumption on the glycemic index of healthy individuals (Aranda-González *et al*., 2014). When stevia glycoside extracts from 2 varieties of *S. rebaudiana* were obtained and provided to human volunteers, it was reported that the extracts had a low glycemic index and had no acute or chronic effect on blood sugar levels.

6.4.1 Repeat-Dose Studies in Animals

In a 90-day repeat-dose oral toxicity study in male and female Sprague-Dawley rats, the safety of rebaudioside A (>95% purity) produced *via* fermentation by *Yarrowia lipolytica* (*Y. lipolytica*)genetically engineered to express the *S. rebaudiana* metabolic pathway was evaluated (Rumelhard *et al*., 2016). This study was conducted in accordance with the FDA Redbook 2000 and OECD 408 guidelines for repeat-dose toxicity studies (OECD, 1998; U.S. FDA, 2000). Male and female Sprague-Dawley rats (20/sex/group) were administered rebaudioside A in the diet at dose levels of 0 (basal diet), 500, 1,000, or 2,000 mg/kg body weight/day for 90 days. No deaths or clinical signs of toxicity were observed throughout the study. A significant decrease in male body weight (5.9%) in and a slight but non-significant reduction in female body weight in the 2,000 mg/kg body weight/day group were reported. The authors associated the changes in body weights with the lower caloric value of the diet containing rebaudioside A in comparison to the basal diet alone and did not consider this finding to be adverse in light of the small magnitude of the difference between the males in the 2,000 mg/kg body weight/day and control groups. No other test article related effects in hematology, coagulation, serum chemistry, and urinalysis parameters, or upon gross pathological and histopathological examinations were observed. Based on the above findings, the authors determined a NOAEL for rebaudioside A (fermented by *Y. lipolytica* expressing the *S. rebaudiana* metabolic pathway) to be 2,000 mg/kg body weight/day, equivalent to 2,057 and 2,021 mg/kg body weight/day for males and females, respectively (about 660 mg /kg body weight/day as steviol equivalents).

A recent 90-day repeat-dose study in rats was identified in which an ethanolic extract from the leaves of the *S. rebaudiana* Bertoni plant was included in the diets of male and female Sprague-Dawley rats (10/sex/group) at concentrations of 0, 1.04, 2.08, or 3.12% (equivalent to 0, 830, 1,670, or 2,490 mg extract/kg body weight/day) (Zhang *et al.*, 2017). Actual intakes of the test substance were 570, 1,163, 1,700 mg/kg body weight for females and 724, 1,464, and 2,238 mg/kg body weight for males (*i.e.,* up to 270 times the manufacturer-recommended daily intake), respectively. There were no mortalities and no treatment-related adverse clinical effects throughout the study. Clinical chemistry and hematological findings revealed no consistent dose-dependent trends. Organ (liver, kidneys, spleen, stomach, duodenum, heart, thymus, adrenals, ovaries, testes) weights, macroscopic evaluations, and microscopic evaluations revealed no treatment-related effects. It is noted that this study did not evaluate the complete set of organs recommended by the OECD (OECD, 1998). The study also uses a test article which does not meet the purity specifications established by JECFA and contained approximately 47.78% polyphenols (mostly isochlorgenic acids) with the remainder consisting of soluble fibers and glucose. Regardless of these limitations, the results of this study support the safety of stevia leaf-derived products.

In a recent study to evaluate the physiological effects of rebaudioside A, groups of 6-month-old male C57BL6/J mice (10/group) were provided either drinking water (control) or drinking water with 0.1% rebaudioside A for approximately 7 months (*i.e.,* until the conclusion of all tests were completed) (Reynolds *et al*., 2017). Upon commencement of dosing with rebaudioside A, the mice were placed in running wheel cages for 32 days. Following the running wheel period, mice were placed in normal cages and allowed to recover for 3 months. At the end of the 3-month period, glucose tolerance, pyruvate tolerance, and insulin tolerance tests were performed with 7- to 10-day recovery periods between each test. After assessing the insulin tolerance of the mice, animals were then placed on a high-fat diet for 2 months to assess obesity susceptibility. Due to increased water consumption relative to the control group, the amount of rebaudioside A consumed was reported to be 5.9 mg/day and, based on supplementary data provided by Reynolds *et al.* (2017), the mean body weights of the mice in the rebaudioside A group varied from 32.5 to 28.5 g between the start and end of the exercise wheel arm of the trial and from 38.8 to 50.8 g between the start and end of the high-fat diet arm. Thus, the dose of rebaudioside A was calculated to range from 116 to 207 mg/kg body weight/day.

While Reynolds *et al*. (2017) did not perform evaluations of endpoints typical in repeat-dose toxicity studies (*e.g.,* histopathology, organ weight analysis), it was reported that rebaudioside A was well tolerated, has no effect on body weight, insulin tolerance, glucose tolerance, pyruvate tolerance, or susceptibility to obesity and does not disrupt circadian rhythms.

6.4.2 Genotoxicity Studies

No mutagenicity was reported in an Ames reverse mutation assay in *Salmonella typhimurium* (*S. typhimurium*)when rebaudioside A produced *via* fermentation by a genetically engineered yeast (*Y. lipolytica*) was tested at a concentration of 5,000 µg/plate in the presence or absence of metabolic activation or in an *in vitro* micronucleus assay when cultured peripheral human lymphocytes were incubated with up to 5,000 µg/mL rebaudioside A for up to 3 hours in the presence or absence of metabolic activation or up to 24 hours in the absence of metabolic activation (Rumelhard *et al.*, 2016). These findings are consistent with conclusions made by previous authoritative bodies, such as JECFA (2010) that steviol glycosides are not genotoxic.

In studies using a crude ethanolic extract obtained from *S. rebaudiana* leaves, the extract was reported to be negative in a reverse mutation assay in *S. typhimurium*, an *in vivo* mouse micronucleus test, and an *in vivo* mouse sperm malformation assay; these findings support the safety of products derived from *S. rebaudiana* Bertoni leaves (Zhang *et al*., 2017).

6.5 Allergenicity Potential

Urban *et al*. (2015) reported that there are several online medical and health resources which have reportedly included food allergy warnings in discussions regarding steviol glycosides. According to Urban *et al*. (2015), these concerns regarding allergenic potential of extracts from *S. rebaudiana* are based on the assertion that the *S. rebaudiana* plant is part of a family of plants which are known to be commonly allergenic. (Urban *et al*., 2015). Following a review of the published literature, Urban *et al*. (2015) reported on 2 case studies in which 2 subjects presented with, in the first case, an allergic reaction (eczema, anaphylaxis), and, in the second case, hypertension and persistent edema following consumption of stevia-sweetened products. In both instances, the purity of the stevia sweetener products is not discussed. Overall, Urban *et al*. (2015) concluded that the limited dataset relating to the allergic potential of steviol glycosides does not provide sufficient evidence to support the food allergy warnings made publicly available through medical and health websites.

Furthermore, 2 authoritative bodies, EFSA (2010) and Health Canada (2012a), have concluded that steviol glycosides, particularly those which are highly-purified, are unlikely to be an allergenic concern for the general population.

6.6 Expert Panel Evaluation

Cargill has concluded that stevia leaf extracts manufactured consistent with cGMP is GRAS for use as general-purpose sweeteners in food and beverages, as described in Part 1.3, on the basis of scientific procedures.

The GRAS determination is based on data generally available in the public domain pertaining to the safety of stevia leaf extracts and based on a unanimous opinion among a panel of experts (“the Expert Panel”), who are qualified by scientific training and experience to evaluate the safety of food ingredients. The Expert

Panel consisted of the following qualified scientific experts: Professor Emeritus I. Glenn Sipes (University of Arizona), Adjunct Professor John A. Thomas (University of Indiana School of Medicine), and Adjunct Associate Professor Stanley M. Tarka Jr. (Pennsylvania State University College of Medicine). The GRAS dossier for stevia leaf extracts was prepared by Intertek Health Sciences, Inc. on behalf of Cargill. The Expert Panel was selected and convened prior to issuance of the FDA's guidance for industry on *Best Practices for Convening a GRAS Panel* (U.S. FDA, 2017b), and therefore no formal written GRAS Panel policy was in place at the time of Expert Panel meeting. However, the notifier confirms that prior to convening the Panel all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, and microbiology, and efforts were placed on identifying conflicts of interests or relevant appearance issues that would potentially bias the outcome of the Expert Panel deliberations; no such conflicts of interests or appearance conflicts were identified. The Expert Panel received a reasonable honorarium as compensation for the Expert Panel's time, and honoraria provided to the Expert Panel were not contingent upon the outcome of the Expert Panel deliberations.

The Expert Panel, convened by Cargill, independently and critically evaluated all data and information presented herein, and concluded that stevia leaf extracts are GRAS for use as a general-purpose sweetener in foods and beverages, as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the Expert Panel and evaluation of such data as it pertains to the proposed GRAS uses of stevia leaf extracts, are presented in Appendix A.

6.7 Conclusions

Based on data and information presented herein Cargill has concluded that stevia leaf extracts, manufactured according to cGMP, for use as general-purpose sweeteners in food and beverages can be determined to be GRAS on the basis of scientific procedures.

Part 7 §170.255 List of Supporting Data and Information

The following generally-available data were cited in this notification and were used to provide the basis for the GRAS status of Cargill's stevia leaf extracts as general-purpose sweeteners in food and beverages:

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Part	Section §	Section Title
170 – Food Additives	170.30	Eligibility for classification as generally recognized as safe (GRAS)
173—Secondary Direct Food Additives Permitted in Food for Human Consumption	173.25	Ion-exchange resins
182—Substances Generally Recognized as Safe	182.1	Substances that are generally recognized as safe
184—Direct Food Substances Affirmed as Generally Recognized as Safe	184.1210	Calcium oxide
	184.1315	Ferrous sulfate

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Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of Stevia Leaf Extracts for Use in Foods as General Purpose Sweeteners

October 6, 2017

INTRODUCTION

Cargill Incorporated (Cargill) intends to market a number of stevia leaf extract products manufactured according to current Good Manufacturing Practices (cGMP) by their suppliers and containing various individual steviol glycosides in any combination or ratio and intended for use in food and beverage products as general-purpose sweetening agents. The intended use of stevia leaf extracts is consistent with the current uses of other related high-intensity sweeteners (HIS) already on the market in the United States (U.S.). All stevia leaf extracts are obtained by water-extraction of leaves from the *Stevia rebaudiana* Bertoni plant according to processes which are consistent with manufacturing methodologies described in previous GRAS notifications as well as in the most recent Chemical and Technical Assessment published by the Joint FAO/WHO Expert Committee on Food Additives [JECFA] (JECFA, 2016a).

At the request of Cargill, an Expert Panel (the "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 on October 6, 2017 to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use as a general-purpose sweetener in traditional foods, stevia leaf extracts would be "Generally Recognized as Safe" (GRAS), based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Dr. I. Glenn Sipes, Ph.D. (University of Arizona College of Medicine), Dr. John Thomas, Ph.D. (University of Indiana School of Medicine), and Stanley Tarka, Ph.D. (The Pennsylvania State University College of Medicine; The Tarka Group, Inc.).

The Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources based on previous steviol glycosides' GRAS notifications published as recently as January 2015 and searches of the published scientific literature conducted from January 2015 through July 2017. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Cargill. The data evaluated by the Panel included information pertaining to the methods of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and comprehensive literature on the safety of stevia leaf extracts and its individual components, as described in the supporting dossier *Documentation Supporting the Evaluation of Stevia Leaf Extracts as Generally Recognized as Safe (GRAS) for Use as General Purpose Sweeteners*.

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the conditions of intended use in traditional foods described herein, stevia leaf extracts, meeting appropriate food-grade specifications, and manufactured and used in accordance with current good manufacturing practice, is GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion, excluding confidential data and information, is provided below.

COMPOSITION, MANUFACTURING AND SPECIFICATIONS

The ingredients that are the subject of this GRAS evaluation is stevia leaf extracts containing not less than 95% steviol glycosides. These naturally-occurring steviol glycosides, of which there are more than 40, are constituents of the *S. rebaudiana* plant and share a molecular structure consisting of a common steviol backbone that is linked on carbons 13 and 19 to mono- di- or oligosaccharide groups (*e.g.*, glucose, rhamnose, xylose, fructose, deoxyglucose, galactose, and/or arabinose) in any combination or orientation. Different pH and temperature conditions can be used during the production of stevia leaf extracts which can result in stevia leaf extracts with different distributions of individual steviol glycosides. In addition to the well-characterized steviol glycosides, as-yet not fully characterized steviol glycosides have also been identified in stevia leaf extracts. These may be produced under particular extraction and/or storage conditions which permit the hydrolysis of sugar moieties or from steviol glycosides containing fewer sugar moieties (Kohda *et al.*, 1976; Kobayashi *et al*., 1977; Chang and Cook, 1983; Kinghorn *et al.,* 1999; Kennelly, 2002; Woelwer-Rieck *et al.*, 2010; Wölwer-Rieck, 2012).

During the manufacture of stevia leaf extracts by Cargill and their suppliers, steviol glycosides are first obtained by water extraction of leaves from the *S. rebaudiana* Bertoni plant. The extract is then centrifuged or filtered using a filter press. The filtrate is then passed through cation and anion exchange columns, adsorption resins, and eluted using organic solvents such as ethanol. The eluent is concentrated by evaporation to remove the solvent and the remaining concentrate is dried to yield the crude extract containing mixed steviol glycosides which are further refined using optional adsorption, filtration, and/or centrifugation to produce different purified steviol glycoside products. This production process is consistent with methodologies described by JECFA (2016a) and uses raw materials and processing aids which are food-grade quality.

The physical and chemical specifications for stevia leaf extracts are based on those published by JECFA (2016b) for steviol glycosides and the Food Chemicals Codex (FCC) for steviol glycosides and are included in Attachment 1 (FCC, 2016). These specifications are also similar to those established in the previous GRAS Notification No. 000619 (Steviol Glycosides) to which the U.S. Food and Drug Administration (FDA) issued a "no questions" letter to the notifier (U.S. FDA, 2016). Since stevia leaf extracts are obtained from a natural source, the potential for microbiological contamination has been considered and limited through product specifications established by Cargill to ensure safety of stevia leaf extracts in food. Routine analyses of stevia leaf extracts are carried out to verify compliance with the chemical specifications and microbiological parameters established by Cargill. Screening for pesticide residues are carried out on stevia leaf extracts to ensure no contamination. The stability of steviol glycosides has been previously established by JECFA and in previous GRAS notifications under conditions of storage and use in foods and beverages across a range of pH values (2.0 to 8.0) and temperatures (5 to 56°C) (JECFA, 2007, U.S. FDA, 2013, 2015a, 2016a). According to JECFA, the compositional similarity among steviol glycosides indicate that stevia leaf extracts would have comparable stability under normal production and storage conditions; stability data reported in previous GRAS notifications support this conclusion.

INTENDED USE AND ESTIMATED EXPOSURE

Stevia leaf extracts are proposed for use as general-purpose sweeteners that will be added to a variety of food products, consistent with the current uses of other related HIS that are already in the market (*e.g.,* aspartame). Published dietary exposure data for other already approved HIS in the U.S. market were adjusted for the relative sweetness intensity to steviol glycosides in order to determine the predicted intake range of stevia leaf extracts (Renwick, 2008). Using these data, the mean intake of stevia leaf extracts is predicted to range from 0.73 mg/kg body weight/day (0.16 mg/kg body weight/day as steviol equivalents) for non-diabetic adults to 3.36 mg/kg body weight/day (2.23 mg/kg body weight/day as steviol equivalents) for diabetic children. Predicted intakes for heavy consumers range from 1.93 mg/kg body weight/day (0.43 mg/kg body weight/day as steviol equivalents) for non-diabetic adults to 4.95 mg/kg body weight/day (3.28 mg/kg body weight/day as steviol equivalents) for non-diabetic children. The highest intake for stevia leaf extracts, 3.28 mg/kg body weight/day, as steviol equivalents, is below the current acceptable daily intake (ADI) for steviol glycosides of 4 mg/kg body weight/day, expressed as steviol, as set by JECFA (2009).

DATA PERTAINING TO SAFETY

The safety of stevia leaf extracts is based on a detailed discussion of the metabolic fate of steviol glycosides, a summary of the conclusions made by global scientific and regulatory authorities regarding the safety of steviol glycosides, two *in vitro* reverse mutation assays, an *in vitro* micronucleus assay in human lymphocytes, an *in vivo* mouse micronucleus test, an *in vivo* mouse sperm malformation assay, and three repeat-dose oral toxicity studies in rats and mice which were not originally identified within the evaluations by JECFA (2009) and the European Food Safety Authority [EFSA] (EFSA, 2010) or in GRAS Notification No. 000619 (U.S. FDA, 2016).

Absorption, Distribution, Metabolism, and Excretion (ADME)

Following ingestion, steviol glycosides passes through the stomach and upper gastrointestinal tract intact (Hutapea *et al*., 1997; Geuns *et al*., 2003, 2007; Koyama *et al*., 2003). Once these steviol glycosides enter the colon, they are subject to complete microbial degradation by members of the *Bacteroidaceae* family, resulting in the release of the aglycone steviol (Wingard *et al*., 1980; Jensen and Canale-Parola, 1985; Hutapea *et al*., 1997; Gardana *et al*., 2003; Koyama *et al*., 2003; Renwick and Tarka, 2008; Chaturvedula and Prakash, 2013; Nikiforov *et al*., 2013; Purkayastha *et al.,* 2014*,* 2015, 2016). The data on the metabolic fate of steviol glycosides were recently assessed by Purkayastha *et al.* (2016) who concluded that steviol glycosides are metabolized to steviol at generally similar hydrolysis rates, irrespective of the types and number of sugar moieties attached to the steviol backbone. Thus, all steviol glycosides, including those present in stevia leaf extract are expected to follow the same metabolic pathways as demonstrated in the above studies.

In metabolic fate studies in rats, the aglycone produced in the colon has been shown to be absorbed systemically *via* the portal vein then transported to the liver where it is metabolized to steviol glucuronide before being excreted in the feces *via* the bile (Wingard *et al*., 1980; Nakayama *et al*., 1986; Sung, 2002; Roberts and Renwick, 2008). Human data present a similar metabolic fate of steviol glycosides with the exception that steviol glucuronide is eliminated *via* the urine due to the lower molecular weight threshold for biliary excretion in rats as compared to humans (Kraemer and Maurer, 1994; Geuns and Pietta, 2004; Simonetti *et al*., 2004; Geuns *et al*., 2006, 2007; Renwick, 2007; Wheeler *et al*., 2008; Roberts *et al.*, 2016). This difference in the route of elimination is of no toxicological significance due to the fact that the water-soluble phase II metabolites are rapidly cleared in both rats and humans. Therefore, toxicology data generated in rats are applicable to assess the safety of steviol glycosides in humans.

History of Use of Steviol Glycosides in Foods and Beverages

The *S. rebaudiana* plant and its steviol glycoside components have been consumed as sweeteners in foods and beverages by humans in countries such as Brazil and Paraguay, as well as by indigenous peoples for decades to hundreds of years (Blumenthal, 1995; Guens, 2003; Ferlow, 2005). Stevioside has been reported to be in commercial use in Asia since at least 1995, and for more than 30 years in Japan (International Sugar Organization, 2001; Ferlow, 2005). There have been no reports of adverse effects following the use of *S. rebaudiana* as a sweetener (Lee *et al.*, 1979; Ferlow, 2005). Very few allergic or hypersensitivity reactions from the use of stevia sweeteners have been reported (Kimata, 2007; Esmail and Kabadi, 2012). These reactions were likely due to impurities present in stevia sweeteners which did not meet the purity specification (≥95%) set by JECFA. In a recent review of the published literature, Urban *et al*. (2015) reported that there is a limited dataset pertaining to the allergic potential of steviol glycosides which does not provide sufficient evidence to support food allergy warnings which have been made publicly on medical and health websites.

Safety Opinions by Scientific and Regulatory Authorities

Numerous JECFA safety reviews over the last few decades have considered the safety of steviol glycosides as food additives (JECFA, 1999, 2006, 2007, 2009, 2016a, 2017) and recently, multiple jurisdictions including the European Union (EU), Australia and New Zealand, and Canada have concluded that preparations containing at least 95% steviol glycosides are safe when used in accordance with cGMP (FSANZ, 2008, 2015, 2017; EU, 2011; Health Canada 2012a,b, 2016, 2017; EFSA, 2015). In addition, multiple GRAS notifications for steviol glycosides from *S. rebaudiana* Bertoni have been submitted to the FDA and received "no questions" letters based on the similar metabolic pathway for all steviol glycosides in rats and humans. The JECFA established an ADI of 0 to 4 mg/kg body weight, as steviol equivalents, based on a no-observed-adverse-effect level (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 a safety factor of 100, to account for intra- and inter-species differences (JECFA, 2009). Initial specifications established by JECFA (2010) stipulated that the purity of steviol glycoside preparations was to be not less than 95% of the 9 named steviol glycosides (stevioside, rebaudioside A, B, C, D, and F, dulcoside A, rubusoside, and steviolbioside). Following the review of new data which was presented in the 82nd meeting of JECFA, a tentative specification was established for "steviol glycosides from *Stevia rebaudiana* Bertoni" which defined steviol glycosides as "all compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni, including glucose, rhamnose, xylose, fructose, and deoxyglucose" (JECFA, 2016b). The ADI was confirmed and extended to all steviol glycosides by JECFA (2016a, 2017). The Expert Panel notes that the inclusion of all steviol glycosides within JECFA's specification for steviol glycosides from *S. rebaudiana* Bertoni demonstrates the safety of steviol glycosides based on the general recognition that all glycosides undergo similar biotransformation following oral administration.

Recent evaluations performed by EFSA (2015) have extended the current specifications for steviol glycosides (consisting of stevioside, rebaudioside A, B, C, D, and F, dulcoside A, rubusoside, and steviolbioside) to include rebaudioside D and M as alternatives to rebaudioside A. EFSA noted that the ADI of 4 mg/kg body weight/day (as steviol) can also be applied to these additional steviol glycosides and that the use of these additional steviol glycosides, "would not be of safety concern" (EFSA, 2015).

Food Standards Australia New Zealand (FSANZ) amended their food standard definitions, to include all minor steviol glycosides extracted from *S. rebaudiana* Bertoni leaf in addition to the 10 already-authorized steviol glycosides (FSANZ, 2017). Following a risk assessment, FSANZ concluded that the ADI for steviol glycosides

from *S. rebaudiana* Bertoni leaf (0 to 4 mg/kg body weight/day, as steviol) is applicable to all steviol glycosides obtained from stevia leaf (FSANZ, 2017). Similarly, following a safety assessment of steviol glycosides, Health Canada decided to expand the food additive description of steviol glycosides to include all steviol glycosides in the *S. rebaudiana* Bertoni plant (Health Canada, 2017).

A paper was published on refinements to the default safety factors used in the determination of the ADI established by JECFA and was summarized by Cargill in the *Documentation Supporting the Evaluation of Stevia Leaf Extracts as Generally Recognized as Safe (GRAS) for Use as General Purpose Sweeteners*. Using the toxicokinetic/toxicodynamic data presented in Roberts *et al*. (2016), the study authors concluded that the chemical-specific adjustment factor (CSAF) for toxicokinetic differences between rats and humans can be estimated to range between 1 and 2.8, rather than the default value of 10 defined by the World Health Organisation (JECFA, 2005). This CSAF provides an ADI between 6 and 16 mg/kg body weight, as steviol equivalents, although the ADI of 0 to 4 mg/kg body weight, as steviol equivalents is still the value assigned by JECFA for stevia leaf extracts.

New Safety Data

Two 90-day repeat-dose study in rats and a 7-month repeat-dose study in mice were identified in the published literature that were not previously included in the evaluation by JECFA (2009), EFSA (2010), or the recent GRAS notification for steviol glycoside preparations (U.S. FDA, 2016). In the first 90-day study, the only significant finding was decreased body weight in males (5.9% decrease relative to the control group) at the highest dose of 2,000 mg rebaudioside A (>95% purity)/kg body weight/day; a similar decrease was observed in female rats; however, the decrease was not statistically significant. These findings were not considered to be the result of a toxic effect but were instead due to the caloric value of the diet in this dose group which was lower than the basal diet alone (Rumelhard *et al*., 2016). Since there were no macroscopic, histological, hematological, or blood biochemistry findings, a NOAEL of 2,000 mg/kg body weight/day was determined for rebaudioside A. In the second 90-day study, rats were provided diets containing an ethanolic extract from *S. rebaudiana* at doses of up to 1,700 and 2,238 mg/kg body weight/day in females and males, respectively (approximately 270 times the manufacturer-recommended daily intake) (Zhang *et al*., 2017). There were no mortalities or treatment-related effects; however, it is noted that the test article did not comply with the purity specifications established by JECFA and did not fully comply with internationally-recognized test guidelines for subchronic toxicity studies since the histological evaluation omitted several organs and tissues. Regardless, the results of this study support the safety of stevia leaf-derived products, including Cargill's stevia leaf extracts. It was reported that rebaudioside A was well tolerated with no effect on body weight, insulin tolerance, glucose tolerance, pyruvate tolerance, susceptibility to obesity, or circadian rhythms when included in drinking water of male mice at doses of up to 5.9 mg/day (calculated as 116 to 207 mg/kg body weight/day, highest dose tested) for 7 months (Reynolds *et al*., 2017). Rumelhard *et al*. (2016) also conducted a reverse mutation assay in *Salmonella typhimurium* and an *in vitro* micronucleus test in peripheral human lymphocytes using rebaudioside A produced *via* fermentation by a genetically engineered yeast. Zhang *et al*. (2017) conducted a reverse mutation assay in *S. typhimurium*, an *in vivo* mouse micronucleus test, and an *in vivo* mouse sperm malformation assay using an ethanolic extract obtained from *S. rebaudiana* leaves. The results of these tests were negative which are consistent with genotoxicity data considered previously by authoritative bodies (JECFA, 2010). Overall, the Panel notes that the findings from these studies further corroborate the safety of steviol glycosides including these data on rebaudioside A and, based on the common metabolic fate, all steviol glycosides for use as general-purpose sweeteners in foods and beverages.

Summary

The common metabolic fate of steviol glycosides in humans is generally recognized and supports the safety of all steviol glycosides (JECFA, 2016a, 2017). Stevia leaf extracts consist of not less than 95% steviol glycosides which are intended for use as general-purpose sweeteners in foods and beverages and are expected to be used at rates consistent with other steviol glycosides already permitted for use in the U.S. The predicted intakes of steviol glycosides (as steviol equivalents) from stevia leaf extracts are below the ADI of 4 mg/kg body weight/day (as steviol equivalents) established by JECFA (2007). The safety of stevia leaf extracts has been established through available safety evaluations of purified steviol glycosides performed by multiple jurisdictions including the EU, Australia and New Zealand, and Canada (FSANZ, 2008, 2015, 2017; EU, 2011; Health Canada 2012a,b, 2016, 2017; EFSA, 2015). "No questions" letters from the FDA have been issued in response to multiple GRAS notifications which have been filed over the past few decades, further corroborating the safety in use of steviol glycosides.

The scientific evidence examined by the Expert Panel demonstrates that under the conditions of intended use, Cargill's stevia leaf extracts would not produce any adverse health effects.

CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that Cargill Incorporated's stevia leaf extracts produced using manufacturing processes designed to provide formulations with different steviol glycoside ratios, meeting appropriate food grade specifications and produced according with current good manufacturing practice, is Generally Recognized as Safe (GRAS) based on scientific procedures under the conditions of intended use in foods specified herein.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

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Attachment 1 Chemical and Microbiological Specifications for Stevia Leaf Extracts

Specification Parameter	Specification
Assay (Steviol glycosides) [a]	NLT 95% wt/wt
Appearance	Loose powder or crystals, white to off-white
Ash	NMT 1.0%
Loss on Drying	NMT 6.0%
Residual Solvents	NMT 0.02% methanol NMT 0.5% ethanol
Lead (as Pb)	NMT 1 mg/kg
Arsenic	NMT 1 mg/kg
Mercury	NMT 1 mg/kg
Cadmium	NMT 1 mg/kg

NLT = not less than; NMT = no more than; wt/wt = by weight

[a] The assay value for steviol glycosides is defined by JECFA as not less than 95% of "*a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties in any of the orientations occurring in the leaves of Stevia rebaudiana Bertoni including, glucose, rhamnose, xylose, fructose, and deoxyglucose*"; additional saccharides (galactose and arabinose) have also been included in this definition (JECFA, 2016b, 2017). Cargill reports the assay value as the sum of rebaudioside A, B, C, D, F, M, stevioside, dulcoside, rubusoside, and steviolbioside.

CERTIFICATE OF ANALYSIS

From:

Minneapolis US SSNA Office

Cargill Incorporated

15407 McGinty Road West

Wayzata MN 55391-2399

Material	:	Delivery	:
Product Description	: Truvia® Stevia RA50	PO Number	:
		Contract Number	:
Batch	: 20170604	Truck/Rail/Container Id	:
Date Manufacture	: 06/04/2017		

Characteristic	Result	UOM	Specification	Method
Total Glycosides (J9+Reb M)	99	% (w/w)	≥95%	STV-002-06
Residual Ethanol	0.1	% (w/w)	≤0.5%	STV-009-01
Residual Methanol	0.00	% (w/w)	≤0.02%	STV-009-01
Appearance	Pass	Visual	White to off-white, loose powder or crystals	STV-003-01
LOD	2	% (w/w)	≤6%	STV-006-02
Ash	0.5	% (w/w)	≤1%	AOAC 945.46
Arsenic	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Cadmium	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Lead	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Mercury	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
APC	<10	CFU/g	<1000 CFU/g	AOAC 966.23
Yeast	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Mold	10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Salmonella	Negative	in 25g	Negative	AOAC-RI 100201

Results listed as typical are not tested on each batch, have not been tested on the listed batch, and represent values or ranges normally found in this material.

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Cargill Incorporated

15407 McGinty Road West

Wayzata MN 55391-2399

Material	:	Delivery	:
Product Description	: Truvia® Stevia RA50	PO Number	:
		Contract Number	:
Batch	: 170614-02	Truck/Rail/Container Id	:
Date Manufacture	: 06/14/2017		

Characteristic	Result	UOM	Specification	Method
Total Glycosides (J9+Reb M)	99	% (w/w)	≥95%	STV-002-06
Residual Ethanol	0.0	% (w/w)	≤0.5%	STV-009-01
Residual Methanol	0.00	% (w/w)	≤0.02%	STV-009-01
Appearance	Pass	Visual	White to off-white, loose powder or crystals	STV-003-01
LOD	3	% (w/w)	≤6%	STV-006-02
Ash	0.1	% (w/w)	≤1%	AOAC 945.46
Arsenic	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Cadmium	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Lead	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Mercury	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
APC	<10	CFU/g	<1000 CFU/g	AOAC 966.23
Yeast	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Mold	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Salmonella	Negative	in 25g	Negative	AOAC-RI 100201

Results listed as typical are not tested on each batch, have not been tested on the listed batch, and represent values or ranges normally found in this material.

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From:

Minneapolis US SSNA Office

Cargill Incorporated

15407 McGinty Road West

Wayzata MN 55391-2399

Material	:	Delivery	:
Product Description	: Truvia® Stevia RA50	PO Number	:
		Contract Number	:
Batch	: 20170702	Truck/Rail/Container Id	:
Date Manufacture	: 07/02/2017		

Characteristic	Result	UOM	Specification	Method
Total Glycosides (J9+Reb M)	98	% (w/w)	≥95%	STV-002-06
Residual Ethanol	0.0	% (w/w)	≤0.5%	STV-009-01
Residual Methanol	0.00	% (w/w)	≤0.02%	STV-009-01
Appearance	Pass	Visual	White to off-white, loose powder or crystals	STV-003-01
LOD	1	% (w/w)	≤6%	STV-006-02
Ash	<0.04	% (w/w)	≤1%	AOAC 945.46
Arsenic	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Cadmium	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Lead	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Mercury	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
APC	<10	CFU/g	<1000 CFU/g	AOAC 966.23
Yeast	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Mold	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Salmonella	Negative	in 25g	Negative	AOAC-RI 100201

Results listed as typical are not tested on each batch, have not been tested on the listed batch, and represent values or ranges normally found in this material.

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From:

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Cargill Incorporated

15407 McGinty Road West

Wayzata MN 55391-2399

Material	:	Delivery	:
Product Description	:ViaTech™	PO Number	:
		Contract Number	:
Batch	:170816-B1	Truck/Rail/Container Id	:
Date Manufacture	: 08/16/2017		

Characteristic	Result	UOM	Specification	Method
Total Glycosides (J9+Reb M)	97	% (w/w)	≥95%	STV-002-06
Residual Ethanol	0.1	% (w/w)	≤0.5%	STV-009-01
Residual Methanol	0.0	% (w/w)	≤0.02%	STV-009-01
Appearance	Pass	Visual	White to off-white, loose powder or crystals	STV-003-01
LOD	1	% (w/w)	≤6%	STV-006-02
Ash	0.2	% (w/w)	≤1%	AOAC 945.46
Arsenic	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Cadmium	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Lead	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Mercury	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
APC	<10	CFU/g	<1000 CFU/g	AOAC 966.23
Yeast	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Mold	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Salmonella	Negative	in 25g	Negative	AOAC-RI 100201

Results listed as typical are not tested on each batch, have not been tested on the listed batch, and represent values or ranges normally found in this material.

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Cargill Incorporated

15407 McGinty Road West

Wayzata MN 55391-2399

Material	:	Delivery	:
Product Description	:ViaTech™	PO Number	:
		Contract Number	:
Batch	:170816-B3	Truck/Rail/Container Id	:
Date Manufacture	: 08/16/2017		

Characteristic	Result	UOM	Specification	Method
Total Glycosides (J9+Reb M)	97	% (w/w)	≥95%	STV-002-06
Residual Ethanol	0.1	% (w/w)	≤0.5%	STV-009-01
Residual Methanol	0.01	% (w/w)	≤0.02%	STV-009-01
Appearance	Pass	Visual	White to off-white, loose powder or crystals	STV-003-01
LOD	1	% (w/w)	≤6%	STV-006-02
Ash	0.1	% (w/w)	≤1%	AOAC 945.46
Arsenic	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Cadmium	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Lead	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Mercury	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
APC	<10	CFU/g	<1000 CFU/g	AOAC 966.23
Yeast	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Mold	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Salmonella	Negative	in 25g	Negative	AOAC-RI 100201

Results listed as typical are not tested on each batch, have not been tested on the listed batch, and represent values or ranges normally found in this material.

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15407 McGinty Road West

Wayzata MN 55391-2399

Material	:	Delivery	:
Product Description	:ViaTech™	PO Number	:
		Contract Number	:
Batch	:170816-B5	Truck/Rail/Container Id	:
Date Manufacture	: 08/16/2017		

Characteristic	Result	UOM	Specification	Method
Total Glycosides (J9+Reb M)	97	% (w/w)	≥95%	STV-002-06
Residual Ethanol	0.1	% (w/w)	≤0.5%	STV-009-01
Residual Methanol	0.00	% (w/w)	≤0.02%	STV-009-01
Appearance	Pass	Visual	White to off-white, loose powder or crystals	STV-003-01
LOD	1	% (w/w)	≤6%	STV-006-02
Ash	0.2	% (w/w)	≤1%	AOAC 945.46
Arsenic	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Cadmium	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Lead	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
Mercury	<0.1	ppm (w/w)	≤1 ppm	USP 730 ICP-MS
APC	<10	CFU/g	<1000 CFU/g	AOAC 966.23
Yeast	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Mold	<10	CFU/g	<50 CFU/g	FDA BAM, 7th Ed.
Salmonella	Negative	in 25g	Negative	AOAC-RI 100201

Results listed as typical are not tested on each batch, have not been tested on the listed batch, and represent values or ranges normally found in this material.

This COA was generated electronically. If you have any questions concerning this document, please feel free to contact Cargill by phone at 1-866-456-8872 or by email at CHN_customerservice@cargill.com.