ORIGINAL SUBMISSION

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GRN 000516

April 18, 2014

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

Subject: GRAS Notification – Steviol Glycoside

Dear Sir:

On behalf of Almendra Limited, of Rayong, Thailand, ToxStrategies, Inc. (its agent) is submitting for FDA review Form 3667 and three copies of the notification as required. The enclosed document provides notice of a claim that the food ingredient described in the enclosed notification is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to foods as a table top sweetener and general purpose non-nutritive sweetener.

If you have any questions or require additional information, please do not hesitate to contact me at 630-352-0303, or dschmitt@toxstrategies.com.

Sincerely,

(b) (6)

Don Schmitt, M.P.H.
Senior Managing Scientist

RECEIVED
APR 28 2014
OFFICE OF
FOOD ADDITIVE SAFETY

ToxStrategies, Inc., 739 Thornapple Dr., Naperville, IL 60650
Office (630) 352-0303 • www.toxstrategies.com

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE

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APR 28 2014
OFFICE OF FOOD ADDITIVE SAFETY

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

GRN 000516

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

PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

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

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

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

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

PART II – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position
Ms. Pnita Chutasmit	Commercial Manager

Company *(if applicable)*
Almendra (Thailand) Limited

Mailing Address *(number and street)*
Amata City Industrial Estate, 7/313 Moo^, Tumbol Mapyangporn, Amphoe Pluakdaeng

City	State or Province	Zip Code/Postal Code	Country
Rayong	Amphoe Pluakdaeng	21140	Thailand

Telephone Number	Fax Number	E-Mail Address
(66) 38-036-387		pc@almendra.com.sg

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

Name of Contact Person	Position
Mr. Donald F. Schmitt	Senior Managing Scientist

Company *(if applicable)*
ToxStrategies, Inc.

Mailing Address *(number and street)*
739 Thornapple Drive

City	State or Province	Zip Code/Postal Code	Country
Naperville	Illinois	60540	United States of America

Telephone Number	Fax Number	E-Mail Address
630-352-0303		dschmitt@toxstrategies.com

PART III – GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

High Purity Steviol Glycoside

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

☐ Electronic Submission Gateway

☒ Paper

☐ Electronic files on physical media with paper signature page

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes 1

Total number of pages 78

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

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

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

☐ a) GRAS Notice No. GRN

☐ b) GRAS Affirmation Petition No. GRP

☐ c) Food Additive Petition No. FAP

☐ d) Food Master File No. FMF

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

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

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

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

☐ Yes *(Proceed to Item 8)*

☒ No *(Proceed to Part IV)*

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

☐ Yes, see attached Designation of Confidential Information

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

☐ No

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

☐ Yes, a redacted copy of the complete submission

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

☐ No

PART IV – INTENDED USE

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

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

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

☐ Yes ☒ No

000004

PART V – IDENTITY

1. Information about the Identity of the Substance

	Name of Substance[1]	Registry Used *(CAS, EC)*	Registry No.[2]	Biological Source *(if applicable)*	Substance Category *(FOR FDA USE ONLY)*
1	High purity steviol glycosides - primarily Rebaudioside A and Stevioside				
2	Rabaudioside A	CAS	58543-16-1	*Stevia rebaudiana* Bertoni leaves	
3	Stevioside	CAS	57817-89-7	*Stevia rebaudiana* Bertoni leaves	

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

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

2. Description

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

Mixture ofsteviol glycosides extracted from the leaves of *Stevia rebaudiana* Bertoni and purified to meet the specifications on page 12 in Table 2.
Chemical structures are provided on pages 8-9 in Figures1-3.
Molecular weights: Rebaudioside A - 967.03 daltons; Stevioside - 804.88 daltons. See page 8.
Chemical formulas: Rebaudioside A - C44H70O23; Stevioside - C38H60O18. See page 8.

3. Synonyms

Provide as available or relevant:

1	Rebaudioside A - 13-[(2-O-β-D-glucopyranosyl-3-O-β-Dglucopyranosyl-β-D- glucopyranosyl) oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester). See pages 7-8.
2	Stevioside - 13-[2-O-β-D-glucopyranosyl-β-Dglucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester. See page 8.
3	000005

Add Continuation Page

PART VI – OTHER ELEMENTS IN YOUR GRAS NOTICE

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

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

Other Information

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

☒ Yes ☐ No

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

☒ Yes ☐ No

PART VII – SIGNATURE

1. The undersigned is informing FDA that Almendra (Thailand) Limited *(name of notifier)* has concluded that the intended use*(s)* of High Purity Steviol Glycoside *(name of notified substance)* described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

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

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

Amata City Industrial Estate, 7/313 Moo6, Tumbol Mapyangporn, Amphoe Pluakdaeng, Rayong, Thailand 21140
(address of notifier or other location)

Almendra (Thailand) Limited *(name of notifier)* agrees to send these data and information to FDA if FDA asks to do so.

OR

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

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
(b) (6)	Donald F. Schmitt, Senior Managing Scientist	mm/dd/yyyy 04/18/2014

PART VIII – LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Appendix A - Specifcations/Certifications for Merck Ethanol	pp. 45 - 51
	Appendix B - Certificates of Analysis - high purity steviol glycosides (5 production batches); and heavy metal, microbiological and pesticide analytical reports.	pp. 52 - 69
	Exhibit I - Report of the Expert Panel	pp. 70 - 78

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

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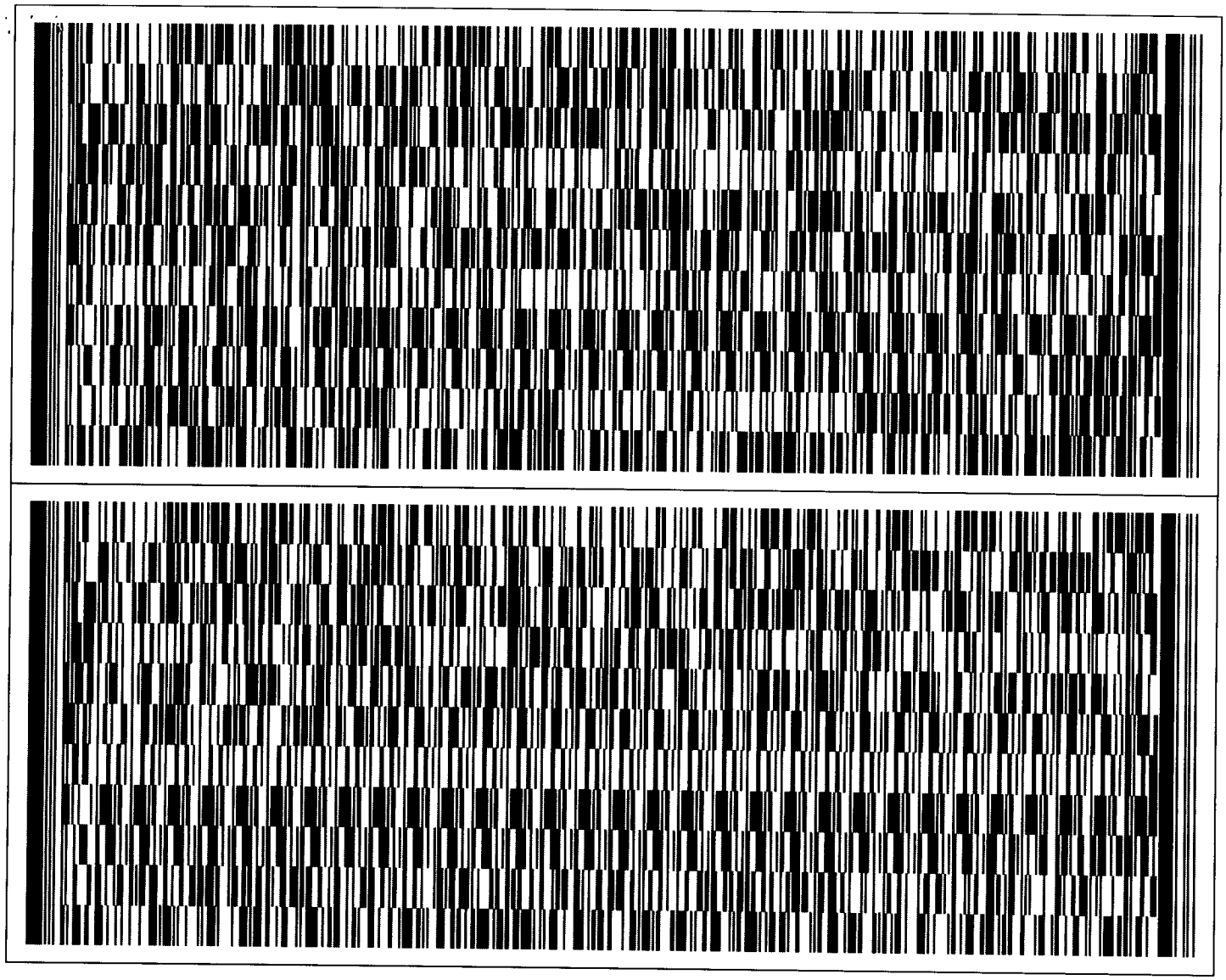

GRAS Determination of Steviol Glycoside for Use in Food

SUBMITTED BY:

Almendra (Thailand) Limited
Amata City Industrial Estate
7/313 Moo6, Tumbol Mapyangporn
Amphoe Pluakdaeng, Rayong
21140 THAILAND

SUBMITTED TO:

U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-200
5100 Paint Branch Parkway
College Park, MD 20740-3835

CONTACT FOR TECHNICAL OR OTHER INFORMATION:

Donald F. Schmitt, MPH
ToxStrategies, Inc.
739 Thornapple Drive
Naperville, IL 60540

April 18, 2014

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FOOD ADDITIVE SAFETY

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List of Acronyms

ADI	Acceptable Daily Intake
ADME	absorption, distribution, metabolism, and excretion
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
cfu	colony forming units
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
g	gram
GI	gastrointestinal
GMP	Good Manufacturing Practice
GRAS	Generally Recognized as Safe
GRN	GRAS Notification
HPLC	High Pressure Liquid Chromatography
JECFA	Joint WHO/FAO Expert Committee on Food Additives
kg	kilogram
LD50	Lethal Dose 50
LDH	lactate dehydrogenase
max	maximum
mg	milligram
mol	mole
NOAEL	No-Observed-Adverse-Effect-Level
NZW	New Zealand White rabbits
ppm	parts per million
Reb A	Rebaudioside A
µg	microgram
US	United States
USP	United States Pharmacopeia
WHO	World Health Organization

1.0 GRAS Exemption Claim

A. Name and Address of Notifier

Almendra (Thailand) Ltd., through its agent ToxStrategies Inc., hereby notifies the Food and Drug Administration that the use of the identified high purity mixture of steviol glycosides described below and which meets the specifications described herein is exempt from pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act because Almendra Ltd. has determined that such use is generally recognized as safe (GRAS) through scientific procedures.

(b) (6)

04/18/2014

Donald F. Schmitt, M.P.H
Senior Managing Scientist
ToxStrategies, Inc.
Agent for Almendra Ltd.

Date

B. Name of GRAS Substance

The name of the substance that is the subject of this GRAS determination is a high purity steviol glycoside mixture (> 95%) containing rebaudioside A (Reb A) and stevioside as its major components.

C. Intended Use in Food

The high purity steviol glycoside mixture (> 95%) containing stevioside and Reb A as its major component(s) is intended for use as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into foods, other than infant formulas and meat and poultry products. It will be added to foods at per serving levels similar to those of highly purified steviol glycoside mixtures and pure steviol glycosides previously notified as GRAS (received "no objection letters") and that will not exceed the amount reasonably required to accomplish its intended technical effect.

D. Basis for GRAS Determination

Almendra Limited's GRAS determination for the intended use of a high purity steviol glycoside preparation primarily composed of Reb A and stevioside is based on scientific procedures as described under 21 CFR § 170.30(b).

The intended uses of the high purity steviol glycoside preparation has been determined to be safe, and Generally Recognized as Safe (GRAS) and the safety of intake exposure under the proposed conditions of use is based on knowledge and

information that is both publicly available and widely accepted by experts qualified by scientific training and experience to evaluate the safety of substances in food. The publicly available safety data combined with the widely disseminated knowledge concerning the chemistry of steviol glycosides and the long history of approval/use of steviol-based additives/supplements provide a sufficient basis for an assessment of the safety of steviol glycosides including stevioside and Reb A for the uses proposed herein.

To date, the FDA has issued "no questions" letters in response to 27 Generally Recognizable As Safe (GRAS) Notifications (GRNs) on both individually highly purified steviol glycoside sweeteners as well as mixtures that meet the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Codex specifications, with four other GRAS Notifications currently pending. Merisant and Cargill were the first to submit GRNs on purified Reb A to the FDA (Merisant, 2008, GRN 252; Cargill, 2008, GRN 253), and subsequent GRNs on these products have been either accepted by FDA with no questions or are pending review. The most recent GRN submitted to FDA for this group of sweeteners (GRN 461) was submitted by Almendra on March 1, 2013, and received a "no questions" response letter from FDA on August 14, 2013 (Almendra, 2013). In addition to containing extensive reviews of the safety information, the early GRNs included expert panel reports that reviewed and discussed in detail the metabolism, toxicology, human health and safety data for both stevioside and Reb A. Based on these GRAS notifications, FDA currently permits the use of steviol glycosides individually and in mixtures as general-purpose sweeteners at the use-levels indicated in the notifications.

Almendra's recent GRAS Notification No. 461 included a summary of a review article by Carakostas et al. (2008) that summarizes the most recent research on rebaudioside A. The summarized findings included the following:

- Steviol glycosides, rebaudioside A, and stevioside are not genotoxic *in vitro*.

- In well-conducted *in vivo* assays, steviol glycosides, rebaudioside A, and stevioside have not been found to be genotoxic.

- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.

- It is well recognized that with the exception of having different numbers and types of sugar moieties, steviol glycosides as a class share the same structural backbone, steviol. As such, all steviol glycosides are expected to follow the same metabolic pathway that has been demonstrated for both rebaudioside A and stevioside. Therefore, results of toxicology studies on either stevioside or rebaudioside A are applicable to assessing the safety of all steviol glycosides. In addition, the use of the ADI established by JECFA that was determined from studies employing stevioside as the main component can be used as the ADI for this highly purified mixture of steviol glycosides.

Given the anticipated dietary patterns and the use concentrations expected in various

foods, JECFA has calculated a widely recognized estimate of daily intake (EDI). Based on a conservative assumption of 100% substitution of steviol glycosides for all sugars, JECFA calculated an EDI of 5 mg/kg bw/day steviol. However, JECFA recognized that these replacement estimates were highly conservative and that the calculated intake of steviol glycosides (as steviol) would more likely be 20 - 30% of these values or 1.0 - 1.5 mg/kg bw/day on a steviol basis. Renwick (2008) also concluded that if only rebaudioside A were used as a total sugar replacement, the levels would be below the JECFA ADI of 0 – 4 mg steviol equivalents/kg bw/day (equivalent to 12 mg Reb A/kg bw/day on a dry weight basis).

Determination of the safety and GRAS status of this high purity steviol glycoside preparation described above for direct addition to food under its intended conditions of use was made through deliberation of an Expert Panel consisting of Stanley M. Tarka, Jr., Ph.D. and Michael Carakostas, DVM, Ph.D., who reviewed a dossier prepared by ToxStrategies as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of this steviol glycoside preparation, and unanimously concluded that the intended use of this steviol glycoside preparation, produced consistent with cGMP and meeting appropriate specifications, as a table top sweetener and general purpose non-nutritive sweetener in food as delineated above is "generally recognized as safe" ("GRAS") based on scientific procedures.

E. Availability of Information

The data and information that serve as the basis of this GRAS determination, as well any information that has become available since the GRAS determination, will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times from ToxStrategies, Inc., Naperville, IL.

2.0 Description of Substance

A. Identity

High purity steviol glycosides are derived from leaves of the *Stevia rebaudiana* Bertoni plant. The composition of the high-purity (>95%) steviol glycoside preparation that is the subject of this GRAS evaluation primarily contains rebaudioside A and stevioside (>80%) with the remainder being other steviol glycosides and meets JECFA's specifications. The scientific literature refers to steviol glycosides as stevia, stevioside, steviol glycosides, and stevia glycoside. Steviol glycosides is the name JECFA established for the family of glycosides derived from the stevia plant. JECFA (WHO, 2000) has provided the following description of steviol glycosides.

> Stevioside is a glycoside of the diterpene derivative steviol (ent-13-hydroxykaur-16-en-19-oic acid). Steviol glycosides are natural constituents of the plant *Stevia rebaudiana Bertoni*, belonging to the Compositae family. The leaves of *S. rebaudiana Bertoni* contain eight different steviol glycosides. Other main constituents are rebaudioside A (tetraglucosylated steviol), rebaudioside C, and dulcosideA. *S. rebaudiana* is native to South America and has been used to sweeten beverages and food for several centuries. The plant has also been distributed to Southeast Asia. Stevioside has a sweetening potency 250-300 times that of sucrose and is stable to heat. In a 62-year-old sample from a herbarium, the intense sweetness of *S. rebaudiana* was conserved, indicating the stability of stevioside to drying, preservation, and storage (Soejarto et al., 1982; Hanson and De Oliveira, 1993, as cited in WHO, 2000).

JECFA (FAO, 2007) has identified the numerous sweetener components of stevia and provided a list of the glycosides and their chemical structures. Currently nine steviol glycosides are recognized by JECFA as part of its established specification. These include stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside, and steviolbioside. Structural information can found below in Section 2.D. It should be noted again that the two major components of this high purity steviol glycoside preparation are Reb A and stevioside.

B. Common Name

High purity steviol glycosides. Steviose SG100 is the common commercial name employed by Almendra Ltd.

C. Common/Chemical Names of Major Components with Chemical Abstracts Service (CAS) Registry Numbers

Rebaudioside A or 13-[(2-O-β-D-glucopyranosyl-3-O-β-Dglucopyranosyl-

β-D- glucopyranosyl) oxy] kaur-16-en-18-oic acid, β-D- glucopyranosyl ester); CAS No. 58543-16-1

Stevioside or 13-[2-O-β-D-glucopyranosyl-β-Dglucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester; CAS No. 57817-89-7

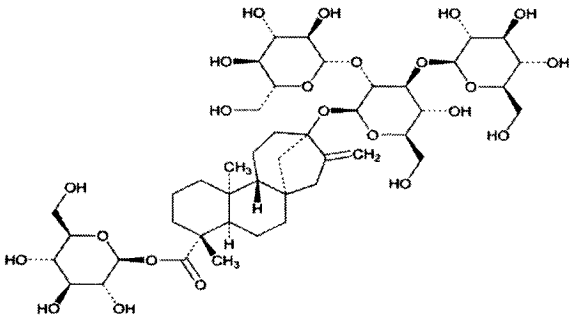
D. Molecular Weight and Empirical and Structural Formulas

Rebaudioside A

Molecular formula: $C_{44}H_{70}O_{23}$

Molecular weight: 967.03

Figure 1. Chemical Structure of Rebaudioside A

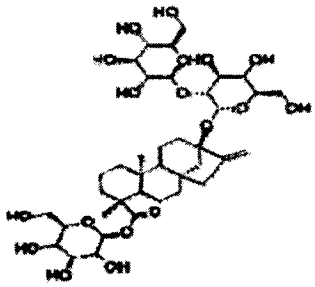


Stevioside

Molecular formula: $C_{38}H_{60}O_{18}$

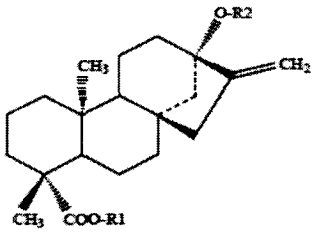
Molecular weight: 804.88

Figure 2. Chemical Structure of Stevioside



JECFA (FAO, 2007a) also published the structures for steviol (the aglycone) and its glycosides and they can be found in Figure 3 below.

Figure 3. Chemical Structures of Steviol Glycosides



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

*Rubusoside CAS No. is incorrect in the JECFA table above and should be 64849-39-4.

E. Production Process

The high purity steviol glycoside (>95%) is prepared in a two-step process. All raw materials and processing aids used in the manufacture of this steviol glycoside preparation are suitable food-grade materials and are used in accordance with applicable U.S. federal regulations as described in Table 1.

Table 1. Raw Materials and Processing Aids Used in the Manufacturing of Steviol Glycoside Preparation-Steviose SG100

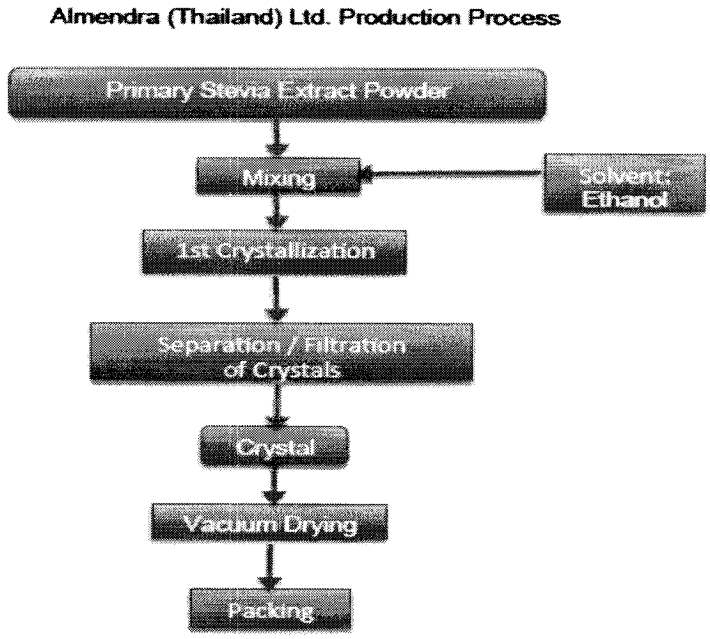
Material	Use	Regulatory Status
Stevia rebaudiana leaves	Reaction substrate	-
Water	Solvent	-
Calcium carbonate	Processing aid	21 CFR §184.1191 (U.S. FDA, 2013)
Ethanol	Processing aid	21 CFR §184.1293 & 21 CFR §172.340 (U.S. FDA, 2013)
Ferric chloride	Processing aid	21 CFR §184.1297, (U.S. FDA, 2013)
Adsorption and ion-exchange resins (strong acidic and strong basic resins)	Processing aid	21 CFR §173.25 (U.S. FDA, 2013)

CFR, Code of Federal Regulations; U.S FDA, United States Food and Drug Administration

The process is as follows: dry *Stevia rebaudiana* (Bertoni) leaves are extracted in hot water with continuous agitation. Nonsoluble material is removed via a flocculation process by adding ferric chloride and/or lime followed by filtration through a filter press. The aqueous extract is then passed through an adsorption resin and subsequently eluted with ethanol. The ethanol is then partially removed by membrane filtration and concentration. The stevia extract powder is then obtained by spray drying.
This first primary stevia powder extract contains 40 - 60% Reb A and more than 90% total steviol glycosides.

This powder extract is employed as the starting material for production of the high purity steviol glycoside (≥95%) preparation. The extract is dissolved in food-grade ethanol and heated. The ethanol used in the purification process meets Ph. Eur, BP, JP, and USP chemical specifications (see Appendix A). The solution is then cooled to allow for crystallization. The crystals are then removed from the solution by centrifugation or other appropriate filtration processes. The crystals are then washed with food-grade ethanol and separated by centrifugation. The solid material dried in vacuum oven to obtain the final high purity steviol glycoside product. The resultant product is analyzed for compliance with Almendra specifications which meet or exceed JECFA specifications for steviol glycosides.

Figure 4. Flow diagram of the manufacturing process for high purity steviol glycoside



F. Product Specifications

Food grade specifications for steviol glycosides have been finalized by JECFA at the 68th meeting (FAO, 2007b) and require not less that 95% of the total of the seven named steviol glycosides, on a dried weight basis. Recently, JECFA has expanded the number of specific glycosides to include Reb D and Reb F (FAO, 2010).

Steviol glycoside preparations are described by JECFA (JECFA, 2010) as white to yellow powders that are freely soluble in water and ethanol. The powders can be odorless or have a slight characteristic odor. Water solutions are 200 to 300 times sweeter than sucrose (FAO, 2007a). The pH of a 1 in 100 solution should be between 4.5 and 7.0. Total ash should not be more than 1% and loss on drying at 105°C for 2 hours should not be more than 6%. Residual ethanol levels should not exceed 5000 ppm. Arsenic and lead levels should not exceed 1 ppm.

Almendra has adopted the following product specifications that meet or exceed JECFA recommendations.

Table 2. Almendra specifications for high purity steviol glycosides

Specification/Analytical Parameter	Specification
Appearance Form	Powder
Appearance Color	White to light yellow
Total Glycoside, (HPLC area, % min)	99.5
Rebaudioside A, (HPLC area, % min)	70 - 83
Stevioside, (HPLC area, % min)	10 - 20
Residual Ethanol (ppm, max)	5000
Water Content (%, max)	3
pH	4.5-7.0
Total Ash (%, max)	0.1
Arsenic (ppm, max)	0.02
Lead (ppm, max)	0.1
Mercury (ppm, max)	0.01
Cadmium (ppm, max)	0.01
Microbiological Purity	
Total Plate Count (cfu/g, max)	1,000
Yeast (cfu/g, max)	100
Mold (cfu/g, max)	100
Heat Resistant Mold (in 50g)	Negative
Salmonella spp. (in 25g)	Negative
Staphylococcus aureus (in 10g)	Negative
Alicyclobacillus acidoterrestris (in 50g)	Negative
Listeria (in 1g)	Negative
Coliform / E.coli (in 1g)	<10
Total Coliforms (in 25g)	Negative
Fecal Coliforms (in 25g)	Negative

Table 3. Composition of five production batches of high purity steviol glycosides

Lot Number	21012013	30012013	02012013	13112012	14112012
Appearance Form	Powder	Powder	Powder	Powder	Powder
Appearance Color	White	White	White	White	White
Total Glycoside, (HPLC area, % min)	99.64	99.83	99.6	99.79	99.78
Rebaudioside A, (HPLC area, % min)	73.98	74.95	72.75	74.80	74.71
Stevioside, (HPLC area, % min)	16.16	15.76	18.68	15.88	16.05
Residual Ethanol (ppm, max)	95.0	219.0	310.0	282.0	302.0
Residual Methanol * (ppm, max)	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Water Content (%, max)	1.70	2.71	1.22	0.82	1.05
pH	6.4	5.7	5.9	5.8	6.0

Total Ash (%, max)	0.080	0.081	0.080	0.089	0.087
Arsenic (ppm, max)	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Lead (ppm, max)	0.01	0.01	0.01	0.01	0.01
Mercury (ppm, max)	0.01	0.01	0.01	0.01	0.01
Cadmium (ppm, max)	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Microbiological Purity					
Total Plate Count (cfu/g, max)	<10	<10	<10	<10	<10
Yeast (cfu/g, max)	<10	<10	<10	<10	<10
Mold (cfu/g, max)	<10	<10	<10	<10	<10
Heat Resistant Mold (in 50g)	Negative	Negative	Negative	Negative	Negative
Salmonella spp. (in 25g)	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus (in 10g)	Negative	Negative	Negative	Negative	Negative
Alicyclobacillus acidoterrestris (in 50g)	Negative	Negative	Negative	Negative	Negative
Listeria (in 1g)	Negative	Negative	Negative	Negative	Negative
Coliform / E.coli (in 1g)	<10	<10	<10	<10	<10
Total Coliforms (in 25g)	Negative	Negative	Negative	Negative	Negative
Fecal Coliforms (in 25g)	Negative	Negative	Negative	Negative	Negative

* Methanol not employed in manufacturing process.

Certificates of Analysis, including the methods of analysis for heavy metals and pesticides for the five representative, non-consecutive lots of high purity steviol glycosides shown above are provided in Appendix B.

G. Stability Data for Steviol Glycosides

The stability of steviol glycosides (including rebaudioside A) has been addressed in Almendra's GRAS Notification No. 461 for rebaudioside A and is summarized here. The stability of the most common steviol glycosides, rebaudioside A and stevioside, has been well studied by companies such as Cargill (GRN 253) and Merisant (GRN 252). Stevioside has also been reported to be stable over the pH range 3 - 9 and can be heated at 100°C for 1 hour, but at pH levels greater than 9 under these conditions it rapidly decomposes (Kinghorn and Soejarto, 1985). These investigators also speculated that at pH 10 steviolbioside would be the major decomposition product produced from stevioside by alkaline hydrolysis. Chang and Cook (1983) investigated the stability of pure stevioside and rebaudioside A in carbonated phosphoric and citric acidified beverages. Some degradation of each sweetening component was noted after 2 months of

storage at 37°C. However, no significant change at room temperature or below following 5 months of storage of stevioside and 3 months of storage of rebaudioside A was noted. Exposure to 1 week of sunlight did not affect stevioside stability but resulted in approximately 20% loss of rebaudioside A. Heating at 60°C for 6 days resulted in 0 - 6% loss of rebaudioside A.

As noted above, Merisant (GRN 252, 2008) conducted stability testing on rebaudioside A (1) as a powder, (2) as a pure sweetener in solution, and (3) on both cola-type and citrus carbonated beverages. No degradation was detected when the powder was stored at 105°C for 96 hours. It was concluded that the powder was stable when stored for 26 weeks at 40±2°C with relative humidity of 75%.

Cargill (GRN 253, 2008; Prakash et al., 2008) conducted extensive stability testing on rebaudioside A as a powder under various storage conditions and under a range of pH and temperatures. Cargill also investigated rebaudioside A stability in several representative food matrices at room temperature and elevated temperatures. Stability profiles were created for tabletop sweetener applications, mock beverages including cola, root beer and lemon-lime, thermally processed beverages, yogurt, and white cake. The results of stability testing revealed some degradation products that had not been detected in bulk rebaudioside A. The degradation products were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana* Bertoni. All the degradation products were found to share the same steviol aglycone backbone structure as found in stevioside and rebaudioside A, but they differed by virtue of the glucose moieties present. The results of stability testing revealed that rebaudioside A is stable in various food matrices following several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, rebaudioside A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, GRN 253, 2008). In photostability studies of the dry powder and mock beverages to ascertain rebaudioside A behavior under defined conditions of fluorescent and near UV light exposure, rebaudioside A was found to be photostable under the defined conditions of analysis (Clos et al., 2008).

GRN No. 461 also cited Sunwin and WILD Flavors' (GRN 304, 2010) investigation of the stability of purified steviol glycosides with rebaudioside A and stevioside as the principal components using a 0.04% solution of 80% Reb A in acidic solutions between pH 2.81 and 4.18. The solutions were stored at 32°C for 4 weeks, and the Reb A content was determined at 1, 2 and 4 weeks. Reb A 80% was found to be very stable at pH 3.17 and above. At pH 2.81, after 4 weeks of storage under accelerated conditions only a 7% loss of Reb A was noted. Sunwin and WILD Flavors also studied the stability of Reb A 80% in simulated beverages using 0.1 % citric acid (pH 3.2). The solutions were pasteurized and stored for 8 weeks at 4° and 32°C, and little difference in sweetness perception was found under these conditions.

The stability data in the scientific literature for stevioside, JECFA's reviews/reports, and the stability testing of rebaudioside A as presented in numerous GRNs by Cargill, Merisant and many other submitters support the stability of Almendra's steviol

glycosides preparation (Steviose SG 100) and provide appropriate stability in foods under the proposed conditions of use which are identical to its many other GRAS Notifications for similar steviol glycoside preparations.

3.0 Historical Consumption and Current Regulated Uses

A. Regulatory History and Uses of Stevia-Derived Sweeteners

Stevia-derived sweeteners have long been permitted as food additives in South America and in several countries in Asia, including China, Japan, and Korea. In addition to the FDA raising no objections to 27 GRAS notices submitted since May 2008 for both highly-purified steviol glycosides and glucosylated steviol glycosides (Table 4), steviol glycosides meeting JECFA specifications have now received food usage approvals in Mexico, Australia, New Zealand, Switzerland, France, Peru, Uruguay, Colombia, Senegal, Russia, Malaysia, Turkey, Taiwan, Thailand, Israel, Canada and Hong Kong (EFSA, 2010; Nutralngredients, 2010; Health Canada, 2012, as cited in Almendra GRN No. 461) as well as Indonesia, India and the Ukraine.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the safety of steviol glycosides at several meetings (2000, 2005, 2006, 2007, and 2009, as cited in GRN No. 461). In 2006, JECFA set a temporary Acceptable Daily Intake (ADI) value for steviol glycosides (expressed as steviol equivalents) of 0 – 2 mg/kg bw/day based on a 2-year rat study (Toyoda et al., 1997). Newer studies presented at a subsequent JECFA meeting demonstrated that ingestion of steviol glycosides daily in hypotensive or diabetic subjects for 4 or 16 weeks, respectively, resulted in no adverse effects at doses of approximately 4 mg (steviol equivalents)/kg bw per day. Based on this, JECFA deleted the previous additional 2X safety factor (going to a 100X safety factor from 200X) and established a permanent Acceptable Daily Intake (ADI) for steviol glycosides (expressed as steviol equivalents) of 0 – 4 mg/kg bw/day (JECFA, 2008, as cited in GRN No. 461). Likewise, the Food Standards Australia New Zealand (FSANZ, 2008) also established an ADI for steviol glycosides of 4 mg/kg bw/day (expressed as steviol) based on the same 2-year carcinogenicity study NOEL used by JECFA.

Prior to the JECFA evaluation, the European Commission's Scientific Committee for Food evaluated stevioside as a sweetener, but concluded that there were too many uncertainties in the toxicology literature to appropriately determine safety (Carakostas et al., 2008). Following the 2008 JECFA assessment, the European Food Safety Authority (EFSA) re-evaluated the safety of steviol glycosides. In 2010, the EFSA Panel on Food Additives and Nutrient Sources Added to Food issued an opinion concluding that steviol glycosides are neither carcinogenic, genotoxic or associated with any reproductive or developmental toxicity. Like JECFA and FSANZ before them, the EFSA Panel considered the 2-year carcinogenicity study published by Toyoda et al. (1997) to be the pivotal study, and established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on application of a 100-fold uncertainty factor to

the NOAEL for stevioside of 967 mg/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day).
To date, and as noted above, the FDA has issued "no questions" letters in response to 27 Generally Recognizable As Safe (GRAS) Notifications (GRNs) on steviol glycoside sweeteners, with four other GRAS Notifications pending. Merisant and Cargill were the first to submit GRNs on purified Reb A to the FDA (Merisant, GRN 252, 2008; Cargill, GRN 253, 2008), and none of the subsequent GRNs on these products have been rejected or withdrawn. The most recent GRN submitted to FDA for a steviol glycoside preparation was submitted by Almendra on February 7, 2013, and received a "no questions" response letter on August 14, 2013 from FDA (Almendra, 2013). In addition to containing extensive reviews of the safety information, the early GRNs included expert panel reports that reviewed and discussed in detail the metabolism, toxicology, and human health and safety data for stevioside and Reb A. Based on these GRAS notifications, FDA currently has no questions regarding the use of steviol glycosides individually and in mixtures as general-purpose sweeteners at the use-levels indicated in these notifications. The sweeteners notified to FDA thus far generally meet or exceed JECFA specifications.

Table 4. FDA GRAS Notice Inventory for Stevia-Derived Sweeteners

GRAS Notification No.	Substance	FDA Status
473	Purified steviol glycosides with rebaudioside X as the principal component	Pending
467	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni	Pending
461	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside A)	FDA has no questions
456	Rebaudioside D purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside D)	Pending
452	Enzyme-modified steviol glycosides	Pending
448	Enzyme-modified steviol glycoside	FDA has no questions
418	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside A)	FDA has no questions
395	Steviol glycosides with rebaudioside A and stevioside as the principal components	FDA has no questions
393	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside A)	FDA has no questions
389	Steviol glycosides with stevioside as the principal component	FDA has no questions

388	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside A)	FDA has no questions
380	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside A)	FDA has no questions
375	Enzyme-modified steviol glycosides	FDA has no questions
369	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni	FDA has no questions
367	Purified steviol glycosides with rebaudioside A and stevioside as the principal components	FDA has no questions
365	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside A)	FDA has no questions
354	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (rebaudioside A)	FDA has no questions
349	Purified steviol glycosides with rebaudioside A and stevioside as the principal components	FDA has no questions
348	Stevioside purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (stevioside)	FDA has no questions
337	Enzyme-modified steviol glycosides preparation (EMSGP)	FDA has no questions
329	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni	FDA has no questions
323	Purified steviol glycosides with rebaudioside A and stevioside as the principal components	FDA has no questions
318	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni	FDA has no questions
304	Purified steviol glycosides with rebaudioside A and stevioside as the principal components	FDA has no questions
303	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni	FDA has no questions
287	Purified steviol glycosides with rebaudioside A and stevioside as the principal components	FDA has no questions
282	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni	FDA has no questions
278	Rebaudioside A purified from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni	FDA has no questions
275	Purified steviol glycosides with rebaudioside A as the principal component	FDA has no questions
253	Rebaudioside A purified from *Stevia rebaudiana* (Bertoni) Bertoni	FDA has no questions
252	Rebaudioside A purified from *Stevia rebaudiana* (Bertoni) Bertoni	FDA has no questions

4.0 Intended Use and Estimated Intake (EDI)

Similar to Almendra's rebaudioside A sweetener that was the subject of GRN No. 461, Almendra's high purity (>95%) steviol glycoside preparation with rebaudioside A and stevioside as the principal components is intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener as defined in 21 CFR 170.3(o)(19) for use in various foods other than infant formulas and meat and poultry products. Intended use levels will vary by food category, but the actual levels are self-limiting due to organoleptic factors. The actual amounts of the steviol glycoside to be added to foods will not exceed the amounts reasonably required to meet its intended technical effect in foods.

Since the information/data contained in Almendra's GRN No. 461 for both food uses and estimated daily intake (Sections IV.B and IV.C) are directly relevant to this GRAS Notification for high purity steviol glycosides, they are being cited here by reference and an abbreviated summary presented below.

The estimated daily intake for steviol glycosides and rebaudioside A has been reported in a variety of publications and has been provided to FDA in multiple GRAS notifications. JECFA (WHO, 2006), FSANZ (2008), Merisant (GRN 252, 2008), and Cargill (GRN 253, 2008) also reviewed and provided various estimates of possible daily intake of steviol glycosides. Cargill considered that with a few minor exceptions, rebaudioside A uses and use levels would be comparable to those of aspartame uses in the US. Using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008), Cargill performed a side-by-side consumption analysis for rebaudioside A versus aspartame. The following tables present these analyses.

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

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

[a] WHO, 2006, reproduced from GRN No. 461.

[b] Calculated assuming twice the sweetness intensity for rebaudioside A and three-fold difference in molecular weight between rebaudioside A and steviol.

JECFA previously evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that were prepared for the European Commission by the Scientific Committee on Food. JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1. The intakes ranged from 1.3 mg/kg bw/day with the African diet to 3.5 mg/kg bw/day with the European diet. Additionally, JECFA also estimated the per capita exposure derived from disappearance (poundage) data supplied by Japan and China. The Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US and they are presented in Table 6. JECFA stated that the estimates were highly conservative and likely overestimated true dietary intakes which would more likely approximate 1.0 - 1.5 mg/kg bw/day on a steviol basis.

Table 6. Estimates of Dietary Exposure to Steviol Glycosides (as Steviol) Assuming Full 100% Replacement of Sugar

Estimate	Exposure (mg kg BW day)
GEMS/Food (International)[a]	1.3–3.5 (for a 60 kg person)
Japan, Per Capita	0.04
Japan, Replacement Estimate[b]	3
US, Replacement Estimate[b]	5

*Reproduced from GRN No. 461. Typically such replacement is, in reality, approximately 30% of dietary sugars.

FSANZ (2008) estimated steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario, which resulted in estimated exposures of 0.3 - 1.0 mg/kg bw/day for the mean and 90th percentile consumer, or 0.5 - 1.5 mg/kg bw/day for rebaudioside A when making both the molecular weight and sweetness equivalency calculations. FSANZ examined consumption in other age groups and concluded that there were no safety concerns for children of any age. Merisant also calculated a dietary estimate for Reb A of 2.0 mg/kg bw/day for the average consumer and 4.7 mg/kg bw/day for a 90th percentile consumer. In another review conducted on behalf of Cargill and included in their GRAS notification, the intake of rebaudioside A when used as a complete sugar replacement was estimated at 1.3 - 3.4 mg/kg bw/day when calculated as Reb A (Renwick, 2008) (See Table 7 below).

Table 7. Summary of Estimated Daily Intake Assessments for Rebaudioside A from JECFA, FSANZ, Merisant, and Cargill

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

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

*Reproduced from GRN 395.

As stated in Almendra GRN No. 461, these different assessments suggest that total daily consumption of rebaudioside A for specified food categories and as a general purpose sweetener is unlikely to exceed 5 mg/kg bw/day (i.e., 1.65 mg steviol equivalents/kg

bw/day), for a total daily dietary exposure of up to 300 mg rebaudioside A for an adult weighing 60 kg. EFSA also calculated the daily intake of steviol glycosides (EFSA, 2010) following the JEFCA guidelines. EFSA (2010) considers that the results from toxicology studies on either stevioside or rebaudioside A are applicable for use in assessing the safety of steviol glycosides as both rebaudioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for each.

It should be noted that in 2011, EFSA revised its dietary exposure assessment of steviol glycosides. For high consumers, revised European exposure estimates to steviol glycosides remain above the established acceptable daily intake (ADI) of 4 mg/kg bw (steviol equivalent). For European children (aged 1-14), revised intake estimates ranged from 1.7 to 16.3 mg/kg bw/day; and for adults, the range was from 5.6 to 6.8 mg/kg bw/day (EFSA, 2011). However, EFSA used a very different intake assessment model in concluding that high consuming children (aged 1-14) and adults could have an intake above the JECFA ADI of 4 mg/kg bw/day. However, the intake assessment procedure used by EFSA is not widely accepted globally because it is considered excessively conservative.

Based on the extensive history of dietary intake assessments by numerous international governments and agencies and the intake assessments contained in over 30 GRAS Notifications to the U.S. FDA, the total daily consumption of steviol glycosides and Reb A for the defined food uses as a general purpose sweetener is expected to be much lower than the acceptable daily intake values established by numerous international regulatory bodies and government agencies.

5.0 Safety

A. Introduction

Almendra's high purity steviol glycoside mixture (> 95%) contains stevioside and rebaudioside A (Reb A) as its major component(s). Because Almendra's high purity steviol glycoside product is a mixture of steviol glycosides, and since the major steviol glycosides within the mixture are closely related in structure, are poorly absorbed, and are processed via the same metabolic pathway (progressive deglycosylation by gut microflora to the aglycone molecule, steviol), these compounds are understood to have very similar toxicology profiles. For this reason, regulatory agencies [e.g., the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA)] have considered toxicology studies conducted on either stevioside or Reb A to be applicable to the safety assessments of steviol glycosides as a larger class of compounds. Therefore, the information summarized in the present safety assessment includes critical information from relevant studies of all high purity steviol glycosides available in the literature (i.e., notably stevioside, Reb A, Reb D, and high purity mixtures).

The safety of high purity steviol glycosides has been extensively reviewed and discussed in the literature and by international regulatory agencies for over a decade. Toxicological issues were reported in earlier animal studies of stevia leaf extracts and crude steviol glycoside mixtures, including adverse reproductive, renal, and cardiovascular effects (reviewed in Abdel-Rahman et al., 2011). These studies were of limited value in the context of safety evaluation since the composition of the extracts and/or mixtures were either of low purity or not reported. More recent studies that focused on purified preparations of stevioside and Reb A showed that purified steviol glycosides do not elicit these adverse effects (reviewed in Carakostas et al., 2008, 2012).

Similarly, there had been some concern over the genotoxic potential of steviol glycoside ingestion based on the fact that the ultimate steviol glycoside hydrolysis product, steviol, elicited positive responses in select *in vitro* assays. The studies at the core of this issue have been extensively explored in comprehensive literature reviews (Brusick, 2008; Urban et al., 2013). Based on an extensive Weight-of-Evidence evaluation of the genotoxicity database for steviol glycosides and steviol, these reviews found that ingestion of steviol glycosides does not pose a risk of genotoxicity in humans, a conclusion shared by a number of food regulation authorities (e.g., JECFA, EFSA, FSANZ).

The JECFA has reviewed the safety of steviol glycosides at several meetings (2000, 2005, 2006, 2007, and 2009). In 2006, JECFA set a temporary Acceptable Daily Intake (ADI) value for steviol glycosides (expressed as steviol equivalents) of 0 – 2 mg/kg bw/day based on a 2-year rat study (Toyoda et al., 1997). The JECFA Committee noted that there was some evidence suggesting steviol glycosides might exert pharmacological effects in hypotensive or diabetic (type 2) subjects. Newer studies presented at a subsequent JECFA meeting demonstrated that ingestion of steviol glycosides daily in hypotensive or diabetic subjects for 4 or 16 weeks, respectively, resulted in no adverse effects at doses of approximately 4 mg (steviol equivalents)/kg bw per day. Based on this, the JECFA Committee deleted the additional 2X safety factor (going to a 100X safety factor from 200X) and established a permanent Acceptable Daily Intake (ADI) for steviol glycosides (expressed as steviol equivalents) of 0 – 4 mg/kg bw/day (JECFA, 2008). Likewise, the Food Standards Australia New Zealand (FSANZ, 2008) also established an ADI for steviol glycosides of 4 mg/kg bw/day (expressed as steviol) based on the same 2-year carcinogenicity study NOEL used by JECFA.

Prior to JECFA's evaluation, the European Commission's Scientific Committee for Food evaluated stevioside as a sweetener, but concluded that there were too many uncertainties in the toxicology literature to appropriately determine safety (Carakostas et al., 2008). Following the 2008 JECFA assessment, the European Food Safety Authority (EFSA) re-evaluated the safety of steviol glycosides. In 2010, the EFSA Panel on Food Additives and Nutrient Sources Added to Food issued an opinion concluding that steviol glycosides are not carcinogenic, genotoxic or associated with any reproductive or developmental toxicity. Like JECFA and FSANZ before them, the EFSA Panel considered the 2-year study published by Toyoda et al. (1997) to be the pivotal study, and established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on

application of a 100-fold uncertainty factor to the NOAEL for stevioside of 967 mg/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day).

To date, the FDA has issued letters of "no question" in response to 27 Generally Recognizable As Safe (GRAS) Notifications (GRNs) on steviol glycoside sweeteners, with four other GRAS Notifications pending. Merisant and Cargill were the first to submit GRNs on purified Reb A to the FDA (Merisant, GRN 252, 2008; Cargill, GRN 253, 2008), and subsequent GRNs on these steviol glycosides have all been accepted for filing and have been issued Letters of No Objection relative to their proposed use by the FDA. The most recent GRN submitted to FDA for a defined steviol glycoside preparation was submitted by Almendra on February 7, 2013, and received a "no questions" response letter on August 14, 2013 from FDA (Almendra, 2013). In addition to containing extensive reviews of the safety information, the early GRNs included expert panel reports that review and discuss in detail the metabolism, toxicology, and human health and safety data for stevioside and Reb A. Based on these GRAS notifications, FDA currently permits the use of steviol glycosides individually and in mixtures as general-purpose sweeteners at the use-levels indicated in the notifications. The steviol glycoside preparations notified to FDA thus far all meet JECFA specifications.

B. Safety Data

Studies of the toxicology, metabolism, and pharmacokinetics of steviol glycosides in animals and humans have been extensively reviewed and summarized in several FDA GRAS Notification dossiers, by multiple international regulatory agencies (JECFA, 2000, 2005, 2006, 2007, 2009; FSANZ, 2008; EFSA, 2010), and in literature reviews (Carakostas et al., 2008, 2012). Since the first GRAS Reports for steviol glycoside products as food additives were submitted to the FDA in 2008, there have been a number of toxicology studies designed and conducted in accordance with current regulatory guidelines on purified (≥ 95%) steviol glycoside compounds. A summary of the most relevant studies on steviol glycoside metabolism and pharmacokinetics, subchronic toxicity, reproductive and developmental toxicity, mutagenicity and genotoxicity, chronic toxicity, and clinical studies is presented below.

1. Metabolism and Pharmacokinetic Studies (ADME)

In vitro and *in vivo* studies demonstrate that stevioside, Reb A, and Reb D are poorly absorbed upon ingestion, and are unaffected by digestive enzymes and acidic juices of the upper gastrointestinal tract in rats and/or pigs (Nakayama et al., 1986; Koyama et al., 2003; Geuns, 2003; Roberts and Renwick, 2008; Nikiforov et al., 2013). These studies show that the structure of steviol glycosides is generally maintained until entry into the large intestine. These relatively large, hydrophilic compounds are then metabolized in the colon to steviol by bacterial glucosidases, which sequentially remove the glucose moieties from steviol glycosides until only the aglycone remains (Renwick and Tarka, 2008; Nikiforov et al., 2013). The steviol molecule cannot be further catabolized, but is absorbed into the portal vein and transported to the liver where it is conjugated with glucuronic acid and glucuronidated. In rats, steviol glucuronide is returned to the gastrointestinal tract via the bile duct where it is metabolized to steviol by bacterial

glucuronidases and excreted in the feces, though there is some enterohepatic re-circulation of steviol in rats.

While steviol glycoside metabolism has been shown to be the same in humans and rats, there are clear species differences in metabolite excretion. For example, Wheeler et al. (2008) investigated the metabolism and pharmacokinetics of stevioside and Reb A in human volunteers. No free steviol was detected in the blood after subjects received either a single oral dose of 5 mg/kg Reb A or 4.2 mg/kg stevioside (equivalent to approximately 1.6 mg/kg of steviol equivalents for both treatment groups), and steviol glucuronide was determined to be the main metabolite in plasma, with a half-life of approximately 14 hours. Steviol epoxide, a transient intermediate and possible mutagen, was not detected in human plasma. As previously observed in rats, steviol was absorbed into the enterohepatic circulation in the volunteers whereupon it was glucuronidated. Unlike the rat studies, which reported the fecal excretion to be the primary route of elimination, steviol glucuronide was the main metabolite found in the urine regardless of the steviol glycoside administered to the subjects, accounting for approximately 60% of the dose. This difference is attributed to a well-known species difference in the molecular weight threshold of biliary elimination (reviewed in Carakostas et al., 2008). However, Wheeler et al. (2008) reported steviol to be the main metabolite detected in human feces in both treatment groups, though this only accounted for a small portion of the administered dose. These results demonstrated that stevioside and Reb A undergo the same metabolic pathways in humans.

As with rats, *in vitro* studies have shown that digestive enzymes of the upper gastrointestinal system are unable to breakdown steviol glycosides; rather, the β-glycosidic bonds of steviol glycosides are not hydrolyzed until acted upon by the microflora of the lower intestine in humans, where steviol is the ultimate metabolite prior to intestinal absorption (Renwick and Tarka, 2008). Additional *in vitro* studies have reported that steviol is 200- to 300-times more permeable to cell membranes than stevioside or Reb A (Geuns et al., 2003), underscoring the importance of the location of steviol glycoside catabolism within the gastrointestinal tract, and explaining why steviol uptake is confined to the enterohepatic circulation.

Since stevioside and Reb A demonstrate similar pharmacokinetics in the rat, and also are metabolized and excreted by similar pathways in humans, regulatory authorities consider the results of toxicological studies on either stevioside or Reb A applicable for the safety assessment of steviol glycosides in general (JECFA, 2006, 2009; FSANZ, 2008; EFSA, 2010). These observations form the basis for the current approach of normalizing steviol glycoside exposures to steviol equivalents, which represents the amount of steviol present in a specific amount of a steviol glycoside. For example, based on the portion of molecular weight comprised by the steviol moiety (mw = 318.5 g/mol), stevioside (mw = 804.4 g/mol) quantities are multiplied by 0.4, and Reb A (mw = 966.4 g/mol) quantities by 0.33 (reviewed by Carakostas et al., 2012). As such, the current Acceptable Daily Intake (ADI) steviol glycosides established by JECFA, FSANZ, and EFSA is 0-4 mg steviol equivalents/kg bw/day (equivalent to 12 mg/kg bw/day Reb A on a dry weight basis).

At the time that this safety evaluation was completed, there had been no additional studies on steviol glycoside metabolism and pharmacokinetics in animals or humans published since the most recent regulatory reviews on the safety of this class of sweeteners (JECFA, 2010; FSANZ, 2008; EFSA, 2010; Almendra, 2013), or since the most recent published reviews (Carakostas et al., 2008, 2012).

2. Acute toxicity

Acute oral toxicity studies with steviol glycosides demonstrated no acute toxicity in either rats, mice or hamsters. Stevioside (96% purity) was not associated with any adverse effects following gavage administration at dose levels of up to 15 g/kg body weight in mice, rats, and hamsters (Toskulkao et al., 1997). Similarly, stevioside, rebaudioside A, or rebaudioside B administered as a single gavage dose of 2 g/kg body weight to male Swiss-Webster mice resulted in no toxic effects (Medon et al., 1982). In addition, oral administration of steviol, the common steviol glycoside metabolite, at a dose of 15 g/kg body weight to rats and mice was not associated with any adverse effects, while the same dosing in hamsters resulted in LD50 values of 5.2 and 6.1 g/kg body weight in males and females, respectively (Toskulkao et al., 1997).

No additional studies on steviol glycoside acute toxicity in animals have been published since the most recent regulatory reviews on the safety of this class of sweeteners (FSANZ, 2008; JECFA, 2010; EFSA, 2010; FDA GRN 418, 2012), or since the most recent published reviews (Carakostas et al., 2008, 2012). A 2012 FDA GRAS notification (Mini Star Intl. GRN418, 2012) included four unpublished acute toxicology studies conducted by Eurofins/Product Safety Laboratories (EPSL) that reported no acute oral toxicity (0.233 – 5 g/kg) or acute dermal toxicity (2 g/kg) in rats, and no primary skin irritation (0.5 g) or primary eye irritation (0.04 g) in rabbits upon oral, dermal, or ocular exposure to rebaudioside A (98% purity) (EPSL, 2011a,b,c,d). These results are supportive of the published findings that steviol glycosides are not acutely toxic in laboratory animals.

3. Subacute Toxicity

One subacute animal assay was conducted as a bridging toxicity study to investigate whether previous toxicity studies on rebaudioside A would be appropriate to support the safety evaluation of rebaudioside D. In a study conducted in accordance with US FDA testing guidelines, Nikiforov et al., (2013) administered 0, 500, 1000, or 2000 mg/kg/day rebaudioside D (purity = 93.5%, with the remaining 6.5% comprised mostly of other steviol glycosides), or 2000 mg/kg/day rebaudioside A (purity = 98.9%), to five groups of 20 Crl:CD(SD) rats (10 male, 10 female) respectively, for 28 consecutive days. The target dose levels had been determined by the authors' previous 90-day dietary toxicity study on rebaudioside A (Nikiforov and Eapen, 2008) (see below). The mean calculated consumption rates for males and females in each test group were 506 and 495, 1027 and 1012, 2042 and 2016, and 2043 and 1965 mg/kg/day, respectively. The authors reported no test article-related clinical observations, changes in terminal body weights, organ

weights, or food consumption, or significant differences in hematological, serum chemistry or urinalysis endpoints between control and treatment groups. Additionally, no test article-related effects were observed during the functional observational battery and motor activity endpoints. Though females in all Reb D dose groups had significantly lower ambulatory activity, this was considered due to quicker habituation and not treatment related; no differences in ambulation were observed in the highest dose Reb D and Reb A treatment group females, and no differences were reported in any of the treatment group males relative to control group males. The authors concluded that this study is appropriate as a bridging study for Reb D, and verifies the safety of Reb D for human consumption (Nikiforov et al., 2013).

4. Subchronic Toxicity

Three primary studies that have formed the basis of conclusions on toxicity associated with subchronic administration of steviol glycosides. In two recent studies, both Curry and Roberts (2008) and Nikiforov and Eapen (2008) administered Reb A in diet to rats over a 90-day period. The primary finding in these subchronic studies was reduced body weight in rats given very high doses of steviol glycosides; no deaths, clinical signs of toxicity, changes in clinical chemistry and hematology parameters, or pathological findings related to treatment were reported. The reduced body weights were observed in female rodents treated with 25,000 and 50,000 mg Reb A/kg diet (Curry and Roberts, 2008), and reduced body weights and body weight gains in male rats administered 36,000 mg Reb A/kg diet/day (Nikiforov and Eapen, 2008). Curry and Roberts (2008) noted a concomitant reduction in feed intake in their study, and the investigators of both studies regarded the reduced body weight as the result of high concentration of a non-nutritive substance in the diet.

Reduced palatability and decreased caloric density of the diet at such high levels of Reb A or stevioside, leading to a decrease in total calorie consumption, are most likely responsible for the observed decreases in body weight and/or body weight gain in these studies (Carakostas et al., 2008, 2012; EFSA, 2010). Therefore, the body weight reduction observed in these studies is not considered an adverse effect of Reb A, and the highest doses in each study (>4,000 mg/kg bw/day and 2,000 mg/kg bw/day, respectively) have been identified as study NOAELs (Curry and Roberts, 2008; Nikiforov and Eapen, 2008; JECFA, 2009; EFSA, 2010).

In an older study, Aze et al. (1991) reported the results of a study in which stevioside (95.6% purity) was administered to rats in five dietary concentrations ranging from 155 – 2,500 mg/kg bw/day over a 90-day period. Terminal body weight reductions were reported at the two highest doses. The authors reported slight but statistically significant increases in blood urea nitrogen (BUN), lactate dehydrogenase (LDH) and single cell necrosis in the liver in all male treatment groups. For the female treatment groups, statistically significant increases in absolute and relative liver weights (three highest dose groups), increases in relative brain and spleen weights (highest dose group), and in absolute and relative kidney weights (two highest dose groups) were reported but were not associated with any histopathological changes. In the absence of clear dose-response relationships, relatively low severity, and limitations to one sex, these observations were

considered by the study authors not to be attributable to the stevioside treatment, resulting in a NOAEL of 2,500-mg/kg bw/day.

Overall, the results of these studies indicate that high purity steviol glycosides are not toxic when ingested in subchronic studies in laboratory animals. This conclusion is further supported by an unpublished subchronic toxicology dog study presented and described in an early FDA GRAS notification for steviol glycoside products (Merisant, GRN 252, 2008). In this study conducted by WIL Research Laboratories, LLC, beagle dogs were fed Reb A (97.5% purity) at dosage levels up to 2000 mg/kg bw/day over a 6-month period (Eapen, 2008). No adverse effects were observed during the study, nor were there significant changes in clinical or pathological endpoints at time of necropsy. Based on these results, the study authors concluded that Reb A does not elicit systemic toxicity in beagle dogs fed a diet containing Reb A at exposures up to 2000 mg/kg bw/day for six months.

5. Reproductive and Developmental Toxicity

Historically, stevia leaf extracts were used by the Paraguayan Guarani Indian women to control contraception (Kinghorn, 2002), and laboratory studies reported that stevia extracts reduced fertility in female rats and mice (Mazzei-Planas and Kuc, 1968; Nunes and Pereira, 1988; Oliveira-Filho et al., 1989; Melis, 1999). In another earlier chronic exposure study, stevia extract demonstrated a potential for adverse effects on the male reproductive system (Yamada et al., 1985). Prior to 2008, these reports indicated to some food safety officials that stevia extracts posed a potential reproductive hazard (FDA, 2007; SCF 1999), although others concluded that the total body of evidence demonstrated that steviol glycosides were not a reproductive hazard to humans (JECFA, 2000, 2006).

To address this concern, Curry et al. (2008) designed and conducted a two-generation reproduction safety study in rats using current internationally accepted guidelines. In this study, Wistar rats were administered high-purity Reb A (97% purity) at concentrations up to 25,000 mg/kg in their diet (corresponding to 2048–2273 mg/kg bw/day). No adverse reproductive effects were observed in either F0 or F1 generations (e.g., no adverse effect on mating performance, fertility, gestation length, or estrus cycles in females, or sperm motility, concentration, or morphology in males). Furthermore, no adverse effects were reported in the offspring of F1 and F2 generations exposed to Reb A, and the study NOAEL was considered to be the highest study dose level tested. These results corroborate the lack of adverse reproductive and developmental effects reported in previous published studies of lesser purity steviol glycoside mixtures (Mori et al., 1981; Yodyingyuad and Bunyawong, 1991; Usami et al., 1995).

High doses of steviol, the aglycone metabolite of steviol glycosides, have been reported to induce maternal and developmental toxicity in hamsters (Wasuntarawat et al., 1998). However, such studies are considered to have little relevance to the safety of steviol glycosides preparations since steviol is not systemically available in humans, as it is rapidly absorbed into the portal system, conjugated with glucuronic acid, and rapidly excreted in the urine.

Based on these studies, JECFA, EFSA, and FSANZ have concluded that high purity steviol glycosides complying with JECFA specifications administered orally are unlikely to have adverse reproductive and developmental effects. This conclusion is further supported by three unpublished reproductive and developmental toxicology animal studies. The first two studies were rat assays presented and described in an early FDA GRAS notification for steviol glycoside products (Merisant, GRN 252, 2008). Again, these studies were conducted by WIL Research Laboratories, LLC, in which rats were administered Reb A (95.7% purity) either via diet or gavage, respectively. The first study was a two-generation dietary reproduction toxicity study in which Reb A was administered at doses of 0, 500, 1000, or 2000 mg/kg bw/day to male and female rats for 70 days prior to mating. No adverse effects were reported at any dose (Sloter, 2008a), and a NOAEL of ≥2000 mg Reb A/kg bw/day was established for rats at both parental (systemic and reproduction toxicity) and neonatal (developmental toxicity) lifestages. In a second study, pregnant dams were administered 0, 500, 1000, or 2000 mg Reb A/kg bw/day on gestational days 0-20 (Sloter, 2008b). No test article-related fetal malformations or developmental variations were observed at any dosage level, resulting in a NOAEL of ≥2000 mg Reb A/kg bw/day for maternal and embryo/fetal developmental toxicity. Finally, the 2010 EFSA safety evaluation of steviol glycosides as food additives cited an unpublished reproduction/developmental study conducted by Charles River Laboratories. It was reported that 97% Reb A in doses up to 1400 mg/kg bw/day had no adverse effects on developing fetuses in NZW rabbits (Charles River Laboratories, 2008), indicating a NOAEL of ≥1400 mg Reb A/kg bw/day for developmental toxicity.

6. Mutagenicity and Genotoxicity

The potential mutagenicity and genotoxicity of steviol glycosides has been an area of intense investigation, the results of which have been rigorously reviewed by food safety authorities (JECFA 2000, 2005, 2006, 2007, 2009; FSANZ, 2008; EFSA, 2010). Additionally, this topic has been the subject of two comprehensive reviews (Brusick et al., 2008; Urban et al., 2013). Overall, *in vitro* and *in vivo* studies have consistently demonstrated that stevioside and Reb A are not mutagenic or genotoxic. Well-documented shortcomings in the methods and data reported in the lone exception – an *in vivo* study reporting DNA breakage in blood cells, spleen, liver and brain of rats administered stevioside in drinking water for 45 days (Nunes et al., 2007) – has led to the determination that these results are irrelevant to animal or human health and safety (Geuns, 2007; Williams, 2007; Brusick, 2008; JECFA, 2009; EFSA, 2010). More recently, a battery of *in vitro* and *in vivo* genotoxicity tests of Reb A (purity 95.6%), designed according to the International Conference on Harmonization recommendations, provided further evidence that steviol glycosides are not genotoxic (Williams and Burdock, 2009; reviewed by JECFA, 2009; EFSA, 2010; Urban et al., 2013).

While the data for steviol glycosides has consistently demonstrated that these compounds lack genotoxic potential, steviol genotoxicity results have been less consistently negative. Steviol produces no positive results in traditional *in vitro* and *in vivo* mutagenicity assays (e.g., doses up to 8000 mg/kg bw/day in rats, mice and hamsters as reported by Temcharoen et al., 2000). However, studies using a forward-mutation method in strain

TM677 (Pezzuto et al., 1985) and chromosome breakage and gene mutation in mammalian cells (Matsui et al., 1989, 1996) have reported positive results for steviol. These *in vitro* models have not been linked to adverse *in vivo* genetic effects, and the conditions required to elicit positive responses in these studies are not useful as predictors of risk, and therefore are not relevant for human health and safety evaluation (Brusick, 2008).

Considering the negative genotoxicity findings for steviol glycosides *in vitro* and *in vivo*, as well as the fact that steviol is rapidly glucuronidated and not available systemically in humans, regulatory authorities have concluded that the genotoxicity database is sufficient in demonstrating that steviol glycosides do not pose a risk of genotoxicity for humans (JECFA, 2006, 2009; FSANZ, 2008; EFSA, 2010; Urban et al., 2013).

Some unpublished studies provide further support for this conclusion. A 2008 FDA GRAS notification (Merisant, GRN 252, 2008) describes three unpublished GLP genotoxicity studies conducted by BioReliance using high purity Reb A (99.5% purity). In the first study, Wagner and Van Dyke (2006) reported no evidence of Reb A mutagenicity in an *in vitro* bacterial reverse mutation with *S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvr*A. Similarly, Clarke (2006) reported no genotoxic potential for Reb A in *in vitro* gene mutation mouse lymphoma L5178Y/TK+/- assays. And finally, Krsmanovic and Huston (2006) reported negative results for Reb A in an *in vivo* mouse micronucleus assay in bone marrow. All three studies yielded negative results, and are therefore supportive of the general conclusions on genotoxicity safety.

7. Chronic Toxicity/Carcinogenicity

Three chronic ingestion studies have been evaluated by food safety authorities in their evaluation of the potential long-term toxicity and carcinogenicity of steviol glycosides. The Toyoda et al. (1997) study is considered the key long-term toxicity and carcinogenicity study by JECFA and other food safety authorities. In this study, stevioside (95.6% purity) was administered to F344 rats for two years at dietary levels of 969 and 1997 mg/kg bw/day for males, and 1120 and 2387 mg/kg bw/day for females (i.e., 2.5% and 5% diet, respectively). Significantly reduced final survival times among males in the high-dose group was attributed to spontaneous large granular lymphocyte leukemia resulting in more rats being sacrificed or dying during the last few weeks of the study. This is a common cancer among senescent F344 rats, and though the incidence for this leukemia increased in a dose-response manner, it was not statistically significant or outside the normal range for age and sex-matched rats. Therefore, neither the leukemia nor the lower final survival in high-dose males was considered treatment-related. The authors concluded stevioside, administered in the diet at a 5% concentration for up to 104 weeks, was not carcinogenic in male or female F344 rats. As with the high-dose treatment animals in the subchronic toxicity studies, Toyoda et al. (1997) reported both reduced bodyweight gain and terminal bodyweights in the high-dose group males and females, which they considered treatment-related. As noted in the Subchronic Toxicity section above, this effect is considered the result of reduced caloric density of the diet and taste aversion (Carakostas et al., 2012).

The study investigators considered the NOAEL for stevioside to be 970 mg/kg/day in males (2.5% dietary exposure) based on the observations in the high dose groups of reduced mean body weights in both sexes and reduced terminal survival rates. Since these high-dose endpoints are a function of reduced palatability and not treatment-related, classifying them as adverse effects was a conservative decision. The impact of this decision is significant, as this study has been used by JECFA as the critical study for setting the ADI. The NOAEL from this study has served as the basis for the permanent JECFA ADI of 0 – 4 steviol equivalents/kg/day (JECFA, 2009; ADI calculation reviewed in Carakostas et al., 2008).

Two other chronic bioassays have been reported in the literature and reviewed by regulatory authorities. Yamada et al. (1985) conducted a two-year study in which F344 rats were administered 50, 150, or 550 mg/kg bw/day *Stevia* extract comprised of > 90% stevioside and Reb A (74.54% and 16.27%, respectively). No treatment-related evidence for carcinogenicity was reported at any dose, though the authors reported some non-neoplastic changes in treated male rats (e.g., decreased spermatogenesis, interstitial cell proliferation in the testes, medullary cell proliferation in the adrenal glands, pulmonary inflammation, renal changes, and increased hematopoiesis in the spleen). However, a review of these responses indicates they lacked statistical significance or dose-dependence, or were common observations in senescent rats (EFSA, 2010). A subsequent two-year rat bioassay, Xili et al. (1992) administered stevioside (85% purity) in the diets of male Wistar rats (128.5, 367.6, and 748.6 mg/kg bw/day) and female (146.3, 416.2, and 838.9 mg kg bw/day). No treatment-related effects were reported for hematology or clinical chemistry parameters at any level, and incidences of non-neoplastic and neoplastic lesions were unrelated to the level of the stevioside in the diet.

Tumor promotion studies further support the conclusion that steviol glycosides are non-carcinogenic, demonstrating that neither stevioside, Reb A, nor steviol promote tumors initiated by genotoxic agents *in vitro* (Okamoto et al., 1983; Konoshima and Takasaki, 2002) or *in vivo* (Kawamori et al., 1995; Hagiwara et al., 1984; Ito et al., 1984) models.

It should be noted that concern has been expressed regarding the classification of steviol glycosides as non-carcinogenic over the fact that chronic toxicity testing of steviol glycosides has not been conducted in multiple species. This issue was addressed in the 2010 EFSA safety evaluation, which reviewed the relevant studies and concluded that there was no need to conduct additional carcinogenicity tests in another animal species (EFSA, 2010). A number of observations have been made that address this issue, and preclude the necessity to conduct additional chronic toxicology studies of steviol glycosides in another species (e.g., mouse). First, metabolism studies conducted in rats and humans demonstrate a common phase I and phase II metabolic mechanism, illustrating that the rat model is sufficiently similar to the human. Second, pharmacokinetics studies in humans show that levels of steviol glycosides and steviol measured in plasma and urine are so low as to indicate that systemic exposure to these compounds is negligible. Third, a plethora of *in vitro* and *in vivo* mutagenicity and genotoxicity assays have consistently demonstrated negative results for steviol

glycosides. Fourth, steviol glycosides are not tumor promoting agents in various experimental models. And finally, negative carcinogenicity data were consistently observed in three studies in multiple strains of rat.

8. Clinical Studies

In addition to the human metabolic and pharmacokinetics studies summarized in Section 1 above, additional human studies have been conducted to evaluate the safety and tolerability of steviol glycosides. Maki et al. (2008a) reported that daily ingestion of 1000 mg of Reb A (mean dose of 10.2 mg Reb A/kg bw/day, or 3.4 mg steviol equivalents/kg bw/day) over a 16-week period did not have an effect on glucose homeostasis or blood pressure in individuals with type-2 diabetes mellitus. In another study, the repeated ingestion of 1,000 mg Reb A/day (mean dose of 14.1 mg Reb A/kg bw/day, or 4.6 mg steviol equivalents/kg bw/day) for 4 weeks did not significantly alter blood pressure measurements in individuals with normal or low systolic blood pressure (Maki et al., 2008b). EFSA noted that the high dose used in each of these studies corresponded to 16.6 mg/kg bw/day of Reb A for an average 60 kg individual (or approximately 5.5 mg steviol equivalents/kg bw/day). Earlier, Maki et al. (2007) had reported in a meeting abstract that a single dose of 1000 mg of Reb A (mean dose of 12.7 mg Reb A/kg bw/day, or 4.2 mg steviol equivalents/kg bw/day) did not affect glucose homeostasis or blood pressure in healthy subjects or those with type-2 diabetes.

The results of the Maki et al. studies are supported by observations made in previous clinical studies (Jeppesen et al., 2006; Ferri et al., 2006; Cavalcante da Silva et al., 2006; Barriocanal et al., 2008), though these earlier studies used extracts of stevia that were either of insufficient or unknown purity. A more recent study published by Anton et al. (2010) compared the impact of pre-meal ingestion of stevia with that of aspartame or sucrose on food intake, satiety, and postprandial glucose and insulin levels in healthy lean and obese individuals between the ages of 18-50. Though hunger and satiety levels did not differ by treatment, stevia significantly lowered post-meal glucose levels compared to sucrose preloads, and post-meal insulin levels compared to both aspartame and sucrose preloads. However, the study was limited by the fact that the authors did not report the composition, purity, or relevant dose metric for the stevia product administered to the volunteers, and it was not clear if the endpoints under investigation were in fact adverse. For these reasons, the results of this study are not likely to impact the current regulatory position regarding the safety of steviol glycoside in humans.

In summary, human clinical studies have demonstrated that steviol glycosides are well tolerated in humans and have not been associated with adverse effects, including in individuals with type-2 diabetes or who are hypotensive.

C. Safety Data Summary

Comprehensive research studies have been conducted and published in support of the evaluation of the safety of the steviol glycosides – especially stevioside and Reb A – and the metabolite steviol, including *in vitro* and *in vivo* animal studies as well as clinical

studies in humans. These studies have undergone extensive scrutiny in both published literature reviews and in the safety evaluation of steviol glycosides conducted by several regulatory authorities. Due to a shared metabolic pathway and ultimate metabolite (steviol), studies on different individual high purity steviol glycosides (e.g., stevioside, Reb A) are considered to be relevant to the safety profile of steviol glycosides as a class. This is best illustrated by the steviol equivalency approach for normalizing the intake of high purity steviol glycoside mixtures established by JECFA. The general consensus among these experts is that high purity steviol glycosides are neither reproductive nor developmental toxicants, are not mutagenic/genotoxic or carcinogenic toxins, and are well tolerated and safe in humans. The 2-year carcinogenicity study in rats published by Toyoda et al. (1997) has been identified by JECFA, FSANZ, and EFSA as the pivotal study for the development of an ADI. The NOAEL in this study was conservatively determined to be 967 mg stevioside/kg bw/day (approximately 388 mg steviol equivalents/kg bw/day). Therefore, the current ADI of 0 – 4 mg steviol equivalents/kg bw/day (equivalent to 12 mg Reb A/kg bw/day on a dry weight basis) represents a conservative estimate of safe exposure.

6.0 Basis for the GRAS Determination

A. Introduction

The regulatory framework for determining whether a substance can be considered generally recognized as safe (GRAS) in accordance with section 201(s) (21 U.S.C. § 321(s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et. Seq.) ("the Act"), is set forth at 21 CFR 170.30, which states:

> General recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.
>
> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data and information.

These criteria are applied in the analysis below to determine whether the use of high purity (>95%) steviol glycosides for use as a table top sweetener and general purpose non-nutritive sweetener for incorporation into foods other than infant formulas

and meat and poultry products is GRAS based upon scientific procedures. All data used in this GRAS determination are publicly available and generally known, and therefore meet the "general recognition" standard under the FD&C Act.

B. Safety Determination

The subject of this GRAS determination is high purity (>95%) steviol glycosides for use as a table top sweetener and general purpose non-nutritive sweetener for incorporation into foods other than infant formulas and meat and poultry products. The publicly available toxicity study data combined with the widely disseminated knowledge concerning the numerous sweetener-related uses of steviol glycosides in food, a long history of consumption of steviol glycosides, and the numerous scientific reviews by international governmental agencies and organizations provide a sufficient basis for an assessment of the safety of high purity steviol glycosides for the uses proposed herein. An ADI for steviol glycosides has been established by JECFA and is based on an extensive database of research studies that were conducted and published in support of the safety of the steviol glycosides (i.e., of note, stevioside and Reb A and the metabolite steviol). The database includes both *in vitro* and *in vivo* animal studies as well as clinical studies in humans. These studies have undergone extensive scrutiny in both published literature reviews and in the safety evaluation of steviol glycosides conducted by several regulatory authorities. Due to a shared metabolic pathway and ultimate metabolite (steviol), studies on different individual high purity steviol glycosides (e.g., stevioside, Reb A) are considered to be relevant to the safety profile of steviol glycosides as a class. This is best illustrated by the steviol equivalency approach for normalizing the intake of high purity steviol glycoside mixtures established by JECFA. The general consensus among experts is that high purity steviol glycosides are not reproductive/developmental, mutagenic/genotoxic or carcinogenic toxins, and are well tolerated in humans. The 2-year carcinogenicity study in rats published by Toyoda et al. (1997) has been identified by JECFA, FSANZ, and EFSA as the pivotal study for the development of an ADI. The NOAEL in this study was conservatively determined to be 967 mg stevioside/kg bw/day (approximately 388 mg steviol equivalents/kg bw/day). Therefore, the current ADI of 0 – 4 mg steviol equivalents/kg bw/day (equivalent to 12 mg Reb A/kg bw/day on a dry weight basis) represents a conservative estimate of safe exposure and is accepted by regulatory bodies around the world.

C. General Recognition of the Safety of High Purity Steviol Glycosides

The intended use of these high purity steviol glycosides has been determined to be safe through scientific procedures as set forth in 21 CFR§170.3(b), thus satisfying the so-called "technical" element of the GRAS determination.

The steviol glycoside preparation that is the subject of this notification is produced using a multi-step process, beginning with the hot-water extraction of *S. rebaudiana* leaves, followed by initial purification and concentration steps to produce the steviol glycoside concentrate. This production process is consistent with the methodologies for the general manufacture of steviol glycosides as described by JECFA. Physical and chemical specifications, identical to those established by JECFA for steviol glycosides were

established for this steviol glycoside preparation. This preparation is obtained from a natural source (*S. rebaudiana* leaves), and the potential presence of microbial contaminants was limited by establishing rigorous microbiological specification parameters. Batch samples of this steviol glycoside preparation are routinely tested to verify compliance with the established chemical and microbiological parameters. Additionally, since this steviol glycoside preparation is obtained from a plant source that may be subjected to various pesticides during cultivation, the final ingredient also was subjected to a multi-residue pesticide screen. Results of the analysis showed the absence of any pesticide residues in this steviol glycoside preparation. Relative to stability in foods and beverages, there are sufficient published data supporting the conclusion that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions.

Almendra intends to market this steviol glycoside preparation (commercially known as Steviose SG 100) as a sweetening agent for use as a general purpose sweetener which will consist of not less than 95% steviol glycosides with rebaudioside A (65-75%) and stevioside (15-25%) representing more than 90% of the finished product and the remainder comprising the following related steviol glycosides, in any combination and ratio: rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, steviolbioside, and rubusoside. Steviol glycosides (steviol conjugated with glucose, xylose, and/or rhamnose) are natural constituents of the *S. rebaudiana* plant. Based on its intended uses as a general purpose sweetening agent, this steviol glycoside preparation will be added to a variety of food products, excluding infant formulas and meat and poultry products, consistent with the current uses of other related high-intensity sweeteners that are already in the market (*e.g.*, aspartame).

Intakes of the steviol glycoside preparation have been estimated based on the extensive history of dietary intake assessments by numerous international governments and agencies and the intake assessments contained in over 30 GRAS Notifications to the U.S. FDA. It is anticipated that the total daily consumption of steviol glycosides from this steviol glycoside preparation for the defined food uses as a general purpose sweetener is expected to be much lower than the acceptable daily intake values established by numerous international regulatory bodies and government agencies.

Steviol glycosides are naturally occurring constituents of the stevia plant, *S. rebaudiana* (Bertoni). As a result of their characteristically sweet taste, extracts of the stevia plant have a long history of human consumption. The safety of steviol glycosides has been the subject of numerous assessments and recently multiple jurisdictions including the U.S., EU, Australia and New Zealand, and Canada have along with a host of other countries concluded that preparations containing at least 95% steviol glycosides are safe when used in accordance with cGMP. Based on the similar metabolic pathway for all steviol glycosides in rats and humans, an ADI of 0 to 4 mg/kg body weight, as steviol equivalents, was established by EFSA, FSANZ, Health Canada, and JECFA on the basis of a NOAEL of 970 mg/kg body weight/day (383 mg/kg body weight/day, as steviol) from a 2-year study in rats (Toyoda *et al.*, 1997) and a safety factor of 100, to account for

intra- and inter-species differences. Therefore it was determined that JECFA's ADI for steviol glycosides also would extend to this steviol glycoside preparation.

Also, because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

Determination of the safety and GRAS status of the high purity steviol glycosides that are the subject of this notification has been made through the deliberations of an Expert Panel convened by Almendra and comprised of Stanley M. Tarka, Jr., Ph.D. and Michael Carakostas, DVM, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that high purity steviol glycosides identified commercially as Steviose SG 100, produced consistent with Good Manufacturing Practice and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concludes that these uses of (high purity steviol glycosides are GRAS based on scientific procedures, and that other experts qualified to assess the safety of dietary supplements would concur with these conclusions. The Panel's GRAS opinion is included as Exhibit 1 to this document.

It is also Almendra's opinion that other qualified scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, Almendra has concluded that this steviol glycoside preparation is GRAS under the intended conditions of use on the basis of scientific procedures; and therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

Almendra Ltd. is not aware of any information that would be inconsistent with a finding that the proposed use of high purity steviol glycosides as a table top sweetener and general purpose non-nutritive sweetener meeting appropriate specifications and used according to Good Manufacturing Practice, is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

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8.0 Appendices

Appendix A. Specifications/Certifications for Merck Ethanol

MERCK

Specification

http://certificates.merck.de

Date of print:
21.09.2010

1.00986.0000 Ethanol absolute suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,USP

	Spec. Values	
Assay		
m/m	≥ 99.2	%
V/V	≥ 99.5	%
Identity (IR)	conforms	
Appearance	conforms	
Clarity of solution	conforms	
Colour of solution	conforms	
Acidity or alkalinity	< 30 ppm	
Density		
d 20 °C/20 °C	0.790 - 0.793	
d 15,56 °C/15,56 °C	" 0.7962	
d 15 °C/15 °C	0.794 - 0.797	
Absorbance		
at 240 nm	" 0.40	
between 250nm and 260nm	" 0.30	
between 270nm and 340nm	" 0.10	
The absorption curve is smooth	conforms	
Volatile impurities (GC)		
Acetaldehyde and Acetal	" 10	ppm
Benzene	" 2	ppm
Methanol	" 100	ppm
Total of other impurities	" 300	ppm
disregard limit	" 9	ppm
residue on evaporation	" 25	mg/l

Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt (Germany): +49 6151 72- 0

Specification

1.00986.0000 Ethanol absolute suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,USP

Other residual solvents (Ph Eur/USP/ICH) excluded by production process.

Residues of metal catalysts or metal reagents acc.to EMEA/CHMP/SWP/4446/2000 are not likely to be present.

Dr. Michael Savelsberg

responsible laboratory manager quality control

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MERCK

Aflatoxin - Certificate

100986 Ethanol absolute suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,USP

The German Regulation on Aflatoxins (Aflatoxinverbotsverordnung), as of July 19th, 2000, addresses the manufacturer of drugs and not the manufacturer or supplier of raw materials used for drug production. In his production process the manufacturer of drugs must not use materials with aflatoxin contents higher than certain limits set by the above-mentioned regulation.

Merck KGaA, as a supplier of raw materials, has neither influence on the storage conditions outside the Merck premises nor on further production conditions and can, therefore, not guarantee the absence of aflatoxins.

Analytical tests regarding the determination of aflatoxins are not carried out routinely, unless specifically mentioned below.

This is to certify that the above-mentioned product is manufactured using raw material of biological origin.
To the best of our knowledge and based on information on the manufacturing procedures, the presence of mould and contamination with aflatoxins is unlikely.

Dr. Michael
Zeiger
Compliance
Certification
OC-QA Quality Assurance Chemicals

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Date: 08.01.2010

MERCK

Allergen - Certificate

100986 Ethanol absolute suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,USP

List of Allergens

Cow's milk protein	Lactose
Chicken	Chicken's egg
Beef	Pork
Fish	Shellfish / Mollusc
Crustaceans	Maize
Rye	Gluten
Soy protein	Soy oil
Nuts	Nut oil
Peanuts	Peanut oil
Sesame	Sesame oil
Legumes/pulses	Lupine
Cinnamon	Vanillin
Coriander	Celery
Umbelliferae	Cocoa
Mustard	Glutamate
Azo dyes	Tartrazine (E102)
Benzoic Acid (E210)	Parabenes (E211-E219)
Latex	

Because of the used raw materials and/or the manufacturing procedure we do not expect the listed allergens in the final product.

The following materials are used as raw material but are not present in the final product: Yeast, Wheat

We point out that Merck KGaA does not perform any testing on allergens in the above-mentioned product.

Based on information from our raw material supplier the sulphite content (E220-E227) is below 10 ppm.

Dr. Michael Zeiger
Compliance
Certification
OC-QA Quality Assurance Chemicals

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Date: 08.01.2010

MERCK

TSE/BSE-Certificate

100986 Ethanol absolute suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,USP

The note for guidance EMEA/410/01 Rev. 2 of the EC considers the requirements of raw materials used for human and veterinary medicinal products. The document introduces risk assessment into the regulatory compliance process for products derived from TSE/BSE-relevant animal species.

We certify that this product is manufactured without the use of raw materials of animal or human origin.

Ethanol is of vegetable origin, produced by fermentation.

During processing the product does not come in contact with animal material.

Therefore, the product does not fall under the scope of the above-mentioned guideline and is not concerned by the TSE/BSE issue.

Dr. Kerstin
Reider
Compliance
Certification
OC-QA Quality Assurance Chemicals

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Date: 25.03.2010

MERCK

GMO - Certificate

100986 Ethanol absolute suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,USP

The European Regulations (EC) 1829/2003 and (EC) 1830/2003 concern the labelling and traceability of genetically modified organisms (GMO) and of food and feed produced from GM organisms.

We hereby declare that the above-mentioned item is neither produced from nor consists of genetically modified organisms. Hence, to the best of our knowledge, there is no objective reason to suspect a risk associated with GMO in the product.

Dr. Michael
Zeiger
Compliance
Certification
OC-QA Quality Assurance Chemicals

This document has been produced electronically and is valid without a signature.

Date: 08.01.2010

RABBI T. HOD-HOCHWALD
Expert for Kosher Foods & Ingredients
Bad Kissingen, Germany
Son of: RABBI ABRAHAM HOCHWALD z"l
Landesrabbiner von Nordrhein
Founder of the Kashruth Organisation

ב"ה

הרב ט. הוד-הוכוולד
מומחה לכשרות מזון וחמרי גלם
באד קיסינגען, גרמניה
בנו של: הרב אברהם הוכוולד ז"ל
הרב למדינת צפון הריין
מייסד מערכת הכשרות

KSI Kosher Supervision

Belgium: Tel/Fax: (+32)-3-281.12.32 **Israel:** Tel/Fax: (+972)-3-922.63.71 **Germany:** Tel: (+49)-172-531.63.71
Address: Grétrystr. 7, B-2018 Antwerp, Belgium **E-mail:** kosher@pandora.be / hodkosher@vodafone.de

Ref 7607 b

י"ג טבת תש"ע - 30/12/2009

תעודת כשרות

הנני מאשר בזה כי בדקתי את הרכיבים ותהליך הייצור והאריזה של המוצר:

Ethanol absolute suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,USP 100986

המיוצר ע"י חברת **מערק** ב-דארמשטאדט, גרמני ואין הוא מכיל, וגם אינו בא במגע עם שום חמרים לא-כשרים בעת הייצור והאריזה.

לפיכך המוצר הנ"ל **כשר** – פרווה לכל ימות השנה פרט לימי חג הפסח

תוקף תעודת כשרות זו הוא לשנה אחת.

ועל זה באתי על החתום היום, י"ג טבת תש"ע:

(b) (6)

Kosher Certificate

I hereby certify that I inspected the ingredients and the production and packing process of:

100986 Ethanol absolute suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,USP

produced by **MERCK KGaA** in Darmstadt (Germany) and I found that it does not contain, nor come in contact with any non-kosher substances in the course of production and packing .

It is therefore **KOSHER -parve for the all year except for the Passover days.**

This certificate is valid for one year, until end of February 2011.

In witness thereof I sign today 30/12/2009

Ra(b) (6) ld
So 'l

Appendix B. Certificates of Analysis

almendra

CERTIFICATE OF ANALYSIS

Product Name:	Steviose SG 100
Batch Number:	(b) (6)
Amount:	-
Manufacturing Date:	January 21, 2013
Expiry Date:	January 20, 2015
COA No :	-
Date :	August 01, 2013

ANALYTICAL RESULTS

Analytical Parameters	Method	Specification	Result
Appearance Form	Visual	Powder	Powder
Appearance Color	Visual	White	White
Total Glycoside (HPLC Area,%)	Jecfa (2010)	99.50	99.64
Rebaudioside A (HPLC Area,%)	Jecfa (2010)	70 - 83	73.98
Stevioside HPLC Area,%)	Jecfa (2010)	10 - 20	16.16
Residual Solvent (ppm)	EPA Method 5021A	≤ 5000	95
Water Content (%)	Karl Fischer	≤ 3.0	1.70

(b) (6)

Ms. Pnita Chutasmit

QA Manager

almendra

CERTIFICATE OF ANALYSIS

Product Name:	Steviose SG 100
Batch Number:	(b) (6)
Amount:	-
Manufacturing Date:	January 30, 2013
Expiry Date:	January 29, 2015
COA No :	-
Date :	August 01, 2013

ANALYTICAL RESULTS

Analytical Parameters	Method	Specification	Result
Appearance Form	Visual	Powder	Powder
Appearance Color	Visual	White	White
Total Glycoside (HPLC Area,%)	Jecfa (2010)	99.50	99.60
Rebaudioside A (HPLC Area,%)	Jecfa (2010)	70 - 83	74.95
Stevioside HPLC Area,%)	Jecfa (2010)	10 - 20	15.76
Residual Solvent (ppm)	EPA Method 5021A	≤ 5000	219
Water Content (%)	Karl Fischer	≤ 3.0	2.71

(b) (6)

Ms. Pnita Chutasmit
QA Manager

almendra

CERTIFICATE OF ANALYSIS

Product Name:	Steviose SG 100
Batch Number:	(b) (6)
Amount:	-
Manufacturing Date:	January 02, 2013
Expiry Date:	January 01, 2015
COA No :	-
Date :	August 01, 2013

ANALYTICAL RESULTS

Analytical Parameters	Method	Specification	Result
Appearance Form	Visual	Powder	Powder
Appearance Color	Visual	White	White
Total Glycoside (HPLC Area,%)	Jecfa (2010)	99.50	99.60
Rebaudioside A (HPLC Area,%)	Jecfa (2010)	70 - 83	72.75
Stevioside HPLC Area,%)	Jecfa (2010)	10 - 20	18.68
Residual Solvent (ppm)	EPA Method 5021A	≤ 5000	310
Water Content (%)	Karl Fischer	≤ 3.0	1.22

(b) (6)

Ms. Pnita Chutasmit
QA Manager

almendra

CERTIFICATE OF ANALYSIS

Product Name:	Steviose SG 100
Batch Number:	(b) (6)
Amount:	-
Manufacturing Date:	November 11, 2012
Expiry Date:	November 10, 2014
COA No :	-
Date :	August 01, 2013

ANALYTICAL RESULTS

Analytical Parameters	Method	Specification	Result
Appearance Form	Visual	Powder	Powder
Appearance Color	Visual	White	White
Total Glycoside (HPLC Area,%)	Jecfa (2010)	99.50	99.79
Rebaudioside A (HPLC Area,%)	Jecfa (2010)	70 - 83	74.80
Stevioside HPLC Area,%)	Jecfa (2010)	10 - 20	15.88
Residual Solvent (ppm)	EPA Method 5021A	≤ 5000	282
Water Content (%)	Karl Fischer	≤ 3.0	0.82

(b) (6)

Ms. Pnita Chutasmit
QA Manager

almendra

CERTIFICATE OF ANALYSIS

Product Name:	Steviose SG 100
Batch Number:	(b) (6)
Amount:	-
Manufacturing Date:	November 14, 2012
Expiry Date:	November 13, 2014
COA No :	-
Date :	August 01, 2013

ANALYTICAL RESULTS

Analytical Parameters	Method	Specification	Result
Appearance Form	Visual	Powder	Powder
Appearance Color	Visual	White	White
Total Glycoside (HPLC Area,%)	Jecfa (2010)	99.50	99.78
Rebaudioside A (HPLC Area,%)	Jecfa (2010)	70 - 83	74.71
Stevioside HPLC Area,%)	Jecfa (2010)	10 - 20	16.05
Residual Solvent (ppm)	EPA Method 5021A	≤ 5000	302
Water Content (%)	Karl Fischer	≤ 3.0	1.05

(b) (6)

Ms. Pnita Chutasmit
QA Manager

OMIC Overseas Merchandise Inspection Co., Ltd. 海外貨物検査株式会社
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TEST REPORT

ORIGINAL

ilac-MRA

Q DMSc Accreditation No. 1666/48

Report No. : CBL59-13627
Sample ID : 59-13627
Batch No. : JBL59/06457
Report Date : November 15, 2012

Customer : ALMENDRA (THAILAND) LTD.
7/313 MOO 6 T. MAPYANGPORN,
A. PLUANGDEAND, RAYONG 21140

Sample Description : FOOD INGREDIENT
Customer's Reference : BATCH (b) (6)
Received Date : October 29, 2012
Sample Condition : Sample is contained in Foil package, sealed.
Analysis Commenced Date : October 29 ,2012
Analysis Completed Date : November 13 ,2012

Test Item	Method	Result	Unit
Alicyclobacillus sp *	Designated by Almendra (Thailand) Ltd.	Not Detected	/g
Fecal Coliform *	APHA ; Chapter 8	<3	(MPN)/g
Listeria spp. *	BAM Online (2003) ; Chapter 10	Not Detected	/25 g
Total Plate Count	Compendium of Method for the Microbiological Examination of Foods (APHA),2001 Chapter 7.	<10 est.	(CFU)/g
Salmonella spp.	ISO 6579:2002 / Amd. 1: 2007	Not Detected	/25 g
Staphylococcus aureus	Compendium of Method for the Microbiological Examination of Foods (APHA), 2001 chapter 39.	<10	(CFU)/g
Escherichia coli	Compendium of Method for the Microbiological Examination of Foods (APHA) ,2001 chapter 8.	<10	(CFU)/g
Coliform	Compendium of Method for the Microbiological Examination of Foods (APHA) ,2001 chapter 8.	<10	(CFU)/g
Yeast	AOAC(2007) 997.02	<10	(CFU)/g
Mold	AOAC(2007) 997.02	<10	(CFU)/g
Moisture *	In-house method OR-215-TM based on AOAC (2007) 934.01	0.74	%
Ash *	In-house method OR-216-TM based on AOAC (2007) 923.03	0.08	%
pH *	AOAC (2007) 943.02	5.41	-
Arsenic (As) *	In-house method based on AOAC(2007) 973.78	Not Detected	mg/kg
Lead (Pb) *	AOAC(2007) 999.11	<0.008	mg/kg
Mercury (Hg) *	In-house method IN-056-TM based on AOAC (2007) 974.14	Not Detected	mg/kg
Cadmium (Cd) *	AOAC(2007) 999.11	0.0047	mg/kg

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№ 201097 BK

OMIC Overseas Merchandise Inspection Co., Ltd. 海外貿物検査株式会社
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Tel No. : +66-2-286-4120-3 Fax No. : +66-2-287-2570-1 URL : http://www.omicnet.com

ORIGINAL

Report No. : CBL59-13627
Sample ID : (b) (6)
Batch No. :

Test Item	Method	Result	Unit
Methanol *	GC/MS (Headspace)	Not Detected	mg/kg
Ethanol *	GC/MS (Headspace)	0.0695	%(w/w)
Organochlorine Group	In-house method CH-094-TM based on Journal of AOAC International Vol. 86, No. 2, 2003		
• 1,1-Dichloro-2,2-Bis (4-Ethylphenyl) Ethane (Perthane) *		Not Detected	mg/kg
• Acetochlor *		Not Detected	mg/kg
• Alachlor *		Not Detected	mg/kg
• Aldrin *		Not Detected	mg/kg
• Allethrin *		Not Detected	mg/kg
• Alpha-BHC *		Not Detected	mg/kg
• Azaconazole *		Not Detected	mg/kg
• Benfluralin *		Not Detected	mg/kg
• Benoxacor *		Not Detected	mg/kg
• Beta-BHC *		Not Detected	mg/kg
• Bifenox *		Not Detected	mg/kg
• Boscalid *		Not Detected	mg/kg
• Bromophos *		Not Detected	mg/kg
• Bromophos-ethyl *		Not Detected	mg/kg
• Bromopropylate *		Not Detected	mg/kg
• Bupirimate *		Not Detected	mg/kg
• Butachlor *		Not Detected	mg/kg
• Captafol *		Not Detected	mg/kg
• Captan *		Not Detected	mg/kg
• Carfentrazone-Ethyl *		Not Detected	mg/kg
• Chlorbenside *		Not Detected	mg/kg
• Chlordane *		Not Detected	mg/kg
• Chlorfenapyr *		Not Detected	mg/kg
• Chlorfenson *		Not Detected	mg/kg
• Chloroneb *		Not Detected	mg/kg
• Chlorothalonil *		Not Detected	mg/kg
• Chlorthal-dimethyl *		Not Detected	mg/kg
• Chlozolinate *		Not Detected	mg/kg
• Cinidone-ethyl *		Not Detected	mg/kg
• Clomeprop *		Not Detected	mg/kg
• Coumafos/Coumaphos *		Not Detected	mg/kg
• DDD *		Not Detected	mg/kg
• DDE *		Not Detected	mg/kg
• DDT *		Not Detected	mg/kg
• Delta-BHC *		Not Detected	mg/kg
• Di-allate *		Not Detected	mg/kg
• Dichlofluanid *		Not Detected	mg/kg
• Dichloran *		Not Detected	mg/kg
• Dichlormid *		Not Detected	mg/kg
• Diclocymet *		Not Detected	mg/kg
• Dicofol *		Not Detected	mg/kg
• Dieldrin *		Not Detected	mg/kg
• Dimethenamid *		Not Detected	mg/kg

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№ 201096 BK

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ORIGINAL

Report No. : CBL59-13627
Sample ID : 59-13627
Batch No. : JBL59/06457

Test Item	Method	Result	Unit
• Dimethipin *		Not Detected	mg/kg
• Endosulfan Sulphate *		Not Detected	mg/kg
• Endosulfan-Alpha *		Not Detected	mg/kg
• Endosulfan-Beta *		Not Detected	mg/kg
• Endrin *		Not Detected	mg/kg
• Endrin aldehyde *		Not Detected	mg/kg
• Endrin ketone *		Not Detected	mg/kg
• Ethalfluralin *		Not Detected	mg/kg
• Etridiazole *		Not Detected	mg/kg
• Fenamidone *		Not Detected	mg/kg
• Fipronil *		Not Detected	mg/kg
• Flufenacet *		Not Detected	mg/kg
• Fluthiacet-methyl *		Not Detected	mg/kg
• Folpet *		Not Detected	mg/kg
• Fthalide *		Not Detected	mg/kg
• Heptachlor *		Not Detected	mg/kg
• Heptachlor epoxide *		Not Detected	mg/kg
• Hexachlorobenzene *		Not Detected	mg/kg
• Isoxaflutole *		Not Detected	mg/kg
• Lindane (gamma-BHC) *		Not Detected	mg/kg
• Mefenpyr-diethyl *		Not Detected	mg/kg
• Methoxychlor *		Not Detected	mg/kg
• Myclobutanil *		Not Detected	mg/kg
• Nitrapyrin *		Not Detected	mg/kg
• Norflurazon *		Not Detected	mg/kg
• Oxabetrinil *		Not Detected	mg/kg
• Pentoxazone *		Not Detected	mg/kg
• Pyridaben *		Not Detected	mg/kg
• Pyridalyl *		Not Detected	mg/kg
• Pyrifenox *		Not Detected	mg/kg
• Quintozene *		Not Detected	mg/kg
• Tecnazene *		Not Detected	mg/kg
• Tetradifon *		Not Detected	mg/kg
• Thiazopyr *		Not Detected	mg/kg
• Thifluzamide *		Not Detected	mg/kg
• Tolylfluanid *		Not Detected	mg/kg
• Tri-Allate *		Not Detected	mg/kg
• Trichlamide *		Not Detected	mg/kg
• Triflumizole *		Not Detected	mg/kg
• Trifluralin *		Not Detected	mg/kg
• Vinclozolin *		Not Detected	mg/kg
Pyrethroid Group	In-house method CH-094-TM based on Journal of AOAC International Vol. 86, No. 2, 2003		
• Bifenthrin *		Not Detected	mg/kg
• Cyfluthrin *		Not Detected	mg/kg
• Cyhalothrin *		Not Detected	mg/kg
• Cypermethrin *		Not Detected	mg/kg
• Deltamethrin, Tralomethrin *		Not Detected	mg/kg

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№ 201095 BK

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ORIGINAL

Report No. : CBL59-13627
Sample ID : 59-13627
Batch No. : JBL59/06457

Test Item	Method	Result	Unit
• Fenpropathrin *		Not Detected	mg/kg
• Fenvalerate *		Not Detected	mg/kg
• Flucythrinate *		Not Detected	mg/kg
• Fluvalinate *		Not Detected	mg/kg
• Permethrin *		Not Detected	mg/kg
• Pyrethrin *		Not Detected	mg/kg
Organophosphorus Group	In-house method CH-093-TM based on Journal of AOAC International Vol. 86, No. 2, 2003		
• Acephate *		Not Detected	mg/kg
• Anilofos *		Not Detected	mg/kg
• Azinphos-methyl *		Not Detected	mg/kg
• Butamifos *		Not Detected	mg/kg
• Cadusaphos *		Not Detected	mg/kg
• Carbophenothion *		Not Detected	mg/kg
• Chlorethoxyphos *		Not Detected	mg/kg
• Chlorfenvinphos *		Not Detected	mg/kg
• Chlorpyrifos *		Not Detected	mg/kg
• Chlorpyrifos-methyl *		Not Detected	mg/kg
• Cyanofenphos *		Not Detected	mg/kg
• Cyanophos *		Not Detected	mg/kg
• Demeton-S-methyl *		Not Detected	mg/kg
• Diazinon *		Not Detected	mg/kg
• Dichlofenthion *		Not Detected	mg/kg
• Dichlorvos, Naled *		Not Detected	mg/kg
• Dicrotophos *		Not Detected	mg/kg
• Dimethoate *		Not Detected	mg/kg
• Dimethylvinphos *		Not Detected	mg/kg
• Dioxathion *		Not Detected	mg/kg
• Disulfoton *		Not Detected	mg/kg
• Edifenphos *		Not Detected	mg/kg
• EPN *		Not Detected	mg/kg
• Ethion *		Not Detected	mg/kg
• Ethoprophos *		Not Detected	mg/kg
• Etrimfos *		Not Detected	mg/kg
• Famphur *		Not Detected	mg/kg
• Fenamiphos *		Not Detected	mg/kg
• Fenchlorfos *		Not Detected	mg/kg
• Fenitrothion *		Not Detected	mg/kg
• Fensulfothion *		Not Detected	mg/kg
• Fenthion *		Not Detected	mg/kg
• Fonofos *		Not Detected	mg/kg
• Formothion *		Not Detected	mg/kg
• Fosthiazate *		Not Detected	mg/kg
• Iprobenfos *		Not Detected	mg/kg
• Isazophos *		Not Detected	mg/kg
• Isocarbofos *		Not Detected	mg/kg
• Isofenphos *		Not Detected	mg/kg
• Isofenphos Methyl *		Not Detected	mg/kg

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№ 201094 BK

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No. 12 - 14 Yen Akas Soi 3 Chongnonsri, Yannawa, Bangkok 10120 Thailand
Tel No. : +66-2-296-4120-3 Fax No. : +66-2-287-2570-1 URL : http://www.omicnet.com

ORIGINAL

Report No. : CBL59-13627
Sample ID : 59-13627
Batch No. : JBL59/06457

Test Item	Method	Result	Unit
• Isoxathion *		Not Detected	mg/kg
• Malathion *		Not Detected	mg/kg
• Mecarbam *		Not Detected	mg/kg
• Mephospholan *		Not Detected	mg/kg
• Methacrifos *		Not Detected	mg/kg
• Methamidophos *		Not Detected	mg/kg
• Methidathion *		Not Detected	mg/kg
• Mevinphos *		Not Detected	mg/kg
• Monocrotophos *		Not Detected	mg/kg
• Omethoate *		Not Detected	mg/kg
• Parathion *		Not Detected	mg/kg
• Parathion-Methyl *		Not Detected	mg/kg
• Phenthoate *		Not Detected	mg/kg
• Phorate *		Not Detected	mg/kg
• Phosalone *		Not Detected	mg/kg
• Phosmet *		Not Detected	mg/kg
• Phosphamidon *		Not Detected	mg/kg
• Piperophos *		Not Detected	mg/kg
• Pirimioxyphos *		Not Detected	mg/kg
• Pirimiphos-Ethyl *		Not Detected	mg/kg
• Pirimiphos-Methyl *		Not Detected	mg/kg
• Profenofos *		Not Detected	mg/kg
• Propaphos *		Not Detected	mg/kg
• Propetamphos *		Not Detected	mg/kg
• Prothiophos *		Not Detected	mg/kg
• Pyrazophos *		Not Detected	mg/kg
• Pyridafenthion *		Not Detected	mg/kg
• Quinalphos *		Not Detected	mg/kg
• Salithion *		Not Detected	mg/kg
• Sulprofos *		Not Detected	mg/kg
• Terbufos *		Not Detected	mg/kg
• Tetrachlorvinphos *		Not Detected	mg/kg
• Thiometon *		Not Detected	mg/kg
• Tolclofos-Methyl *		Not Detected	mg/kg
• Triazophos *		Not Detected	mg/kg
• Tribufos *		Not Detected	mg/kg
• Trichlorfon *		Not Detected	mg/kg
• Vamidothion *		Not Detected	mg/kg
Carbamate Group	In-house method CH-101-TM based on Journal of AOAC International. Vol. 86, No.2, 2003,p. 412-431.		
• 1-Naphthol *		Not Detected	mg/kg
• Aldicarb *		Not Detected	mg/kg
• Aldicarb sulfoxide *		Not Detected	mg/kg
• Aldoxycarb *		Not Detected	mg/kg
• Bendiocarb *		Not Detected	mg/kg
• Bufencarb *		Not Detected	mg/kg
• Carbaryl *		Not Detected	mg/kg
• Carbofuran *		Not Detected	mg/kg

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№ 201093 BK

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Tel No. : +66-2-296-4120-3 Fax No. : +66-2-287-2570-1 URL : http://www.omicnet.com

ORIGINAL

Report No. : CBL59-13627
Sample ID : 59-13627
Batch No. : JBL59/06457

Test Item	Method	Result	Unit
• Etrofol *		Not Detected	mg/kg
• Fenobucarb *		Not Detected	mg/kg
• Isoprocarb *		Not Detected	mg/kg
• Methiocarb *		Not Detected	mg/kg
• Methomyl *		Not Detected	mg/kg
• Metolcarb *		Not Detected	mg/kg
• Oxamyl *		Not Detected	mg/kg
• Promecarb *		Not Detected	mg/kg
• Propoxur *		Not Detected	mg/kg
• Thiodicarb *		Not Detected	mg/kg
• XMC *		Not Detected	mg/kg
• Xylylcarb (MPMC) *		Not Detected	mg/kg

* : This item is out of scope of accreditation.
BAM : Bacteriological Analytical Manual Online.
est. : estimated count
APHA : Compendium of Methods for the Microbiological Examination of food ,4th Edition ,2001

The sample(s) will be held for thirty days from the date of the report.
Reported result refers to submitted sample(s) only.
This report shall not be reproduced except in full, without written approval of the laboratory.

Overseas Merchandise Inspection Co., Ltd.
Bangkok Laboratory

(b) (6) [redacted]

(KONGSAK SUPASOON)
Laboratory Manager

(b) (6) [redacted]

(KUNAPORN SANGUANKAEW)
Supervisor of Chromatography Section

(b) (6) [redacted]

(PIRAPHON PANTAMAS)
Organic/Inorganic Section Manager

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№ 201100 BK

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ORIGINAL

TEST REPORT

Accreditation No. 1066/48

Report No. : CBL59-19294
Sample ID : 59-19294
Batch No. : JBL59/08988
Report Date : February 04, 2013

Customer : ALMENDRA (THAILAND) LTD.
7/313 MOO 6 T. MAPYANGPORN,
A. PLUANGDEAND, RAYONG 21140

Sample Description : 1ST CAKE P08 (SG100)
Customer's Reference : BATCH NO. 21012013
Received Date : January 23, 2013
Sample Condition : Sample is contained in Foil package, sealed.
Analysis Commenced Date : January 24 ,2013
Analysis Completed Date : January 30 ,2013

Test Item	Method	MDL	Result	Unit
Moisture (Karl Fischer) *	AOCS(1997) Ca 2e-84	-	1.70	%
Arsenic (As)	In-house method OR-190-TM based on AOAC (2007) 986.15	0.006	Not Detected	mg/kg
Ash *	In-house method OR-216-TM based on AOAC (2007) 923.03	-	0.05	%
Cadmium (Cd) *	AOAC(2007) 999.11	0.0001	Not Detected	mg/kg
Ethanol *	GC/MS (Headspace)	-	0.0095	%(w/v)
Lead (Pb) *	AOAC(2007) 999.11	0.002	0.010	mg/kg
Mercury (Hg)	In-house method IN-056-TM based on AOAC (2007) 974.14	0.002	0.012	mg/kg
Methanol *	GC/MS (Headspace)	5	Not Detected	mg/kg
pH *	AOAC (2007) 943.02	-	6.36	-
Organophosphorus Group	In-house method CH-093-TM based on Journal of AOAC International Vol. 86, No. 2, 2003			
• Acephate *		0.01	Not Detected	mg/kg
• Anilofos *		0.03	Not Detected	mg/kg
• Azinphos-methyl *		0.05	Not Detected	mg/kg
• Butamifos *		0.005	Not Detected	mg/kg
• Cadusaphos *		0.007	Not Detected	mg/kg
• Carbophenothion *		0.01	Not Detected	mg/kg
• Chlorethoxyphos *		0.005	Not Detected	mg/kg
• Chlorfenvinphos *		0.01	Not Detected	mg/kg
• Chlorpyrifos *		0.01	Not Detected	mg/kg
• Chlorpyrifos-methyl *		0.01	Not Detected	mg/kg
• Cyanofenphos *		0.01	Not Detected	mg/kg
• Cyanophos *		0.005	Not Detected	mg/kg
• Demeton-S-methyl *		0.005	Not Detected	mg/kg

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ORIGINAL

Report No. : CBL59-19294
Sample ID : 59-19294
Batch No. : JBL59/08988

Test Item	Method	MDL	Result	Unit
• Diazinon *		0.005	Not Detected	mg/kg
• Dichlofenthion *		0.01	Not Detected	mg/kg
• Dichlorvos, Naled *		0.003	Not Detected	mg/kg
• Dicrotophos *		0.015	Not Detected	mg/kg
• Dimethoate *		0.01	Not Detected	mg/kg
• Dimethylvinphos *		0.01	Not Detected	mg/kg
• Dioxathion *		0.02	Not Detected	mg/kg
• Disulfoton *		0.005	Not Detected	mg/kg
• Edifenphos *		0.01	Not Detected	mg/kg
• EPN *		0.01	Not Detected	mg/kg
• Ethion *		0.002	Not Detected	mg/kg
• Ethoprophos *		0.005	Not Detected	mg/kg
• Etrimfos *		0.005	Not Detected	mg/kg
• Famphur *		0.03	Not Detected	mg/kg
• Fenamiphos *		0.005	Not Detected	mg/kg
• Fenchlorfos *		0.01	Not Detected	mg/kg
• Fenitrothion *		0.01	Not Detected	mg/kg
• Fensulfothion *		0.01	Not Detected	mg/kg
• Fenthion *		0.01	Not Detected	mg/kg
• Fonofos *		0.005	Not Detected	mg/kg
• Formothion *		0.005	Not Detected	mg/kg
• Fosthiazate *		0.02	Not Detected	mg/kg
• Iprobenfos *		0.01	Not Detected	mg/kg
• Isazophos *		0.01	Not Detected	mg/kg
• Isocarbofos *		0.01	Not Detected	mg/kg
• Isofenphos *		0.01	Not Detected	mg/kg
• Isofenphos Methyl *		0.01	Not Detected	mg/kg
• Isoxathion *		0.01	Not Detected	mg/kg
• Malathion *		0.02	Not Detected	mg/kg
• Mecarbam *		0.01	Not Detected	mg/kg
• Mephospholan *		0.005	Not Detected	mg/kg
• Methacrifos *		0.003	Not Detected	mg/kg
• Methamidophos *		0.003	Not Detected	mg/kg
• Methidathion *		0.01	Not Detected	mg/kg
• Mevinphos *		0.005	Not Detected	mg/kg
• Monocrotophos *		0.01	Not Detected	mg/kg
• Omethoate *		0.02	Not Detected	mg/kg
• Parathion *		0.01	Not Detected	mg/kg
• Parathion-Methyl *		0.01	Not Detected	mg/kg
• Phenthoate *		0.01	Not Detected	mg/kg
• Phorate *		0.005	Not Detected	mg/kg
• Phosalone *		0.03	Not Detected	mg/kg
• Phosmet *		0.05	Not Detected	mg/kg
• Phosphamidon *		0.02	Not Detected	mg/kg
• Piperophos *		0.01	Not Detected	mg/kg
• Pirimioxyphos *		0.02	Not Detected	mg/kg
• Pirimiphos-Ethyl *		0.01	Not Detected	mg/kg
• Pirimiphos-Methyl *		0.01	Not Detected	mg/kg
• Profenofos *		0.01	Not Detected	mg/kg

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ORIGINAL

Report No. : CBL59-19294
Sample ID : 59-19294
Batch No. : JBL59/08988

Test Item	Method	MDL	Result	Unit
• Propaphos *		0.01	Not Detected	mg/kg
• Propetamphos *		0.005	Not Detected	mg/kg
• Prothiophos *		0.01	Not Detected	mg/kg
• Pyrazophos *		0.03	Not Detected	mg/kg
• Pyridafenthion *		0.01	Not Detected	mg/kg
• Quinalphos *		0.01	Not Detected	mg/kg
• Salithion *		0.005	Not Detected	mg/kg
• Sulprofos *		0.005	Not Detected	mg/kg
• Terbufos *		0.005	Not Detected	mg/kg
• Tetrachlorvinphos *		0.01	Not Detected	mg/kg
• Thiometon *		0.005	Not Detected	mg/kg
• Tolclofos-Methyl *		0.005	Not Detected	mg/kg
• Triazophos *		0.01	Not Detected	mg/kg
• Tribufos *		0.01	Not Detected	mg/kg
• Trichlorfon *		0.005	Not Detected	mg/kg
• Vamidothion *		0.03	Not Detected	mg/kg
Carbamate Group	In-house method CH-101-TM based on Journal of AOAC International. Vol. 86, No.2, 2003,p. 412-431.			
• 1-Naphthol *		0.005	Not Detected	mg/kg
• Aldicarb *		0.005	Not Detected	mg/kg
• Aldicarb sulfoxide *		0.005	Not Detected	mg/kg
• Aldoxycarb *		0.005	Not Detected	mg/kg
• Bendiocarb *		0.005	Not Detected	mg/kg
• Bufencarb *		0.005	Not Detected	mg/kg
• Carbaryl *		0.005	Not Detected	mg/kg
• Carbofuran *		0.005	Not Detected	mg/kg
• Etofol *		0.005	Not Detected	mg/kg
• Fenobucarb *		0.005	Not Detected	mg/kg
• Isoprocarb *		0.005	Not Detected	mg/kg
• Methiocarb *		0.005	Not Detected	mg/kg
• Methomyl *		0.005	Not Detected	mg/kg
• Metolcarb *		0.005	Not Detected	mg/kg
• Oxamyl *		0.001	Not Detected	mg/kg
• Promecarb *		0.005	Not Detected	mg/kg
• Propoxur *		0.001	Not Detected	mg/kg
• Thiodicarb *		0.001	Not Detected	mg/kg
• XMC *		0.005	Not Detected	mg/kg
• Xylylcarb (MPMC) *		0.005	Not Detected	mg/kg
Organochlorine Group	In-house method CH-094-TM based on Journal of AOAC International Vol. 86, No. 2, 2003			
• 1,1-Dichloro-2,2-Bis (4-Ethylphenyl) Ethane (Perthane) *		0.2	Not Detected	mg/kg
• Acetochlor *		0.06	Not Detected	mg/kg
• Alachlor *		0.04	Not Detected	mg/kg
• Aldrin *		0.004	Not Detected	mg/kg
• Allethrin *		0.01	Not Detected	mg/kg
• Alpha-BHC *		0.004	Not Detected	mg/kg

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ORIGINAL

Report No. : CBL59-19294
Sample ID : 59-19294
Batch No. : JBL59/08988

Test Item	Method	MDL	Result	Unit
• Azaconazole *		0.02	Not Detected	mg/kg
• Benfluralin *		0.008	Not Detected	mg/kg
• Benoxacor *		0.016	Not Detected	mg/kg
• Beta-BHC *		0.008	Not Detected	mg/kg
• Bifenox *		0.016	Not Detected	mg/kg
• Boscalid *		0.001	Not Detected	mg/kg
• Bromophos *		0.003	Not Detected	mg/kg
• Bromophos-ethyl *		0.014	Not Detected	mg/kg
• Bromopropylate *		0.012	Not Detected	mg/kg
• Bupirimate *		0.04	Not Detected	mg/kg
• Butachlor *		0.06	Not Detected	mg/kg
• Captafol *		0.04	Not Detected	mg/kg
• Captan *		0.006	Not Detected	mg/kg
• Carfentrazone-Ethyl *		0.001	Not Detected	mg/kg
• Chlorbenside *		0.005	Not Detected	mg/kg
• Chlordane *		0.004	Not Detected	mg/kg
• Chlorfenapyr *		0.006	Not Detected	mg/kg
• Chlorfenson *		0.006	Not Detected	mg/kg
• Chloroneb *		0.04	Not Detected	mg/kg
• Chlorothalonil *		0.01	Not Detected	mg/kg
• Chlorthal-dimethyl *		0.006	Not Detected	mg/kg
• Chlozolinate *		0.004	Not Detected	mg/kg
• Cinidone-ethyl *		0.002	Not Detected	mg/kg
• Clomeprop *		0.005	Not Detected	mg/kg
• Coumafos/Coumaphos *		0.01	Not Detected	mg/kg
• DDD *		0.006	Not Detected	mg/kg
• DDE *		0.004	Not Detected	mg/kg
• DDT *		0.006	Not Detected	mg/kg
• Delta-BHC *		0.006	Not Detected	mg/kg
• Di-allate *		0.01	Not Detected	mg/kg
• Dichlofluanid *		0.02	Not Detected	mg/kg
• Dichloran *		0.001	Not Detected	mg/kg
• Dichlormid *		0.012	Not Detected	mg/kg
• Diclocymet *		0.001	Not Detected	mg/kg
• Dicofol *		0.04	Not Detected	mg/kg
• Dieldrin *		0.005	Not Detected	mg/kg
• Dimethenamid *		0.01	Not Detected	mg/kg
• Dimethipin *		0.001	Not Detected	mg/kg
• Endosulfan Sulphate *		0.006	Not Detected	mg/kg
• Endosulfan-Alpha *		0.006	Not Detected	mg/kg
• Endosulfan-Beta *		0.006	Not Detected	mg/kg
• Endrin *		0.01	Not Detected	mg/kg
• Endrin aldehyde *		0.02	Not Detected	mg/kg
• Endrin ketone *		0.008	Not Detected	mg/kg
• Ethalfluralin *		0.008	Not Detected	mg/kg
• Etridiazole *		0.008	Not Detected	mg/kg
• Fenamidone *		0.1	Not Detected	mg/kg
• Fipronil *		0.008	Not Detected	mg/kg
• Flufenacet *		0.02	Not Detected	mg/kg

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№ 209129 BK

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ORIGINAL

Report No. : CBL59-19294
Sample ID : 59-19294
Batch No. : JBL59/08988

Test Item	Method	MDL	Result	Unit
• Fluthiacet-methyl *		0.001	Not Detected	mg/kg
• Folpet *		0.02	Not Detected	mg/kg
• Fthalide *		0.004	Not Detected	mg/kg
• Heptachlor *		0.006	Not Detected	mg/kg
• Heptachlor epoxide *		0.004	Not Detected	mg/kg
• Hexachlorobenzene *		0.002	Not Detected	mg/kg
• Isoxaflutole *		0.05	Not Detected	mg/kg
• Lindane (gamma-BHC) *		0.003	Not Detected	mg/kg
• Mefenpyr-diethyl *		0.016	Not Detected	mg/kg
• Methoxychlor *		0.01	Not Detected	mg/kg
• Myclobutanil *		0.09	Not Detected	mg/kg
• Nitrapyrin *		0.003	Not Detected	mg/kg
• Norflurazon *		0.02	Not Detected	mg/kg
• Oxabetrinil *		0.003	Not Detected	mg/kg
• Pentoxazone *		0.016	Not Detected	mg/kg
• Pyridaben *		0.003	Not Detected	mg/kg
• Pyridalyl *		0.002	Not Detected	mg/kg
• Pyrifenox *		0.002	Not Detected	mg/kg
• Quintozene *		0.004	Not Detected	mg/kg
• Tecnazene *		0.004	Not Detected	mg/kg
• Tetradifon *		0.008	Not Detected	mg/kg
• Thiazopyr *		0.02	Not Detected	mg/kg
• Thifluzamide *		0.008	Not Detected	mg/kg
• Tolylfluanid *		0.01	Not Detected	mg/kg
• Tri-Allate *		0.008	Not Detected	mg/kg
• Trichlamide *		0.001	Not Detected	mg/kg
• Triflumizole *		0.002	Not Detected	mg/kg
• Trifluralin *		0.008	Not Detected	mg/kg
• Vinclozolin *		0.008	Not Detected	mg/kg
Pyrethroid Group	In-house method CH-094-TM based on Journal of AOAC International Vol. 86, No. 2, 2003			
• Bifenthrin *		0.02	Not Detected	mg/kg
• Cyfluthrin *		0.04	Not Detected	mg/kg
• Cyhalothrin *		0.02	Not Detected	mg/kg
• Cypermethrin *		0.04	Not Detected	mg/kg
• Deltamethrin, Tralomethrin *		0.02	Not Detected	mg/kg
• Fenpropathrin *		0.02	Not Detected	mg/kg
• Fenvalerate *		0.02	Not Detected	mg/kg
• Flucythrinate *		0.004	Not Detected	mg/kg
• Fluvalinate *		0.01	Not Detected	mg/kg
• Permethrin *		0.04	Not Detected	mg/kg
• Pyrethrin *		0.03	Not Detected	mg/kg

*: This item is out of scope of accreditation.

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№ 209130 BK

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ORIGINAL

Report No. : CBL59-19294
Sample ID : 59-19294
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Exhibit I. Report of the Expert Panel

OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE STATUS (GRAS) OF A PURIFIED STEVIOL GLYCOSIDE EXTRACT

Introduction

An independent panel of experts, qualified by scientific training and experience to evaluate the safety of food and food ingredients was requested by Almendra (Thailand) Ltd to determine the safety and Generally Recognized As Safe (GRAS) status of a high-purity steviol glycoside mixture. The intended use of the steviol glycoside mixture is as a high-intensity non-nutritive general-purpose sweetener, the same as the existing GRAS uses of steviol glycoside sweeteners. The maximum uses and use levels would also be the same as the existing GRAS uses of steviol glycoside sweeteners.

A safety review based on the existing scientific literature on the safety of steviol glycosides (through October 2013) was conducted by ToxStrategies, Inc. and is summarized in their attached dossier. The safety of steviol glycosides has also been extensively reviewed in 27 GRAS Notifications (GRN) that FDA has responded to with "no questions" letters. The Expert Panel members reviewed the dossier prepared by ToxStrategies and other pertinent information and agreed to the conclusions described below.

Description

The Almendra high-purity steviol glycoside mixture consists of 65-75% rebaudioside A and 15-25% stevioside with a total steviol glycoside content of greater than 99.5%. All steviol glycosides contain a common "core" diterpene derivative known as steviol. Steviol glycosides are naturally occurring constituents of the plant *Stevia rebaudiana Bertoni*. Rebaudioside A has a molecular weight of 967.03 and consists of four glucose side chains around the steviol core. Stevioside consists of three glucose side chains with a molecular weight of 804.88. The conversion factors for converting rebaudioside A and stevioside to steviol equivalents are 0.33 and 0.40, respectively.

Manufacturing Process

The manufacturing (purification) process for steviol glycosides extracted from *S. rebaudiana* has been described in scientific publications and previous GRAS notifications submitted to FDA. Briefly, dry *S. rebaudiana* leaves are extracted in hot water and non-soluble material is removed via flocculation and filtration. The aqueous extract is passed through a resin, which is then eluted with ethanol. A semi-purified stevia extract powder containing approximately 90% steviol glycosides is then obtained via filtration and spray drying.

The final purified steviol glycoside ingredient is obtained by dissolving the semi-purified product in heated ethanol followed by crystallization and centrifugation or filtration.

The resulting powder consists almost entirely of steviol glycosides, which meet or exceed JECFA and Almendra specifications.

Functionality

Stevia has been used as an herbal sweetener in several countries, mostly in Asia, for many years (Carakostas et al., 2012). Purified steviol glycosides have been approved as a general-purpose sweetener in the EU and in over an additional 20 individual countries. Steviol glycosides have also been recognized as general-purpose sweeteners in 27 GRNs listed on FDA's website. Sweetness potency and temporal profile of rebaudioside A clearly show its high-intensity sweetness functionality (Prakash et al., 2008). Rebaudioside A is approximately 200 times sweeter than sucrose and approximately as sweet as aspartame when tested at a 6% sucrose equivalency in water.

Currently, the most common commercially available source of purified steviol glycosides is tabletop sweetener products. However, steviol glycosides have been successfully tested in a large number of foods and beverages (Prakash et al., 2008). Steviol glycosides are stable in a wide variety of food applications including those where it is exposed to baking temperatures.

Intended Use and Intake Assessment

As a high-intensity sweetener, use levels of steviol glycosides are limited by organoleptic factors. Due the similar sweetness intensity compared to aspartame, steviol glycosides will often be used at similar levels in foods and beverages as aspartame. Typical rebaudioside A concentrations in various foods and beverages have been estimated by Prakash et al. (2008). The Almendra steviol glycoside product will be used as a general purpose sweetener for foods and beverages in the same manner as their previous steviol glycoside product described in GRN No. 461.

Intake assessments for steviol glycosides have been largely based on the assumption that direct replacement of other high-intensity sweeteners was the most accurate basis for exposure. Renwick (2008) reviewed exposure to steviol glycosides by using intake information for aspartame and other high intensity sweeteners from a variety of published sources. The results of Renwick's (2008) assessment predicted average intakes of rebaudioside A for children and adults of 0.43 and 0.70 mg steviol equivalents/kg body weight/day, respectively. High-percentile consuming adults and children were predicted to consume 1.12 and 1.65 mg steviol equivalents/kg body weight/day. Children with diabetes were predicted to consume 1.5 mg steviol equivalents/kg body weight/day.

JECFA used an assessment based on the total replacement of dietary sugar, which resulted in a predicted intake of steviol glycosides ranging from 0.9 mg/kg body weight/day in Africa to 7.1 mg/kg body weight/day on a steviol equivalent basis (Carakostas et al., 2008). JECFA considered it unlikely that steviol glycosides would completely replace sugar and estimated that a 20-30% replacement estimate was a more likely exposure.

Almost every regulatory authority that has reviewed steviol glycoside safety and exposure has generally accepted some version of the JECFA intake assessment and concluded that dietary exposure to steviol glycosides was less than 4 mg steviol equivalents/kg body weight/day for all population groups. The European Food Safety Authority (EFSA) used a different intake assessment model and concluded that high consuming children aged 1- 14 and adults could have an intake above the JECFA ADI of 4 mg/kg body weight/day. However, the intake assessment procedure used by EFSA is not widely accepted globally because it is considered excessively conservative.

Safety Data

The safety of purified steviol glycoside extracts has been extensively reviewed in several scientific publications including both journals and book chapters. In addition, the JECFA review, the written conclusions of several noted food regulatory authorities and reviews in 27 GRNs posted on FDA's website are also available to the public. All these assessments generally agree that steviol glycosides are safe for human consumption with a wide margin of safety. As detailed in the Almendra dossier, several food safety authorities initially had reservations about the safety of steviol glycosides because specifications were unclear and several open questions requiring scientific studies remained unanswered. When these issues were resolved, steviol glycoside safety has been widely accepted by food safety authorities around the world. Only the highlights of steviol glycoside safety will be outlined here.

Metabolism

All steviol glycosides are metabolized to a common core metabolite, steviol by bacteria in the lower intestinal tract. Several studies have shown that intact rebaudioside A and stevioside are not metabolized or absorbed in the stomach or small intestine of rats, pigs or humans. In rats, steviol is absorbed from the colon, converted to a glucuronide in the liver and re-excreted via the bile into the intestinal tract. Colonic bacteria de-conjugate the glucuronide and steviol is excreted in feces. In humans, steviol is also absorbed from the colon, conjugated in the liver to a glucuronide and then mostly excreted via the kidney in urine. The larger and more complex steviol glycosides are less likely to be completely metabolized to steviol by colonic bacteria and can be excreted unabsorbed in the feces.

The similar metabolism and pharmacokinetics of stevioside and rebaudioside A have led to the widely accepted conclusion that all steviol glycosides are metabolized in rats and humans via similar pathways. Safety studies conducted with one steviol glycoside have been accepted as applicable to all steviol glycosides.

Subacute Toxicity

Results of a 28-day study reported by Nikiforov (2013) on rebaudioside D are outlined in the Almendra dossier. No adverse effects were reported in male or female rats fed a diet containing rebaudioside D at levels up to 2000 mg/kg/day. Curry and Roberts (2008) also reported results from a 4-week study in Wistar rats fed diets containing purified

rebaudioside A at doses up to 100,000 ppm in the diet, equivalent to doses of over 14,000 mg/kg/day. No toxicologically significant effects were observed in any treated group, but body weights were reduced in rats fed 50,000 ppm and above due to a reduction in caloric value of the diet caused by the presence of the non-caloric sweetener. Some unusual physiological effects on osmotic homeostasis were observed in the higher dose groups due to the extreme doses. Most importantly though was the finding that male reproductive organs were unaffected by even extreme doses of rebaudioside A.

Subchronic Toxicity

Several 90-day studies in rats and a six-month study in dogs given steviol glycosides in their diet have been reported. Some body weight effects caused by the reduced caloric value of the diet at higher doses were noted, but no toxicologically significant findings have been reported. Curry and Roberts (2008) have reported the highest NOEL in a 90-day rat study of >4,000 mg/kg/day.

Reproduction and Developmental Toxicity

Reproduction toxicity concerns were among several issues that prevented the approval of steviol glycoside sweeteners in many countries for years. This was partly due to the publication of several studies using crude or poorly characterized stevia extracts that reported adverse reproduction system effects. However, there were also other contemporary publications of reproduction safety studies that reported no adverse reproduction safety effects. Curry et al. (2008) reported on a two-generation reproduction safety study in rats conducted according to then current international guidelines for such studies. They reported no adverse effects on a wide range of reproduction endpoints across two-generations of offspring. In addition, unpublished reports in GRN 252 for a two-generation reproduction safety study and a developmental safety study in rats also showed no adverse effects in rats at rebaudioside A doses of up to 2000 mg/kg/day. A second unpublished developmental safety report was cited in the EFSA evaluation. No adverse effects on developing offspring were reported in NZW rabbits at doses up to 1400 mg/kg/day.

Mutagenicity and Genotoxicity

There is a long history of controversy about the genotoxicity of steviol glycosides, which has been extensively reviewed. Details of these issues are outlined in the Almendra dossier. Steviol glycosides have been consistently shown to be non-genotoxic across a wide range of in-vitro and in-vivo assays. Steviol has some potential to show genotoxicity in a few specific genotoxicity assays that are considered unsuitable for predicting risk to humans. In addition, systemic exposure to steviol in humans is extremely limited as absorbed steviol is quickly converted to a glucuronide in the liver.

Chronic Toxicity and Carcinogenicity

The Almendra dossier outlines the results from three chronic bioassays conducted with stevioside in rats, the most important of which is the publication by Toyoda et al. (1997). The Toyoda study used a steviol glycoside extract containing predominantly stevioside but which met current JECFA specifications for purity. Tumors reported in male and female F344 rats in this study were typical of the background incidence of neoplasia in

this strain of rat. The authors concluded that dietary exposure to stevioside at levels up to 5% were not carcinogenic under the conditions of the study. However, Toyoda et al. (1997) did note that rats given a diet containing 5% stevioside did show reduced body weight and reduced survival rates at study termination, which they considered to be a treatment-related effect. The NOAEL for the study was reported to be 2.5% stevioside in the diet (equivalent to 970 mg/kg/day). As the longest study, this NOAEL became the basis for the JECFA ADI of 4 mg/kg/day after the application of a 100-fold safety factor and conversion of the stevioside NOAEL to steviol equivalents. Contrary to other published studies where reduced body weight was considered not to be a treatment-related effect by study authors, peer reviewers and regulatory authorities, the Toyoda et al. (1997) study was very conservative in its conclusions, which has driven a very conservative ADI.

Clinical Studies

JECFA and other food safety authorities expressed concerns about the potential for steviol glycosides to unexpectedly reduce blood pressure or adversely affect subjects with Type II diabetes for many years, which prevented the development of a JECFA ADI. While several clinical studies published prior to late 2008 provided partial answers, two clinical studies meeting the full requirements of JECFA were reported by Maki et al. (2008a; 2008b) and resolved these questions. No effects on blood pressure were observed in individuals with normal, low-normal or low blood pressure given 1000 mg of rebaudioside A per day for 4 weeks. In a separate study, no adverse effects on glucose homeostasis or blood pressure were observed in normal or diabetic subjects given 1000 mg of rebaudioside A per day for 16 weeks.

Some concerns have been expressed about the potential of all high-intensity sweeteners to promote weight gain through an effect on gastrointestinal hormones that reduces satiety and promotes increased caloric consumption. Anton et al. (2010) compared a commercial stevia product (probably a mixed purified steviol glycoside) with aspartame. No toxicologically important endpoints were evaluated in the study, though some differences in post-meal glucose and insulin levels between subjects given a sucrose or aspartame pre-load and stevia were observed.

Conclusions of the Expert Panel

We, the undersigned members of the Expert Panel, have individually and collectively critically reviewed the published and ancillary information pertinent to the identification, use and safety of a purified steviol glycoside extract. We conclude that this purified steviol glycoside extract produced by Almendra under the conditions described in the attached dossier and meeting JECFA and Almendra specifications is safe.

We also conclude that the intended use of this purified steviol glycoside extract, meeting the specifications described above, as a general purpose high-intensity sweetener is Generally Recognized As Safe (GRAS) based on scientific procedures.

_____ _____

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SUBMISSION END