

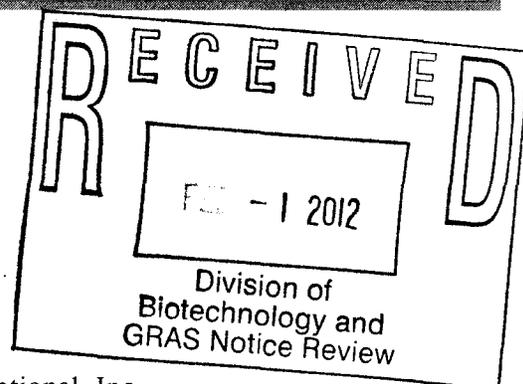
ORIGINAL SUBMISSION

Product Solutions Research, Inc.

1477 Drew Avenue, Suite 102
Davis, CA 95618, USA
Phone: 530.758.0080
E-Mail: sangeetais@gmail.com

January 31, 2011

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



Re: GRAS Notification for SWEETN' UP™ Stevia submitted by MiniStar International, Inc.

Dear Sir/Madam:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), MiniStar International, Inc., through Product Solutions Research as its agent, hereby provides notice of a claim that the food ingredient Rebaudioside A preparation (SWEETN' UP™) described in the enclosed notification document 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.

As required, please find enclosed three copies of the notification. We have also included an electronic copy of this document on a CD which is in PDF format for your further reference.

The data and information that are the basis for the notifier's GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at reasonable times at a specific address set out in the notice or will be sent to FDA upon request. Please feel free to contact me by phone at 530.758.0080 or by email at sangeetais@me.com.

Thank you.

Sincerely,

(b) (6)



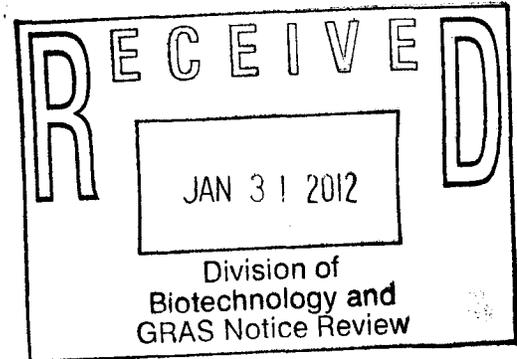
Sangeeta Patel, PhD
Chief Scientific Officer
Product Solutions Research, Inc.

Agent for:

MiniStar International, Inc.
21118 Commerce Pointe Drive
City of Industry, CA 91789, USA

Product Solutions Research
PO Box 4013
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productsolutionsis@gmail.com
Date January 27, 2012

Ms Moraima Ramos-Valle
Food and Drug Administration
University Station (HFS-200)
4300 River Road
College Park MD 20740
T: 301-436-1248
E: moraima.ramos-valle@fda.hhs.gov



Dear Ms. Ramos,

This letter is a follow up to your phone call on January 26th 2012. Any additional information, including data regarding SWEETN' UP brand stevia on behalf of Ministar International is available for your review to support this determination. I can be reached by phone at (530) 758.0080 or email at sangeetais@me.com

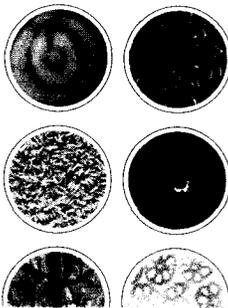
Respectfully,

(b) (6)



Sangeeta Patel PhD

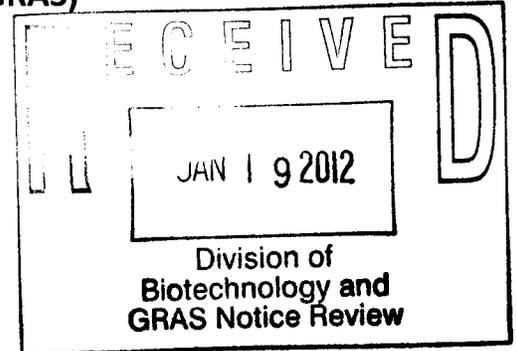
PRODUCT SOLUTIONS RESEARCH



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**NOTICE TO THE U.S. FOOD AND DRUG ADMINISTRATION THAT THE USE
OF SWEETN' UP™ STEVIA - REB A 98% DERIVED FROM
STEVIA REBAUDIANA AS A FOOD INGREDIENT IS
GENERALLY RECOGNIZED AS SAFE (GRAS)**



SUBMITTED BY:

MiniStar International, Inc.
21118 Commerce Pointe Drive
City of Industry, CA 91789 U.S.A.

PREPARED BY:

Product Solutions Research, Inc.
1477 Drew Avenue, Suite 102
Davis, CA 95618 U.S.A.

January 12, 2012

**NOTICE TO THE U.S. FOOD AND DRUG ADMINISTRATION THAT THE USE
OF SWEETN' UP™ STEVIA - REB A 98% DERIVED FROM
STEVIA REBAUDIANA AS A FOOD INGREDIENT IS
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SUBMITTED BY:

MiniStar International, Inc.
21118 Commerce Pointe Drive
City of Industry, CA 91789 U.S.A.

PREPARED BY:

Product Solutions Research, Inc.
1477 Drew Avenue, Suite 102
Davis, CA 95618 U.S.A.

January 12, 2012

**GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%**

Product Solutions Research, Inc.

1477 Drew Avenue, Suite 102
Davis, CA 95618, USA
Phone: 530.758.0080
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January 03, 2011

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

Re: GRAS Notification for SWEETN' UP™ Stevia submitted by MiniStar International, Inc.

Dear Sir/Madam:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), MiniStar International, Inc., through Product Solutions Research as its agent, hereby provides notice of a claim that the food ingredient Rebaudioside A preparation (SWEETN' UP™) described in the enclosed notification document 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.

As required, please find enclosed three copies of the notification. We have also included an electronic copy of this document on a CD which is in PDF format for your further reference. If you have any questions or require additional information, please feel free to contact me by phone at 530.758.0080 or by email at sangeetais@me.com.

Thank you.

Sincerely,

(b) (6)



Sangeeta Patel, PhD
Chief Scientific Officer
Product Solutions Research, Inc.

Agent for:

MiniStar International, Inc.
21118 Commerce Pointe Drive
City of Industry, CA 91789, USA

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

As defined herein, MiniStar International, Inc. has determined that Sweetn' Up™ Rebaudioside A (Reb A) ≥ 98% derived from *Stevia rebaudiana* (Bertoni, 1899) is Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the Federal Food, Drug and Cosmetic Act. This determination, made by experts qualified by scientific training and experience, is based on scientific procedures as described in the following sections, under the conditions of its intended use in food. Therefore, the use of Sweetn' Up™ Reb A ≥ 98% is exempt from the requirement of premarket approval.

(b) (6)

Signed: _____

Philip Bromley
Director of Scientific Affairs
MiniStar International, Inc.

Date: _____

01/21/12

B. Name and Address of Notifier

MiniStar International, Inc.,
21118 Commerce Pointe Drive,
City of Industry, CA 91789

MiniStar International, Inc. (MiniStar) accepts responsibility for the GRAS determination that has been made for the Reb A product (Sweetn' Up™) as described in this notification.

C. Name of GRAS Substance

Rebaudioside A derived from *Stevia rebaudiana* (Bertoni). The trade name of the substance is Sweetn' Up™.

D. Conditions of Use

Sweetn' Up™ preparation, primarily containing Reb A (≥ 98%), is intended to be added as a general purpose non-nutritive sweetener into various food categories, other than in

¹See 62 FR 18938 (17 April 1997) which is accessible at:

<http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm>

**GRAS Assessment – MINISTAR International, Inc.
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practices (cGMP) in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect and such that intakes are consistent with the acceptable daily intake (ADI) of 0 – 4 mg/kg body weight/day of steviol glycosides (as steviol equivalents) established in 2008 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Food Standards Australia New Zealand (FSANZ).

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, Reb A ≥ 98% preparation (Sweetn' Up™) derived from *Stevia rebaudiana* intended for use in foods by MiniStar International, Inc., has been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below. This determination is based on sufficient qualitative and quantitative scientific evidence, including a variety of animal studies and human clinical studies, as well as *in vitro* studies further corroborating its safety.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be available for review and copying at reasonable times at the office of Product Solutions Research, Inc., 1477 Drew Avenue, Suite 102, Davis, CA 95618, U.S.A.

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II. BACKGROUND

A. Purpose

At the request of MiniStar International, Inc. (MiniStar), Product Solutions Research, Inc. has undertaken an independent safety evaluation of MiniStar's stevia preparation that will be marketed as Sweetn' Up™. Sweetn' Up™ is composed of Reb A (≥98.0%). The objective of this safety assessment is to determine the intended food uses of Sweetn' Up™ as a general-purpose non-nutritive sweetener is Generally Recognized As Safe, i.e., GRAS, under the intended conditions of use.

B. Preface

For the present GRAS assessment, MiniStar provided substantial background information to Product Solutions Research, Inc. to undertake this process. In particular, the information provided addressed the safety and toxicity of steviol glycosides; the history of use of stevia in food; and compositional details, specifications, and method of manufacturing of Sweetn' Up™. MiniStar was asked to provide adverse reports, as well as those that supported conclusions of safety. The composite safety/toxicity studies, in concert with exposure information, were used in the present GRAS determination. Additionally, an independent search of the scientific and regulatory literature extending through January 3, 2012 was performed and used for the GRAS assessment. The references that were considered relevant to the objective are discussed in Section VIII.

C. Regulatory History of Steviol Glycosides

In the United States, steviol glycosides have been used as a dietary supplement since 1995 and later Reb A was made available (December 2008) as a food additive (sweetener). As a sweetener, steviol glycoside preparations are available under multiple trade names including: Only Sweet, PureVia, Reb-A, Rebiana, SweetLeaf, and Truvia. As of January 3, 2012, FDA has received approximately 15 GRAS notifications seeking authorization for the addition of stevioside or steviol glycosides as a food ingredient; with the FDA issuing "no questions" letters in each case.

In October 2008, the Food Standards Australia New Zealand (FSANZ) completed evaluation of an application for use of all steviolglycosides as a food additive and has recommended to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) to amend the Australia New Zealand Food Standards Code to allow its use in food (FSANZ, 2008). In August 2008, Switzerland's Federal Office for Public Health cited the favorable actions of JECFA in issuing its approval for the use of stevia as a sweetener (Switzerland Office of Public Health, 2008).

In the European Union, the European Food Safety Authority (EFSA) gave a positive safety assessment for steviol glycosides in April 2010 (EFSA 2011). The Directorate General for

GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%

Health and Consumers (DG Sanco) stated in June 2011 that stevia is proposed for approval by the EC in November or December of 2011 (Byrne, 2011). The Joint Expert Committee on Food Additives (JECFA) has also reviewed steviol glycosides at several of its biannual meetings. In 2006, at its 63rd meeting, JECFA established a temporary ADI (acceptable daily intake) of 0 – 2 mg/kg (on a steviol basis) and subsequently based on review of additional studies in 2008 the ADI was raised to 0 – 4 mg/kg bw/day (on a steviol basis) (WHO, 2008). Recently, in 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009) for its uses in food. In the past decade, approvals for steviol glycosides for use as a food additive (sweetener) have been received in France (2009), Mexico (2009), Russian Federation (2008), Australia/New Zealand (2008), Switzerland (2008), Japan (2010) and the US (2008).

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III. INFORMATION ABOUT THE IDENTITY OF REBAUDIOSIDE A AND SWEETN' UP™

A. Common Name and Source Material

The common or usual name for the product is Reb A, a steviol glycoside naturally found in the plant *Stevia rebaudiana*. Sweetn' Up™ consisting of Reb A (≥ 98% purity) is the commercial name used by MiniStar in referring to the notified substance. In the scientific literature, steviol glycosides have been referred to as stevia, stevioside, steviol glycosides, and stevia glycoside. JECFA adopted the term, steviol glycosides, for the family of steviol derivatives with sweetness properties that are derived from the stevia plant. Presently, the term “stevia”, is used more narrowly to describe the plant or crude extracts of the plant, while stevioside is the common name for another one of the specific glycosides that is extracted from stevia leaves.

S. rebaudiana is a perennial shrub of the Compositae family, known from northeastern Paraguay, Brazil, and other South American regions for over 1500 years (Geuns, 2003a,b,c; Ferlow, 2006). The leaves of *S. rebaudiana* contain at least 10 different steviol glycosides: stevioside, rebaudioside A, B, C, D, E, and F, dulcoside A, rubusoside, and steviolbioside. Rebaudioside A accounts for approximately 2 to 4% of the plant leaf composition by weight (SCF, 1999a,b).

B. Description

Rebaudioside A (≥ 98% purity) manufactured by MiniStar is a white fine powder with characteristic odor. The product description is similar to that described by Food Chemical Codex (FCC) and JECFA. Recently, FCC (2009) published a monograph on Reb A with its description and specifications. As per FCC, Reb A is a white to off-white, hygroscopic fine crystal, granule, or powder with a sweet taste. It is freely soluble in ethanol:water 50:50 (v/v) and is sparingly soluble in water and in ethanol. Rebaudioside A is obtained from the leaves of the *Stevia rebaudiana* plant in a multistep separation and purification process. As described in FCC, principle steps of manufacturing include extraction of steviol glycosides from the leaves using an aqueous or aqueous alcoholic (ethanol or methanol) solvent, and purification of Reb A from the resulting mixture of steviol glycosides by resin absorption followed by recrystallization from an aqueous or aqueous alcoholic (ethanol or methanol) solvent. It is primarily composed of Reb A, a glycoside of the *ent*-kaurenoide diterpenoid aglycone known as steviol (FCC, 2009).

As per JECFA (FAO, 2010), steviol glycosides are described as a white to yellow powder, odorless to having a slight characteristic odor, and exhibiting a sweetness that is 200 – 300 times greater than sucrose. The ingredient must consist of a minimum of 95% of 9 specific steviol glycosides. The steviol glycosides are freely soluble in water and ethanol, and the 1 in 100 solutions exhibit pH values ranges from 4.5 to 7.0.

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Sweetn' Up™ - Stevia Reb A ≥ 98%

Of the nine different steviol glycosides, the two principal sweeteners of stevia extracts have been identified as Reb A and stevioside. The chemical information and structures are shown below (Table 1a, 1b; Figure 1).

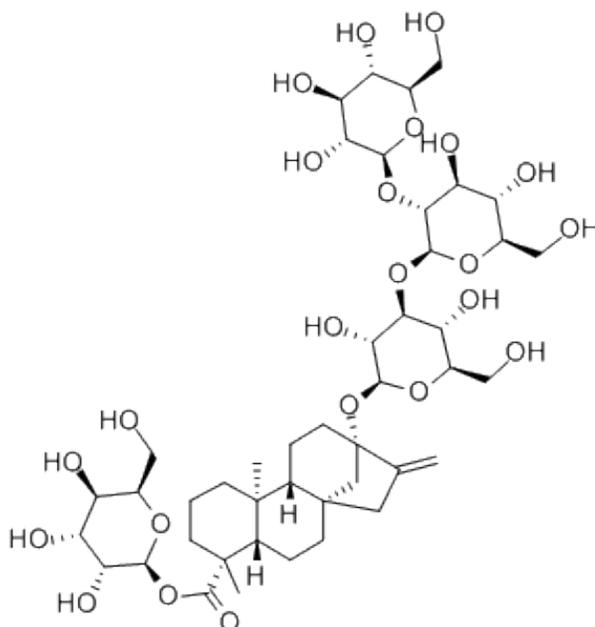
Table 1a. Chemical Information of Rebaudioside A

Chemical Name:	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-p-D-glucopyranosyl) oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
Synonyms:	(4R)-13-[(2-O-β-D-Glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid β-D-glucopyranosyl ester; (4α)-13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]kaur-6-en-8-oic acid β-D-glucopyranosyl ester
Chemical Formula:	C ₄₄ H ₇₀ O ₂₃
Formula Weight:	967.03
CAS Registry Number:	58543-16-1

Table 1b. Chemical Information of Stevioside

Chemical Name:	13-[2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
Synonyms:	glucopyranosyl ester; (4α)-β-D-glucopyranosyl 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oate
Chemical Formula:	C ₃₈ H ₆₀ O ₁₈
Formula Weight:	804.88
CAS Registry Number:	57817-89-7

Figure 1. Chemical Structure of Rebaudioside A



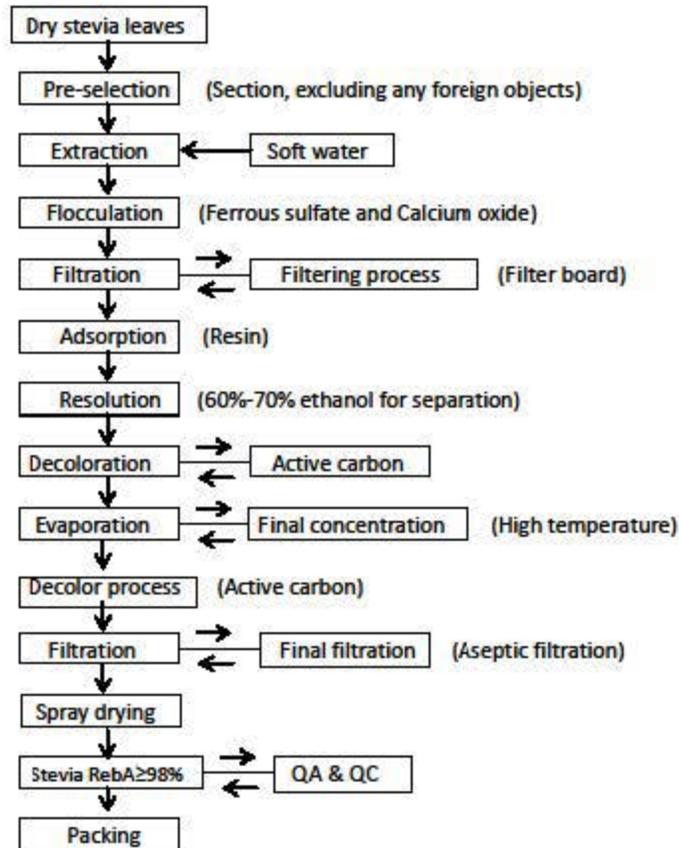
C. Manufacturing Process

The flow-scheme diagram in Figure 2 describes an overview of MiniStar's Reb-A manufacturing process. All chemical reagents used in the process, the adsorption resin and the ion exchange resins are food grade. Raw materials for the production of steviol glycosides are the leaves of non-genetically modified cultivars of *S. rebaudiana*. Leaves from selected varieties of stevia plants are used for Reb A production. In brief, Reb A is obtained through aqueous extraction and multiple purification steps. *S. rebaudiana* leaves are dried, crushed and subjected to extraction with hot water. Ferrous sulfate and calcium oxide are added to the extract solution to precipitate plant substances such as pectin and pigment, followed by filtration steps. The filtrate is passed through adsorption resin to trap the steviol glycoside components. Subsequently, the glycosides adsorbed on the resin are eluted with ethanol. The ethanol extract is desalted by passing through ion exchange resin and decolorized using active carbon. The extract is evaporated at high temperature. The concentrate is decolorized a second time using active carbon and spray dried. The content of the Reb A in the final MiniStar product is ≥ 98%.

The ethanol and other processing aids (ferrous sulfate, calcium oxide, and active carbon) used in the purification process complies with FCC 5th Edition specifications, and the ion exchange resins used in the manufacturing comply with 21 CFR 173.25. The MiniStar Reb A is prepared in accordance with current Good Manufacturing Practices (cGMP) at Qufuxiangzhou Stevia Products Co., Ltd., No. 11, Yulong Road, Qufu, China, 273100.

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Figure 2. Production Flow Chart for Manufacturing Stevia Rebaudioside A ≥ 98%



D. Product Characteristics

1. Specifications for MiniStar International, Inc.'s Sweetn' up™

MiniStar has established food grade specifications for its Reb A product (Sweetn' Up™) that meet or exceed JECFA recommendations which also comply with FCC (2009) specifications for Reb A.

A comparison of the specifications provided by MiniStar for five batches is summarized in Table 2. This data demonstrate that the MiniStar's specifications for Sweetn' Up™ meet both the FCC and JECFA specifications. Consistency of Reb A manufacturing has been demonstrated by analyses of five batches of Reb A and Certificates of Analysis from the vendor (see Appendix A). Heavy metal analysis for three batches is included from the vendor (Appendix B).

Pesticide analysis was conducted, and none of the pesticides or their residues were detected in the samples (Appendix C). In addition, MiniStar's Stevia Reb A manufacturer, Qufuxiangzhou Stevia Products Co., Ltd., (Qufu, China), received National Organic Program (NOP) certification (Certificate No. 9149) with the Certification of Environmental Standards GmbH (CERES) under the scope of Processing and Trade. NOP Certificate was also included in Appendix C. NOP certification is in accordance with the European Regulation (EC) 834/07, the US National Organic Program (NOP), and CERES standard interpretation, and shows that MiniStar's stevia extract (steviol glycosides Reb A) are certified organic under the US National Organic Program 7 CFR Part 205, and that this certification applies only to the organic mode of production.

Detailed identity-related analyses for Reb A and other steviol glycosides are presented in Appendix D.

a. Sweetn' Up Steviol Glycosides and JECFA Specifications

Expanded JECFA 2010 specifications of steviol glycoside is provided in Appendix E. Steviol glycoside food grade specifications prepared at the 73rd JECFA meeting in 2010, recommended that a minimum requirement of 95% of the total of the 9 steviol glycosides on a dried weight basis. The 9 steviol glycosides are: stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside, and steviolbioside. Rebaudioside A and stevioside are major components of interest because of their sweetening property. The other 7 glycosides found in preparations of steviol glycosides accepted by the JECFA specifications with the 95% requirement are typically found at much lower levels than stevioside or rebaudioside A. Previously in 2008, JECFA specifications for steviol glycosides, excluded rebaudioside D and rebaudioside F (FAO, 2008).

GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%

Table 2. JECFA and FCC Specifications and Batch Analysis for Sweetn' Up™

Parameter	JECFA Specifications Steviol Glycosides ¹	FCC Specifications Rebaudioside A	Batch No.				
			20110118	20110213	20110308	20110421	20110512
Appearance	White to light yellow	—	White fine powder				
Odor	Odorless or slight characteristic	—	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Solubility	Freely soluble in water	Freely soluble in water:ethanol (50:50)	Freely soluble in water:ethanol				
Chemical Tests							
Total Steviol Glycosides(% dry basis)	Not less than 95%	Not more than 5% ²	99.18%	99.21%	99.28%	99.17%	99.23%
Rebaudioside A	NA	Not less than 95 %	98.15%	98.08%	98.25%	98.12%	98.15%
Loss on Drying	Not more than 6% (105°C, 2h)	Not more than 6%	2.53%	2.61%	2.72%	2.63%	2.68%
Ash	Not more than 1%	Not more than 1%	0.02%	0.03%	0.02%	0.02%	0.03%
pH (1% solution)	Between 4.5 and 7.0	Between 4.5 and 7.0	6.0	6.0	6.0	6.0	6.0
Lead	Not more than 1 mg/kg	Not more than 1 mg/kg	<1 ppm				
Arsenic	Not more than 1 mg/kg	Not more than 1 mg/kg	<1 ppm				
Residual solvents							
Methanol	Not more than 200 mg/kg	Not more than 0.02%	< 100 ppm				
Ethanol	Not more than 5000 mg/kg	Not more than 0.5%	< 500 ppm				
Microbiological Data							
Total Plate Count (cfu/g)	NA	NA	< 1000	< 1000	< 1000	< 1000	< 1000
Coliform (cfu/g)	NA	NA	Negative	Negative	Negative	Negative	Negative
Yeast & Mold (cfu/g)	NA	NA	Negative	Negative	Negative	Negative	Negative
Salmonella (cfu/g)	NA	NA	Negative	Negative	Negative	Negative	Negative
Staphylococcus (cfu/g)	NA	NA	Negative	Negative	Negative	Negative	Negative

¹Specifications prepared at the 73rd JECFA (2010) meeting and published in FAO/JECFA Monographs 10 (2010); ²Excludes Reb A but includes additional two glycosides Reb D and Reb F; NA = Not Applicable.

GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%

In accordance with 2010 JECFA specifications on steviolglycosides, MiniStar's highly purified Sweetn' Up™ is comprised of 99.6% (w/w) Reb A, 0.437% (w/w) stevioside and small amounts of other glycosides, exceeding total steviolglycosides composition compared to those in Merisant and Cargill materials.

2. Stability Data

As described in multiple GRAS notices submitted to FDA, the stability of Reb A in several food products has been supported and confirmed by a series of studies. Chang and Cook (1983) investigated the stability of pure stevioside and Reb A in carbonated phosphoric and citric acidified beverages. Although these investigators noted some degradation of stevioside and Reb A after 2 months of storage at 37° C, no significant change at room temperature or below was noted following 5 months of storage of stevioside and 3 months of storage of Reb A. Exposure to 1 week of sunlight did not affect stevioside, but resulted in approximately 20% loss of Reb A. Heating at 60° C for 6 days resulted in 0 – 6% loss of Reb A. In another study, Kinghorn and Soejarto (1985) reported that stevioside is a stable molecule over the pH range 3 – 9 and can be heated at 100° C for 1 hour. However, a rapid decomposition was noted at pH levels greater than 9 under these conditions. These investigators suggested that at pH 10 steviolbioside would be the major decomposition product.

As described in its GRAS notification to FDA, Merisant (2008) conducted experiments with Reb A: (1) as a powder, (2) as a pure sweetener in solution, and (3) on both cola-type and citrus carbonated beverages. The results of these experiments did not reveal any degradation when the powder was stored at 105° C for 96 hours. It was concluded that the powder was stable when stored for 26 weeks at 40 ± 2° C with relative humidity of 75 ± 5%. Both published and unpublished testing results from Merisant revealed that Reb A in carbonated citric acid beverages and phosphoric acid beverages did not significantly degrade during prolonged storage at refrigeration, normal ambient, or elevated ambient temperatures. Minimal loss of Reb A was detected after storage at 60° C, with considerable degradation noted after 13 hours at 100° C for carbonated beverage solutions and pure sweetener solutions (Merisant, 2008).

As described in its GRAS notification to FDA, Cargill (2008) extensively studied stability of Reb A as a powder under various storage conditions and under a range of pH and temperatures, and in several representative food matrices at room and elevated temperatures. Stability was tested for tabletop sweetener applications, mock beverages including cola, root beer and lemon-lime, thermally processed beverages, yogurt and white cake. The results of these investigations revealed some degradation products that were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana*. All the degradation products were found to share the same steviol aglycone backbone structure, but differed by virtue of the glucose moieties present. The results of stability testing suggest that Reb A is stable in various food matrices. The extent and rate of degradation is dependent on pH, temperature, and time. When

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placed in beverages, Reb A is most stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C.

Ministar's purified Reb A ≥98% is comparable in composition to the Merisant and Cargill materials, and the stability characteristics are expected to be equivalent to that exhibited by the available studies. Thus, the high purity Reb A products from MiniStar are well-suited for the intended food uses as described in this monograph.

IV. PROPOSED FOOD USES

A. Food Categories and Intended Use Levels

MiniStar International, Inc. intends to market Sweetn' Up™ for incorporation into various food categories as a general purpose sweetener and in various foods other than infant formulas and meat and poultry products. The intended uses and use levels for rebaudioside A are similar to those for similar products considered to be GRAS. Sweetn' Up™ will function as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19) which states "Non-nutritive sweeteners: Substances having < 2% of the caloric value of sucrose per equivalent unit of sweetening capacity". The use levels will vary by food category, but the levels are self-limiting due to organoleptic characteristics and consumer taste considerations. However, the amounts of Sweetn' Up™ to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation (see 21 CFR 182.1(b)(1)). The intended use levels of purified Reb A by MiniStar International, Inc. and the food categories in which it is used are identical to those described by Cargill (2008).

B. Food Uses Described by JECFA, FSANZ and in GRAS notices

JECFA and FSANZ reviewed estimates of possible consumption of mixed steviol glycosides. Additionally, Merisant and Cargill estimated the consumption of rebaudioside A in their GRAS notices to the FDA. Merisant listed expected levels of use for Reb A largely based on food consumption survey data from the 2003 – 2004 National Health and Nutrition Examination Survey (NHANES) for various food applications in their GRAS Notification. On a per user basis, the mean and 90th percentile daily consumption of Reb A was estimated at 2.0 and 4.7 mg/kg bw/day, respectively.

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Table 3. JECFA Reported Food Uses of Steviol Glycosides with Calculated Steviol Equivalents

Food Category	Maximum Use Level Reported/Calculated		
	Steviol Glycosides* (mg/kg food)	Rebaudioside A [#] (mg/kg food)	Rebaudioside A [#] (mg Steviol equivalents/kg of food)
Desserts	500	250	83
Cold Confectionary	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

*Report derived from WHO, 2006.

[#]Calculated for MiniStar product assuming twice the sweetness intensity for Reb A and 3-fold difference in molecular weight between rebaudioside A and steviol. Adapted from GRAS notice 278.

C. Estimated Daily Intake

MiniStar intends to incorporate Sweetn' Up™ into the same foods and at levels proportional to those mentioned in previous FDA GRAS notices, particularly those by Merisant and Cargill. As both notices were reviewed by the FDA, it is likely that the FDA considered cumulative intake from both notices. There are no new food uses proposed for Sweetn' Up™. The substance mentioned in Merisant and Cargill's GRAS notices have been reported to contain ≥ 98% Reb A, while the subject of present GRAS determination also contains ≥ 98% Reb A. The application of Reb A to the same foods and at the same levels is not expected to notably affect the intake of Reb A in the diet of the public from introduction into the market by another supplier who will have to compete in essentially the same market and foods.

In some of the FDA GRAS notices that were submitted subsequent to Merisant and Cargill, consumer intake estimates provided by JECFA (Table 4) were used to measure the potential human exposures of the subject steviol glycosides in foods as reported in the US and in other countries. As Reb A is about twice as sweet as the mixed glycosides, these levels can be adjusted accordingly. MiniStar International, Inc. intends to use Reb A in a number of food categories at levels that comply with GMP uses. Based on the above and considering the JECFA estimates on a per user basis, the mean daily consumption of Reb A has been estimated to be 2.0 mg/kg bw/day, and that for the 90th percentile consumer it is estimated to be 4.7 mg/kg bw/day.

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Table 4. Summary of FDA GRAS Notices on Rebaudioside A

GRAS Notice No.	Notifier	Intended use	Estimated Daily Intake (90th Percentile)	Safety Assessment Basis
GRN 252	Whole Earth Sweetener Company, LLC (Chicago, IL)	As a sweetener in a variety of food categories, and as a table top sweetener, formulated to provide 30 mg of rebaudioside A per gram finished product	5 mg/kg bw/day	Scientific Procedures*
GRN 253	Cargill, Inc. (Wayzata, MN)	General-purpose sweetener in foods, at levels determined by current good manufacturing practices	3.4 mg/kg body weight/day for children with diabetes. Predicted intakes for heavy intake consumers ranged from 3.4 mg/kg bw/day for non-diabetic adults to 5.0 mg/kg bw/day for non-diabetic children.	Scientific Procedures*
GRN 275	McNeil Nutritionals, LLC (Fort Washington, PA)	As a tabletop sweetener, including packet, tablet and granular powder form	McNeil based EDI on two estimates – MRCA ¹ :50 mg/d and 18 mg/d (steviol equivalents) NHANES ² : 98 mg/d and 35 mg/d (steviol equivalents)	Scientific Procedures*
GRN 278	Blue California (Rancho Santa Margarita, CA)	As a sweetener in baked products, beverages (non-alcoholic), breakfast cereals, coffee and tea, confections and frostings, dairy product analogs, fats and oils, frozen dairy desserts, fruit and water ices, milk (whole and skim), milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, snack foods, soft candy, sugar substitutes, sweet sauces, toppings and syrups, meal replacement food products, and medical foods, as well as table top sweetener	Range of 1.3 – 4.7 mg/kg bw/day	Scientific Procedures*

Table 4. Summary of FDA GRAS Notices on Rebaudioside A cont.

GRAS Notice No.	Notifier	Intended use	Estimated Daily Intake (90 th Percentile)	Safety Assessment Basis
GRN 282	Sweet Green Fields (Bellingham, WA)	As a general-purpose sweetener in a variety of food products, including cereals and energy bars, and diet soft drinks, fruit juice drinks, and iced teas	3.4 mg/kg bw/day	Scientific Procedures*
GRN 287	Wisdom Natural Brands (Gilbert, AZ)	General-purpose sweetener in foods, excluding meat and poultry products and infant formulas, at levels determined by current good manufacturing practice	Per FSANZ estimates: 1.0 mg/kg bw/day (steviol equivalents); Per GRN000252 1.6 mg/kg bw/day (steviol equivalents); Per GRN000253 provides an EDI for rebaudioside A of 0.4 – 1.1 mg/kg bw/day (steviol equivalents) ³	Scientific Procedures*
GRN 303	SunwinUSA, LLC (Frisco, TX) and WILD Flavors (Erlanger, KY)	As a table top sweetener and for incorporation into various food categories as a general purpose sweetener	Per GRN000252 and GRN000253 provides an EDI for rebaudioside 0.4 – 1.6 mg/kg bw/day (steviol equivalents)	Scientific Procedures*
GRN 318	Pyure Brands, LLC (Naples, FL)	General-purpose sweetener in foods, at levels determined by good manufacturing practice, as well as use as a table top sweetener	Per GRN000252 and GRN000253 provides an EDI for rebaudioside 0.4 – 1.6 mg/kg bw/day (steviol equivalents)	Scientific Procedures*
GRN 323	PureCircle USA, Inc. (Oak Brook, IL)	General-purpose sweetener in foods, excluding meat and poultry products and infant formulas, at levels determined by good manufacturing practice, as well as use as a table top sweetener	Per JEFCA estimates: Ranged from 1.3 – 3.5 mg/kg bw/day steviol glycosides (as steviol). JECFA concluded that the replacement estimates were highly conservative and that a more probable EDI of steviol glycosides (as steviol) would be 20 – 30% of these values or 1.0 to 1.5 mg/kg bw/day	Scientific Procedures*
GRN 329	GLG Life Tech, Ltd. (Vancouver, BC)	General-purpose sweetener in foods, excluding meat and poultry products, at levels determined by current good manufacturing practices	3.4 mg/kg bw/day	Scientific Procedures*

Table 4. Summary of FDA GRAS Notices on Rebaudioside A cont.

GRAS Notice No.	Notifier	Intended use	Estimated Daily Intake (90 th Percentile)	Safety Assessment Basis
GRN 348	GLG Life Tech, Ltd. (Vancouver, BC)	General-purpose sweetener in foods, excluding meat and poultry products, at levels determined by current good manufacturing practices	3.4 mg/kg bw/day	Scientific Procedures*
GRN 354	Guilin Layn Natural Ingredients, Corp. (Gullin, China)	General-purpose sweetener in foods, excluding meat and poultry products and infant formulas, at levels determined by good manufacturing practice, as well as use as a table top sweetener	Per JECFA, GRN 000252 and GRN 000253: Range of 0.4 – 1.6 mg/kg bw/day (steviol equivalents); 0.5 to 0.8 mg/kg bw/day (expressed as steviol).	Scientific Procedures*
GRN 365	BrazTek International Inc. (El Dorado Hills, CA)	General-purpose sweetener in foods, excluding meat and poultry products and infant formulas, at levels determined by good manufacturing practice, as well as use as a table top sweetener	BrazTek considers that the safe use of its rebaudioside A is supported by the fact that it is substantially equivalent to rebaudioside A, notified as GRAS to FDA on May 20, 2008 (GRN 000253).	Scientific Procedures*
GRN 369	ZhuchengHaotian Pharm Co., Ltd. C/O Shanghai Freeman Americas, LLC (Piscataway, NJ)	General-purpose sweetener in foods, excluding meat and poultry products, at levels determined by current good manufacturing practices	3.4 mg/kg bw/day	Scientific Procedures*
GRN 380	GLG Life Tech, Ltd. (Vancouver, BC)	General purpose sweetener in foods, excluding meat and poultry products, at levels determined by good manufacturing practices, as well as use as a table top sweetener	Per JECFA, GRN 000329 and GRN 000348: yield EDIs for Reb A in the range of 0.4 – 1.6 mg/kg bw/day, expressed as steviol equivalents.	Scientific Procedures*

¹Data based on a 14-day survey from the Market Research Corporation of America (MRCA).

²Data based on a 2-day survey from the United States Department of Health and Human Services 2003 – 2004 National Health and Nutrition Examination Survey (NHANES).

*Safety assessment based on published and unpublished studies pertaining to the safety evaluation of rebaudioside A, including studies on rebaudioside A, stevioside, steviol, and crude stevia extracts. Among the published studies considered were acute toxicity studies in rats, mice, and hamsters; sub-chronic toxicity studies in rats; chronic toxicity/carcinogenicity studies in rats; and reproductive/developmental toxicity studies in rats and hamsters. Published clinical studies and published and unpublished absorption, distribution, metabolism and excretion studies in animals and humans we also considered. Additional studies include published *in vitro* and *in vivo* mutagenicity/genotoxicity studies.

V. DATA PERTAINING TO SAFETY

A. Reviews by FDA, JECFA, FSANZ, EFSA & Others

The safety related data on stevia and steviol glycosides have been assessed by a number of reviewers (Brusick, 2008a,b; Carakostas et al., 2008; Geuns, 2003a,b,c; Huxtable, 2002) and most notably by regulatory agencies such as FDA, JECFA (WHO, 2000, 2006, 2007, 2008), Food Standards Australia New Zealand (FSANZ, 2008) and recently by European Food Safety Authority (2010) for use in food. Majority of the reviews published before 2008, primarily focused on mixtures of steviol glycosides and were not specific for purified Reb A. The early reviews focused on concerns seen in various toxicology studies, such as the decrease in fertility with crude stevia preparations and the mutagenic activity of the principle metabolite steviol. Subsequently, well-designed studies with pure test material were conducted. JECFA also encouraged investigating the clinical effects of steviol glycosides on blood pressure in hypertensive subjects and glucose metabolism in diabetic individuals. Based on these new data JECFA established a temporary ADI in 2006 that was subsequently elevated and finalized.

1. Summary of FDA GRAS notices

As of January 3, 2012, FDA¹ has approximately 15 GRAS notices on steviol glycosides in which the agency has issued “FDA has no question” letters. The early notices to FDA on highly purified Reb A were submitted independently by Merisant and Cargill in May 2008. In response to these notices, on December 17, 2008, the agency issued “no question” letters for each notice. Since December 2008, a series of GRAS notifications were submitted to FDA for stevia-derived sweetener products by several companies. So far FDA has not rejected any submissions and none of the submitted notice has been withdrawn. The firms that received a “no question” letter from FDA are available on the agencies website.²

The most recent notice to FDA on rebaudioside A that received a “no question” letter on July, 8, 2011 from FDA was submitted in December 2011 (GRN, 365). A summary of the notices that received “no question” letters from FDA is presented in Table 4. In all these GRAS notices to the FDA, biological data, metabolism, and safety of rebaudioside A and

¹ Accessible at: <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true>

² GRAS notification 252 was submitted by Merisant, GRAS notification 253 was submitted by Cargill, GRAS notification 275 was submitted by McNeil Nutritionals, GRAS notification 278 was submitted by Blue California, GRAS notification 282 was submitted by Sweet Green Fields, GRAS notification 287 was submitted by Wisdom Natural Brands, GRAS notifications 303 and 304 were submitted by Sunwin and Wild Flavors, GRAS notification 318 was submitted by Pyure Brands, GRAS notification 323 was submitted by PureCircle USA, and GRAS notification 329 was submitted by GLG Life Tech; information pertaining to these notifications are listed on FDA's website at <http://www.accessdata.fda.gov/scripts/fc/fcnNavigation.cfm?rpt=grasListing>, along with their respective “no question” letters.

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steviol glycosides have been extensively presented and discussed. The most recent review and response from the FDA occurred in 2011. Given the similarity between the FDA notices and the present GRAS determination, it is instructive to review the FDA's responses to these notices and the information presented in these notices on rebaudioside A from a safety perspective. All this information suggests that the agency is comfortable with the GRAS determination of Reb A at the intended use levels mentioned in these notices.

2. Summary of JECFA Reviews

JECFA reviewed the safety of steviol glycosides in 2000, 2005, 2006, 2007, and 2009 and established an ADI for steviol glycosides (expressed as steviol equivalents) of 4 mg/kg bw/day. The JECFA ADI of 4 mg/kg bw/day for steviol equivalents corresponds to 12 mg/kg bw/day for Reb A. At most recent evaluation of steviol glycoside (69th meeting) the committee summarized its evaluation as follows:

From a long-term study with stevioside, which had already been discussed by the Committee at its fifty-first meeting, a NOEL of 970 mg/kg bw/day was identified. At its sixty-third meeting, the Committee set a temporary ADI of 0 – 2 mg/kg bw for steviol glycosides, expressed as steviol, on the basis of this NOEL for stevioside of 970 mg/kg bw/day (383 mg/kg bw/day expressed as steviol) and a safety factor of 200, pending further information. The further information was required because the Committee had noted that stevioside had shown some evidence of pharmacological effects in patients with hypertension or with type 2 diabetes at doses corresponding to about 12.5 – 25.0 mg/kg bw/day (5 – 10 mg/kg bw/day expressed as steviol).

The results of the new studies presented to the Committee at its present meeting have shown no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw/day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. The Committee concluded that the new data were sufficient to allow the additional safety factor of 2 and the temporary designation to be removed and established an ADI for steviol glycosides of 0 – 4 mg/kg bw expressed as steviol.

The Committee noted that some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides, but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.

3. Summary of FSANZ Review

Food Standards Australia New Zealand (FSANZ) completed a review of the safety of steviol glycosides for use as a sweetener in foods in 2008. The FSANZ assessed the risk of steviol glycosides and concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure and blood glucose in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day. The agency stated that the adequacy of the existing database and a new study in humans provides a basis for revising the uncertainty factors that were used by the JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore, a full ADI of 4 mg/kg bw/day (expressed as steviol equivalents), derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study, was established. This determination is consistent with the outcome of the recent JECFA meeting in June 2008, where steviol glycosides were reconsidered, and the temporary ADI was raised to 4 mg/kg bw/day.

As regards the dietary exposure assessment, FSANZ estimated that for the majority of consumers, the ADI is not exceeded when steviol glycosides were added to the range of foods considered. Two scenarios were modeled: (1) a full sugar replace scenario and (2) a 30% market share uptake scenario. Based on the full sugar replacement scenario, the estimated exposure for high consumers (children aged 2 – 6 years) at the 90th percentile was at the ADI. However, this estimate is based on very conservative assumptions. When a dietary exposure estimate was undertaken with concentrations of steviol glycosides that reflect a more realistic level of use (a 30% market share uptake scenario), it was estimated that dietary exposure for high consumers (children aged 2 – 6 years) at the 90th percentile was only 55% of the ADI. Due to the conservative assumptions in the dietary exposure calculations, the agency concluded that there are no public health and safety concerns for steviol glycosides when used as a food additive at the maximum levels considered by the agency.

4. Summary of EFSA Review

On March 10, 2010, EFSA adopted a scientific opinion on the safety of steviol glycosides (mixtures that comprise not less than 95% of stevioside and/or Reb A) as a food additive. Earlier in 1984, 1989 and 1999, the Scientific Committee for Food (SCF) evaluated stevioside as a sweetener and concluded that there was insufficient data to assess the safety. However, following JECFA's 2008 assessment, EFSA reevaluated the safety of steviol glycosides at the request of European Commission.

For the safety assessment of steviol glycoside, the EFSA Panel considered several *in vitro* and *in vivo* animal studies and human tolerance studies on the steviol glycosides, Reb A

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and stevioside and their metabolite steviol. After considering all the data available, the Panel concluded that steviol glycosides are not carcinogenic, genotoxic or associated with any reproductive/developmental toxicity. The Panel considered a carcinogenicity study with stevioside of high purity (>95%) as pivotal for the evaluation. The NOAEL in the carcinogenicity study was 2.5% stevioside in the diet, equal to 967 mg stevioside/kg bw/day (approximately 388 mg steviol equivalents/kg bw/day), the highest dose tested. Additionally, humans studies demonstrated that daily doses of the steviol glycosides up to 1000 mg/person/day, equivalent to 16.6 mg/kg bw/day for a 60 kg person (corresponding to approximately 330 mg steviol equivalents/person/day or to 5.5 mg steviol equivalents/kg bw/day) were well-tolerated by individuals with normal glucose metabolism or type-2 diabetes mellitus. The Panel 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 2.5% in the diet, equal to 967 mg stevioside/kg bw/day (approximately 388 mg steviol equivalents/kg bw/day), from a 2-year carcinogenicity study in the rat. Conservative estimates of steviol glycosides exposure both in adults and in children suggest that it is likely that the ADI would be exceeded at the maximum proposed use levels.

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B. Toxicity Data on Rebaudioside A

Although majority of the published safety data has been reviewed in the above described FDA notices as well as in other regulatory agency assessment, an attempt has been made to describe some of the relevant data in the following section. Some of the recent investigations on Reb A included additional sub-chronic toxicity studies, mutagenicity studies, reproduction and developmental studies, comparative pharmacokinetic studies with stevioside in rats and humans and human clinical studies.

1. Absorption, Distribution, Metabolism and Excretion (ADME) Studies

In a comparative toxicokinetics and metabolism study on rebaudioside A, stevioside, and steviol, Roberts and Renwick (2008) reported that orally administered single doses of the radio-labeled compounds in rats were rapidly absorbed with plasma concentration-time profiles following similar patterns for stevioside and Reb A. Elimination of radioactivity from plasma was complete within 72 hours of administration. All plasma samples had similar metabolite profiles. The predominant radioactive component in all samples was steviol, with lower amounts of steviol glucuronide(s) and low levels of one or two other metabolites. Rebaudioside A, stevioside, and steviol were metabolized and excreted rapidly, with the majority of the radioactivity eliminated in the feces within 48 hours. Urinary excretion accounted for <2% of the administered dose for all compounds in both intact and bile duct-cannulated rats, and the majority of the absorbed dose was excreted via the bile. After administration of the compounds to intact and bile duct-cannulated rats, radioactivity in the feces was present primarily as steviol. The predominant radioactive compound detected in the bile of all cannulated rats was steviol glucuronide(s), indicating de-conjugation in the lower intestine. The overwhelming weight of data on toxicokinetics and metabolism indicate that Reb A and stevioside are handled in an almost identical manner in the rat after oral dosing.

In another study, pharmacokinetic investigation was done as a toxicokinetic phase of a dietary study to determine the potential of Reb A toxicity in rats at levels up to 2000 mg/kg bw/day (Sloter, 2008). Rebaudioside A and total steviol were detected in peripheral blood during daily administration of 2000 mg/kg bw/day of Reb A at extremely low levels, with mean plasma concentrations of approximately 0.6 and 12.0 ug/mL, respectively. Estimates of absorbed dose for Reb A and total steviol were approximately 0.02% and 0.06%, respectively, based on the amounts measured in urine collected over 24 hours in comparison to daily administered dietary dose to rats. Mean fecal Reb A and measured hydrolysis products expressed as Total Reb A Equivalents compared to daily administered dose results in an estimate of percent of dose recovered at 84%.

In a randomized, double blind, cross-over study in healthy male subjects, Wheeler et al. (2008) assessed the comparative pharmacokinetics of steviol and steviol glucuronide

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following single oral doses of Reb A and stevioside. Following administration of Reb A or stevioside, steviol glucuronide appeared in the plasma of all subjects, with median T_{max} values of 12 and 8 hours post-dose, respectively. Steviol glucuronide was eliminated from the plasma, with similar $t_{1/2}$ values of approximately 14 hours for both compounds. Administration of Reb A resulted in a significantly (approximately 22%) lower steviol glucuronide geometric mean C_{max} value (1472 ng/ml) than administration of stevioside (1886 ng/mL). The geometric mean AUC_{0-t} value for steviol glucuronide after administration of Reb A (30,788 ng*hr/mL) was approximately 10% lower than after administration of stevioside (34,090 ng*hr/mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72-hour collection period, accounting for 59% and 62% of the Reb A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that both Reb A and stevioside were hydrolyzed to steviol in the gastrointestinal tract prior to absorption. The majority of circulatory steviol was in the form of steviol glucuronide indicating rapid first-pass conjugation prior to urinary excretion. Only a small amount of steviol was detected in urine (Reb A: 0.04%; stevioside: 0.02%). Researchers concluded that Reb A and stevioside underwent similar metabolic and elimination pathways in humans with steviol glucuronide excreted primarily in the urine and steviol in the feces. No safety concerns were noted as determined by reporting of adverse events, laboratory assessments of safety or vital signs.

2. Sub-chronic Toxicity Studies

Two repeated-dose studies were conducted by the oral route in Wistar rats for 4 and 13 weeks (Curry and Roberts, 2008). In their 4-week study, Reb A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000 and 100,000 ppm. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In their 13-week study, Wistar rats were administered Reb A at dietary concentrations of 0, 12,500, 25,000 and 50,000 ppm. Reductions in body weight gain attributable to initial taste aversion and lower caloric density of the diet were observed in high-dose male and female groups. Inconsistent reductions in serum bile acids and cholesterol were attributed to physiological changes in bile acid metabolism due to excretion of high levels of Reb A via the liver. All other hepatic function test results and liver histopathology were within normal limits. Significant changes in other clinical pathology results, organ weights and functional observational battery test results were not observed. Macroscopic and microscopic examinations of all organs, including testes and kidneys, were unremarkable with respect to treatment-related findings. The NOAEL in the 13-week toxicity study was considered to be 50,000 ppm or approximately 4161 and 4645 mg/kg bw/day in male and female rats, respectively.

In another study, Reb A (99.5% purity) was administered to Sprague-Dawley rats in the diet at target exposure levels of 500, 1000, and 2000 mg/kg bw/day for 90 days (Nikiforov and Eaton, 2008). There were no treatment-related effects on the general condition and behavior of the animals as determined by clinical observations, functional

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observational battery, and locomotor activity assessments. Evaluation of clinical pathology parameters revealed no toxicologically relevant, treatment-related effects on hematology, serum chemistry, or urinalysis. Macroscopic and microscopic findings revealed no treatment-related effects on any organ evaluated. Lower mean body weight gains were noted in males in the 2000 mg/kg bw/day group throughout the study, which was considered by the authors to be test article related. Given the small magnitude of the difference as compared to controls, this effect was not considered to be adverse. The NOAEL was determined as ≥ 2000 mg/kg bw/day.

In a 90-day dietary toxicity study, Reb A (99.5% purity) was given to Cri:CD(SD) rats at the doses of 500, 1000 and 2000 mg/kg bw/day (Eapen, 2007). Each group consisted of 20/animals/sex. There were no treatment-related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters. There were no treatment-related macroscopic, organ weight, or microscopic findings. Significantly lower body weight gains were noted in the 2000 mg/kg bw/day group in males but not females. The body weight in males was 9.1% lower than the control group at the end of the dosing period (study week 13). The investigators did not consider this result to be adverse due to the small magnitude of difference from the control group value, and they suggested that the reduction was most likely due to the large proportion of the diet represented by the test material. The assigned NOAEL was ≥ 2000 mg/kg bw/day.

In a 6-month dietary toxicity study, Beagle dogs were given Reb A (97.5% purity) at dosage levels of 0, 500, 1000 or 2000 mg/kg bw/day (Eapen, 2008). All groups consisted of 4 males and 4 females. During the course of the study, there were no unscheduled deaths and no treatment-related clinical observations were noted. Home cage, open field observations and functional observations and measurements were unaffected by the administration of Reb A. There were no differences in hematology findings, serum chemistry findings, or urinalysis findings between groups. In addition, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. Based on the results of this study, the authors concluded that no systemic toxicity of Reb A was observed at dosage levels up to 2000 mg/kg bw/day and the assigned NOAEL was ≥ 2000 mg/kg bw/day.

3. Reproductive and Developmental Toxicity Studies

In a study conducted by Curry et al. (2008b), male and female Han Wistar rats were not adversely affected by Reb A (97% purity) treatment as shown in the survival and general condition of the F₁ and F₂ offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation. The NOAEL for reproductive effects was 25,000 ppm. The NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm or 2048 to 2273 mg/kg bw/day (Curry et al., 2008b). Reb A (97% purity) was administered via the diet to male and female Han Wistar rats at 0, 7500, 12,500, and 25,000 ppm for two generations. Reb A treatment

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was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. No treatment-related effects of Reb A were observed in either the F₀ or F₁ generations on reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology. The survival and general condition of the F₁ and F₂ offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by Reb A treatment. The NOAEL for reproductive effects was 25,000 ppm. The NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm or 2048 to 2273 mg/kg bw/day.

In an unpublished two-generation dietary reproduction study, four groups of male and female Cri:CD(SD) rats (30/sex/group) were offered either basal diet or Reb A (purity 95.7%) in the diet for at least 70 consecutive days prior to mating (Sloter, 2008). Reb A doses were 0, 500, 1000 and 2000 mg/kg bw/day for the F_a and F₁ generations. F_a animals were approximately 7 weeks of age at the initiation of test diet exposure. The test diet was offered to the offspring selected to become the F₁ generation following weaning beginning on postnatal day 21. The F_a and F₁ males continued to receive Reb A throughout mating and continuing through the day of euthanasia. The F_a and F₁ females continued to receive Reb A throughout mating, gestation and lactation, until day of euthanasia. There were no effects on reproduction in males or females (estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints). A dose level ≥ 2000 mg/kg bw/day (highest dose administered) was assigned to be the NOAEL for parental systemic and reproductive toxicity.

4. Mutagenicity and Genotoxicity Studies

Reb A was evaluated for genotoxicity in several *in vitro* and *in vivo* assays covering mutation, chromosome damage and DNA strand breakage with negative results (Pezzuto et al, 1985; Nakajima, 2000a,b; Sekihashi et al., 2002) as reviewed by Brusick (2008b). These studies indicate that Reb A is unlikely to cause mutagenic or genotoxic effects.

An unpublished chromosome aberration assay of Reb A in cultured mammalian cells was submitted for JECFA review (Nakajima, 2000a). The JECFA review of this study indicated that no increases in chromosome aberrations were found.

In their GRAS Notification, Merisant submitted three unpublished studies on Reb A including a bacterial mutagenicity study (Wagner and Van Dyke, 2006), a mouse lymphoma study (Clarke, 2006) and a mouse micronucleus study (Krsmanovic and Huston, 2006). All three studies indicated no mutagenic or genotoxic activity of Reb A.

5. Clinical Studies

Two separate randomized, double-blind, placebo-controlled clinical studies were conducted by Maki et al. (2008a,b) on Reb A (97% purity, 1000 mg) for 4 weeks and 16 weeks to evaluate its hemodynamic effects.

In the four week clinical study, 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) were provided either consumption 1000 mg/day Reb A (97% purity) or placebo in (Maki et al., 2008a). Subjects were predominantly female (76%, Reb A and 82%, placebo) with a mean age of 41 (range 18 to 73) years. At baseline, mean resting, seated SBP/DBP was 110.0/70.3 mm Hg and 110.7/71.2 mm Hg for the Reb A and placebo groups, respectively. Compared with placebo, Reb A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-hour ambulatory blood pressure responses. Researchers concluded that these results indicated that consumption of as much as 1000 mg/day of Reb A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

In the 16 weeks clinical trial (conducted at six research sites in the US) men and women (33 – 75 years of age) with type 2 diabetes mellitus were divided into two groups and orally consumed either 1000 mg Reb A (97% purity, n= 60) or placebo (n = 62) (Maki et al., 2008b). Mean± standard error changes in glycosylated hemoglobin levels did not differ significantly between the Reb A ($0.11 \pm 0.06\%$) and placebo ($0.09 \pm 0.05\%$; $p = 0.355$) groups. Changes from baseline for Reb A and placebo, respectively, in fasting glucose (7.5 ± 3.7 mg/dL and 11.2 ± 4.5 mg/dL), insulin (1.0 ± 0.64 μ U/mL and 3.3 ± 1.5 μ U/mL), and C-peptide (0.13 ± 0.09 ng/mL and 0.42 ± 0.14 ng/mL) did not differ significantly ($p > 0.05$ for all). Assessments of changes in blood pressure, body weight, and fasting lipids indicated no differences by treatment. Reb A was well tolerated, and records of hypoglycemic episodes showed no excess versus placebo. Researchers suggested that chronic use of 1000 mg Reb A did not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

C. Acute Toxicology Studies on Sweetn' Up™

MiniStar International, Inc. sponsored 4 toxicology studies on Sweetn' Up™ that were performed at a GLP certified laboratory Eurofins/Product Safety Laboratories (Dayton, NJ, USA) (see Appendix F). Acute oral toxicity, acute dermal toxicity, primary skin irritation and primary eye irritation studies were conducted (see Appendices F1, F2, F3, F4). (Eurofins/Product Safety Laboratories, 2011a,b,c,d).

An acute oral toxicity test (Up and Down Procedure) was conducted with rats to determine the potential for Stevia Leaf 98% Reb-A Powder Extract to produce toxicity from a single dose via the oral route (Eurofins/Product Safety Laboratories, 2011a). A Main Test was conducted using a default starting dose level of 175 mg/kg which was

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administered to one healthy female rat by oral gavage. Following the Up and Down procedure, six additional animals were dosed at levels of 233, 781, 2231 or 5000 mg/kg. Females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males. All animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days after dosing. Body weights were recorded prior to administration and again on Days 7 and 14 (termination) following dosing. Necropsies were performed on all animals at terminal sacrifice. Under the conditions of this study, the acute oral LD₅₀ of the test substance is greater than 5000 mg/kg of body weight in female rats.

An acute dermal toxicity test was conducted with rats to determine the potential for Stevia Leaf 98% Reb-A (Sweetn' Up™) Powder Extract to produce toxicity from a single topical application (see Appendix F.2) (Eurofins/Product Safety Laboratories, 2011b). Under the conditions of this study, the single dose acute dermal LD₅₀ of the test substance is greater than 2000 mg/kg of body weight in male and female rats. Two thousand milligrams of the test substance per kilogram of body weight was applied to a patch and then applied to the skin of ten healthy rats for 24 hours. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Body weights were recorded prior to application and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice. All animals survived exposure to the test substance and appeared active and healthy during the study. Although three females lost weight through Day 7, all animals gained body weight over the 14-day observation period. There were no other signs of gross toxicity, dermal irritation, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

Primary skin irritation test was conducted with rabbits to determine the potential for Stevia Leaf 98% Reb-A (Sweetn' Up™) Powder Extract to produce irritation after a single topical application (see Appendix F.3) (Eurofins/Product Safety Laboratories, 2011c). Under the conditions of this study, the test substance is classified as non-irritating to the skin. Five-tenths of a gram of the test substance was moistened with distilled water and then applied to the skin of three healthy rabbits for 4 hours. Following exposure, dermal irritation was evaluated by the method of Draize et al. (1944). There was no dermal irritation observed at any treated dose site during the study. The Primary Dermal Irritation Index (PDII) calculated for this test substance was 0.0.

A primary eye irritation test was conducted with rabbits to determine the potential for Stevia Leaf 98% Reb-A (Sweetn' Up™) Powder Extract to produce irritation from a single instillation via the ocular route (see Appendix F.4) (Eurofins/Product Safety Laboratories, 2011d). Under the conditions of this study, the test substance is classified as minimally irritating to the eye. One-tenth of a milliliter (0.04 grams) of the test substance was instilled into the right eye of three healthy rabbits. The left eye remained untreated and served as a control. Ocular irritation was evaluated by the method of Draize et al.

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(1944). There was no corneal opacity or iritis observed in any treated eye during this study. One hour after test substance instillation, two animals exhibited positive conjunctivitis. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by 48 hours.

VI. SUMMARY

MiniStar International Inc. intends to use standardized rebaudioside A product extracted and purified from the leaves of *Stevia rebaudiana* as a food ingredient. The purified concentrate is a white fine powder with a characteristic odor. The product will be marketed under the trade name Sweetn' Up™. The product specifications established by MiniStar for its food grade Reb A product (Sweetn' Up™) meets or exceed that of JECFA recommendations and also comply with FCC specifications. The compositional analysis of the product revealed that it primarily contains Reb A (>98%). MiniStar intends to use Reb A product as a general-purpose sweetener in foods, excluding meat and poultry products and infant formulas, at levels determined by good manufacturing practice, as well as use as a table top sweetener. The intended use levels of Sweetn' Up™ and proposed food categories are similar to those described in FDA GRAS notices. The available information from JECFA and the GRAS notices indicate that the mean and 90th percentile daily consumption of Reb A is likely to be 2.0 and 4.7 mg/kg bw/day, respectively.

The safety of steviol glycosides and Reb A has been extensively reviewed by regulatory agencies around the world. Additionally, the safety and toxicity of these glycosides has been extensively investigated in several preclinical and clinical studies. Some of the recent studies on Reb A and stevioside formed the basis of the two initial GRAS notifications to FDA each by Cargill (GRN 253) and Merisant (GRN 252). JECFA has also critically and extensively evaluated the use of steviol glycosides in foods and established the ADI for steviol glycosides of adequate purity as defined by JECFA specifications to be 4 mg/kg bw/person as steviol equivalents, which corresponds to 12 mg/kg bw/day for Reb A on a dry weight basis.

The FDA has reviewed and issued “no question” letters to approximately 15 GRAS notices on Reb A. In these notices, biological data, metabolism, and safety of Reb A and steviol glycosides have been extensively summarized and discussed. The most recent notice to FDA on Reb A that received a “no question” letter on July, 8, 2011 from FDA was submitted in December 2011 (GRN 365). The acceptance of these GRAS notices suggests that the agency is comfortable with the GRAS determination of Reb A at the intended use levels mentioned in these notices. Given the similarity between the FDA notices and the present GRAS determination, the data and information from these notices is applicable to the Merisant product. The available information suggest that MiniStar product is equivalent to those in several GRAS notices and is safe for human consumption as described.

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On the basis of scientific procedures⁴ the consumption of Reb A as a general-purpose sweetener in foods, at levels determined by good manufacturing practice, as well as use as a table top sweetener are considered safe. Rebaudioside A (Sweetn' Up™) used in the proposed food applications is produced according to current good manufacturing practices (cGMP).

VII. CONCLUSION

Based on a critical review of the publicly available data summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that consumption of Reb A (>98% pure) (Sweetn' Up™) as a general-purpose sweetener in foods, at levels determined by good manufacturing practice, as well as use as a table top sweetener when not otherwise precluded by a Standard of Identity as described in this monograph and resulting in the 90th percentile estimated intake of 4.7 mg rebaudioside A/kg body weight/day is Generally Recognized As Safe (GRAS).

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that rebaudioside A (>98% pure) (Sweetn' Up™), when used as described, is GRAS based on scientific procedures.

Signatures

(b) (6)

Sangeeta Patel, PhD

Date

1/12/12

(b) (6)

Madhusudan Soni, PhD, FACN

Date

01/12/12

⁴21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

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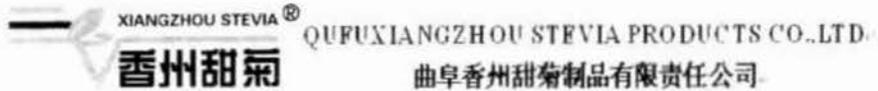
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APPENDIX A:

Certificates of Analysis on MiniStar's Sweetn' Up™



Address: No 11, Yulong Road, Qufu, China 273100
FDA Bioterrorism Registration Number: 16072880290
Tel: 0086-537-4487369 Fax: 0086-537-4400999

Certificate Of Analysis

Product Name: Rebaudioside A 98% PE Manufacture Date: 2011.01.18
Latin Name: Stevia Rebaudiana Expire Date: 2013.01.17
Batch No: 20110118 Batch Quantity: 400kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance	White fine powder	White fine powder	Visual
Odor	Characteristic	Characteristic	Gustation
CHEMICAL TESTS			
Total Steviol(Gluco)sides (% dry basis)	≥99	99.18	HPLC
Rebaudioside A%	≥98	98.15	HPLC
Loss on Drying (%)	≤4.00	2.53	CP/USP
Solubility	Soluble in water and ethanol	Conforms	Visual inspection
Ash (%)	≤0.05	0.02	CP (1g/580°C/2hrs)
pH	5.5-7.0	6.0	CP/USP
Specific Optical Rotation	-30°—-38°	-33°	CP/USP
Specific Absorbance	≤0.01	0.005	CP/USP
Lead (ppm)	≤1	<1	ICP/MS
Arsenic (ppm)	≤1	<1	ICP/MS
Cadmium (ppm)	≤1	<1	ICP/MS
Mercury (ppm)	≤1	<1	ICP/MS
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Yeast&Mold(cfu/g)	≤100	≤100	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP
Solvent Residues			
Methanol (ppm)	<100	<100	CP/USP
Ethanol (ppm)	<500	<500	CP/USP

Storage: Cool and dry place, keep away from strong light and heat
Package: 25kgs drum or carton (two heat-sealed foil food grade bags inside)
Country of Origin : China
Note: NON-GMO NON-ALLERGEN

INSPECTION: (b) (6)

RECHECK: (b) (6)





QUFUXIANGZHOU STEVIA PRODUCTS CO.,LTD.

曲阜香州甜菊制品有限责任公司

Address: No 11, Yulong Road, Qufu, China, 273100

FDA Bioterrorism Registration Number: 16072880290

Tel: 0086-537-4487369 Fax: 0086-537-4400999

Certificate Of Analysis

Product Name: Rebaudioside A 98% PE Manufacture Date: 2011.02.13

Latin Name: Stevia Rebaudiana Expire Date: 2013.02.12

Batch No: 20110213 Batch Quantity: 400kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance	White fine powder	White fine powder	Visual
Odor	Characteristic	Characteristic	Gustation
CHEMICAL TESTS			
Total SteviolGluconides (% dry basis)	≥99	99.21	HPLC
Rebaudioside A%	>98	98.08	HPLC
Loss on Drying (%)	≤4.00	2.61	CP/USP
Solubility	Soluble in water and ethanol	Conforms	Visual inspection
Ash (%)	≤0.05	0.03	CP (1g/580°C/2hrs)
pH	5.5-7.0	6.0	CP/USP
Specific Optical Rotation	-30°—-38°	-33°	CP/USP
Specific Absorbance	≤0.01	0.005	CP/USP
Lead (ppm)	≤ 1	< 1	ICP/MS
Arsenic (ppm)	≤ 1	< 1	ICP/MS
Cadmium (ppm)	≤ 1	<1	ICP/MS
Mercury (ppm)	≤ 1	< 1	ICP/MS
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Yeast&Mold(cfu/g)	≤ 100	≤ 100	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP
Solvent Residues			
Methanol (ppm)	<100	<100	CP/USP
Ethanol (ppm)	<500	<500	CP/USP

Storage: Cool and dry place, keep away from strong light and heat

Package: 25kgs drum or carton (two heat-sealed foil food grade bags inside)

Country of Origin : China

Note: NON-GMO NON-ALLERGEN



INSPECTION: (b) (6)

RECHECK: (b) (6)



QUFUXIANGZHOU STEVIA PRODUCTS CO.,LTD.

曲阜香州甜菊制品有限责任公司

Address: No 11, Yulong Road, Qufu, China, 273100

FDA Bioterrorism Registration Number: 16072880290

Tel: 0086-537-4487369 Fax: 0086-537-4400999

Certificate Of Analysis

Product Name: Rebaudioside A 98% PE Manufacture Date: 2011.03.08

Latin Name: Stevia Rebaudiana Expire Date: 2013.03.07

Batch No: 20110308 Batch Quantity: 450kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance	White fine powder	White fine powder	Visual
Odor	Characteristic	Characteristic	Gustation
CHEMICAL TESTS			
Total SteviolGlucosides (% dry basis)	≥99	99.28	HPLC
Rebaudioside A%	≥98	98.25	HPLC
Loss on Drying (%)	≤4.00	2.72	CP/USP
Solubility	Soluble in water and ethanol	Conforms	Visual inspection
Ash (%)	≤0.05	0.02	CP (1g/580°C/2hrs)
pH	5.5-7.0	6.0	CP/USP
Specific Optical Rotation	-30°--38°	-33°	CP/USP
Specific Absorbance	≤0.01	0.005	CP/USP
Lead (ppm)	≤ 1	< 1	ICP/MS
Arsenic (ppm)	≤ 1	< 1	ICP/MS
Cadmium (ppm)	≤ 1	<1	ICP/MS
Mercury (ppm)	≤ 1	< 1	ICP/MS
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Yeast&Mold(cfu/g)	≤ 100	≤ 100	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP
Solvent Residues			
Methanol (ppm)	<100	<100	CP/USP
Ethanol (ppm)	<500	<500	CP/USP

Storage: Cool and dry place, keep away from strong light and heat

Package: 25kgs drum or carton (two heat-sealed foil food grade bags inside)

Country of Origin: China

Note: NON-GMO NON-ALLERGEN

INSPECTION: (b) (6)

RECHECK: (b) (6)

GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%



QUFUXIANGZHOU STEVIA PRODUCTS CO.,LTD.

曲阜香州甜菊制品有限责任公司

Address: No 11, Yulong Road, Qufu, China, 273100

FDA Bioterrorism Registration Number: 16072880290

Tel: 0086-537-4487369 Fax: 0086-537-4400999

Certificate Of Analysis

Product Name: Rebaudioside A 98% PE Manufacture Date: 2011.04.21

Latin Name: Stevia Rebaudiana Expire Date: 2013.04.20

Batch No: 20110421 Batch Quantity: 450kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance	White fine powder	White fine powder	Visual
Odor	Characteristic	Characteristic	Gustation
CHEMICAL TESTS			
Total SteviolGluconides (% dry basis)	≥99	99.17	HPLC
Rebaudioside A%	≥98	98.12	HPLC
Loss on Drying (%)	≤4.00	2.63	CP/USP
Solubility	Soluble in water and ethanol	Conforms	Visual inspection
Ash (%)	≤0.05	0.02	CP (1g/580°C/2hrs)
pH	5.5-7.0	6.0	CP/USP
Specific Optical Rotation	-30°--38°	-33°	CP/USP
Specific Absorbance	≤0.01	0.005	CP/USP
Lead (ppm)	≤ 1	< 1	ICP/MS
Arsenic (ppm)	≤ 1	< 1	ICP/MS
Cadmium (ppm)	≤ 1	< 1	ICP/MS
Mercury (ppm)	≤ 1	< 1	ICP/MS
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Yeast&Mold(cfu/g)	≤ 100	≤ 100	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP
Solvent Residues			
Methanol (ppm)	<100	<100	CP/USP
Ethanol (ppm)	<500	<500	CP/USP

Storage: Cool and dry place, keep away from strong light and heat

Package: 25kgs drum or carton (two heat-sealed foil food grade bags inside)

Country of Origin : China

Note: NON-GMO NON-ALLERGEN

INSPECTION: (b) (6)

RECHECK: (b) (6)


QUFUXIANGZHOU STEVIA PRODUCTS CO.,LTD.
曲阜香州甜菊制品有限责任公司
 Address: No 11, Yulong Road, Qufu, China, 273100
 FDA Bioterrorism Registration Number: 16072880290
 Tel: 0086-537-4487369 Fax: 0086-537-4400999

Certificate Of Analysis

Product Name: Rebaudioside A 98% PE Manufacture Date: 2011.05.12
 Latin Name: Stevia Rebaudiana Expire Date: 2013.05.11
 Batch No.: 20110512Batch Quantity: 450kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance	White fine powder	White fine powder	Visual
Odor	Characteristic	Characteristic	Gustation
CHEMICAL TESTS			
Total SteviolGlucosides (% dry basis)	≥99	99.23	HPLC
Rebaudioside A%	≥98	98.15	HPLC
Loss on Drying (%)	<4.00	2.68	CP/USP
Solubility	Soluble in water and ethanol	Conforms	Visual inspection
Ash (%)	<0.05	0.03	CP (1g/580°C/2hrs)
pH	5.5-7.0	6.0	CP/USP
Specific Optical Rotation	-30°--38°	-33°	CP/USP
Specific Absorbance	<0.01	0.005	CP/USP
Lead (ppm)	≤1	<1	ICP/MS
Arsenic (ppm)	≤1	<1	ICP/MS
Cadmium (ppm)	≤1	<1	ICP/MS
Mercury (ppm)	≤1	<1	ICP/MS
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Yeast&Mold(cfu/g)	≤100	≤100	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP
Solvent Residues			
Methanol (ppm)	<100	<100	CP/USP
Ethanol (ppm)	<500	<500	CP/USP

Storage: Cool and dry place, keep away from strong light and heat
 Package: 25kgs drum or carton (two heat-sealed foil food grade bags inside)
 Country of Origin : China
 Note: NON-GMO NON-ALLERGEN

INSPECTION: (b) (6) _____ RECHECK: (b) (6) _____

**GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%**

APPENDIX B:

Heavy Metal Analysis



Advanced Botanical Consulting & Testing, Inc.

1169 Warner Ave., Tustin, CA 92780, Phone: (714) 259-0384 Fax: (714) 259-0385

Ministar International, Inc.
21118 Commerce Pointe Drive
City Of Industry, CA 91789
Tel: (909) 598-3963
Fax: (909) 598-5733

ATTN: Mr. Nick Huang/Craig
PO#: Samples

Client Sample ID: Stevia 98% Reb-APE
Product code: STE091
Lot #: 20110421
Lab #: 075500

Received Date: 12/01/2011

Report Date: 12/09/2011

Analyses	Results
Arsenic (Arsenic)	0.014ppm
Cadmium (Cd)	0.002ppm
Mercury (Hg)	0.006ppm
Lead (Pb)	0.035ppm

Method: ICP/MS

(b) (6)

Analyzed by: _____

Chemist

(b) (6)

Approved by: _____

Wendi Wang, PhD, President



Advanced Botanical Consulting & Testing, Inc.

1169 Warner Ave., Tustin, CA 92780, Phone: (714) 259-0384 Fax: (714) 259-0385

Ministar International, Inc.
21118 Commerce Pointe Drive
City Of Industry, CA 91789
Tel: (909) 598-3963
Fax: (909) 598-5733

ATTN: Mr. Nick Huang/Craig
PO#: Samples

Client Sample ID: Stevia 98% Reb-APE
Product code: STE091
Lot #: 20100910
Lab #: 075498

Received Date: 12/01/2011

Report Date: 12/09/2011

Analyses	Results
Arsenic (Arsenic)	0.044ppm
Cadmium (Cd)	0.001ppm
Mercury (Hg)	0.015ppm
Lead (Pb)	0.061ppm

Method: ICP/MS

Analyzed by: (b) (6) Chemist
Approved by: (b) (6) Wendi Wang, PhD, President

#2227 P.008/008

12/12/2011 09:11

GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%



QUFUXIANGZHOU STEVIA PRODUCTS CO.,LTD.

曲阜香州甜菊制品有限责任公司

Address: No 11, Yulong Road, Qufu, China, 273100

FDA Bioterrorism Registration Number: 16072880290

Tel: 0086-537-4487369 Fax: 0086-537-4400999

Certificate Of Analysis

Product Name: Rebaudioside A 98% PE Manufacture Date: 2011.04.21

Latin Name: Stevia Rebaudiana Expire Date: 2013.04.20

Batch No: 20110421 Batch Quantity: 400kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance	White fine powder	White fine powder	Visual
Odor	Characteristic	Characteristic	Gustation
CHEMICAL TESTS			
Total SteviolGlucosides (% dry basis)	≥99	99.21	HPLC
Rebaudioside A%	≥98	98.08	HPLC
Loss on Drying (%)	≤4.00	2.61	CP/USP
Solubility	Soluble in water and ethanol	Conforms	Visual inspection
Ash (%)	≤0.05	0.03	CP (1g/580°C/2hrs)
pH	5.5-7.0	6.0	CP/USP
Specific Optical Rotation	-30°--38°	-33°	CP/USP
Specific Absorbance	≤0.01	0.005	CP/USP
Lead (ppm)	≤ 1	<0.035	ICP/MS
Arsenic (ppm)	≤ 1	<0.014	ICP/MS
Cadmium (ppm)	≤ 1	<0.002	ICP/MS
Mercury (ppm)	≤ 1	<0.006	ICP/MS
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Yeast&Mold(cfu/g)	≤ 100	≤ 100	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP
Solvent Residues			
Methanol (ppm)	<100	<100	CP/USP
Ethanol (ppm)	<500	<500	CP/USP

Storage: Cool and dry place, keep away from strong light and heat

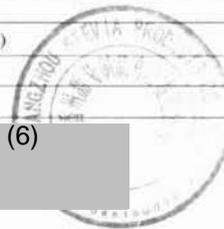
Package: 25kgs drum or carton (two heat-sealed foil food grade bags inside)

Country of Origin: China

Note: NON-GMO NON-ALLERGEN

INSPECTION: (b) (6)

RECHECK: (b) (6)



GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%


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香州甜菊

QUFUXIANGZHOU STEVIA PRODUCTS CO.,LTD.
曲阜香州甜菊制品有限责任公司

Address: No. 11, Yulong Road, Qufu, China, 273100
 FDA Bioterrorism Registration Number: 16072880290
 Tel: 0086-537-4487369 Fax: 0086-537-4400999

Certificate Of Analysis

Product Name: Rebaudioside A 98% PE Manufacture Date: 2010.09.10
 Latin Name: Stevia Rebaudiana Expire Date: 2012.09.09
 Batch No: 20100910 Batch Quantity: 450kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance	White fine powder	White fine powder	Visual
Odor	Characteristic	Characteristic	Gustation
CHEMICAL TESTS			
Total Steviol/Glucosides (% dry basis)	≥99	99.28	HPLC
Rebaudioside A%	≥98	98.25	HPLC
Loss on Drying (%)	≤4.00	2.72	CP/USP
Solubility	Soluble in water and ethanol	Conforms	Visual inspection
Ash (%)	≤0.05	0.02	CP (1g/580°C/2hrs)
pH	5.5-7.0	6.0	CP/USP
Specific Optical Rotation	-30°--38°	-33°	CP/USP
Specific Absorbance	≤0.01	0.005	CP/USP
Lead (ppm)	≤ 1	<0.061	ICP/MS
Arsenic (ppm)	≤ 1	<0.044	ICP/MS
Cadmium (ppm)	≤ 1	<0.001	ICP/MS
Mercury (ppm)	≤ 1	<0.015	ICP/MS
Microbiological Data			
Total Plate Count(cfu/g)	≤1000	<1000	CP/USP
Yeast&Mold(cfu/g)	≤ 100	≤ 100	CP/USP
Coliform(cfu/g)	Negative	Negative	CP/USP
Salmonella(cfu/g)	Negative	Negative	CP/USP
Staphylococcus(cfu/g)	Negative	Negative	CP/USP
Solvent Residues			
Methanol (ppm)	<100	<100	CP/USP
Ethanol (ppm)	<500	<500	CP/USP

Storage: Cool and dry place, keep away from strong light and heat
Package: 25kgs drum or carton (two heat-sealed foil food grade bags inside)
Country of Origin : China
Note: NON-GMO NON-ALLERGEN

INSPECTION (b) (6)

REC'D (b) (6)



GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%


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QUFUXIANGZHOU STEVIA PRODUCTS CO.,LTD.
曲阜香州甜菊制品有限责任公司

Address: No 11, Yulong Road, Qufu, China, 273100
 FDA Bioterrorism Registration Number: 16072880290
 Tel: 0086-537-4487369 Fax: 0086-537-4400999

Certificate Of Analysis

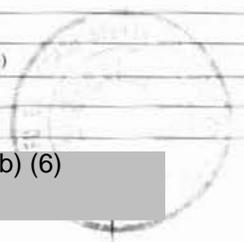
Product Name: Rebaudioside A 98% PE Manufacture Date: 2011.05.30
 Latin Name: Stevia Rebaudiana Expire Date: 2013.05.29
 Batch No: 20110530 Batch Quantity: 450kg

ITEM	SPECIFICATION	TEST RESULTS	Standards
Appearance	White fine powder	White fine powder	Visual
Odor	Characteristic	Characteristic	Gustation
CHEMICAL TESTS			
Total Steviol Glucosides (% dry basis)	≥99	99.23	HPLC
Rebaudioside A%	≥98	98.15	HPLC
Loss on Drying (%)	<4.00	2.68	CP/USP
Solubility	Soluble in water and ethanol	Conforms	Visual inspection
Ash (%)	≤0.05	0.03	CP (1g/580°C/2hrs)
pH	5.5-7.0	6.0	CP/USP
Specific Optical Rotation	-30°--38°	-33°	CP/USP
Specific Absorbance	≤0.01	0.005	CP/USP
Lead (ppm)	≤1	<0.015	ICP/MS
Arsenic (ppm)	≤1	<0.006	ICP/MS
Cadmium (ppm)	≤1	<0.001	ICP/MS
Mercury (ppm)	≤1	<0.003	ICP/MS
Microbiological Data			
Total Plate Count (cfu/g)	≤1000	<1000	CP/USP
Yeast & Mold (cfu/g)	≤100	≤100	CP/USP
Coliform (cfu/g)	Negative	Negative	CP/USP
Salmonella (cfu/g)	Negative	Negative	CP/USP
Staphylococcus (cfu/g)	Negative	Negative	CP/USP
Solvent Residues			
Methanol (ppm)	<100	<100	CP/USP
Ethanol (ppm)	<500	<500	CP/USP

Storage: Cool and dry place, keep away from strong light and heat
Package: 25kgs drum or carton (two heat-sealed foil food grade bags inside)
Country of Origin: China
Note: NON-GMO NON-ALLERGEN

INSPECTION (b) (6)

RECHECK (b) (6)



GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%

APPENDIX C:

Pesticide Analysis and National Organic Program (NOP) Certificate



**Advanced Botanical Consulting &
Testing, Inc.**

1169 Warner Ave., Tustin, CA 92780, Phone: (714) 259-0384 Fax: (714) 259-0385

Ministar International, Inc.
21118 Commerce Pointe Drive
City Of Industry, CA 91789
Tel: (909) 598-3963
Fax: (909) 598-5733

ATTN: Mr. Nick Huang/Craig
PO#: N/A

Client Sample ID: Stevia 98% Reb-APE
Product code: STE091
Lot #: 08520110902
Lab #: 075454

Received Date: 12/01/2011

Report Date: 12/05/2011

Results

Summary: Sample meets USP 34<561> & European Pharmacopelal Pesticide Residue Limits for all Compounds. No Quintozene residues detected

Compound	Concentration (mg/Kg) Found in Sample	ABC Detection Limit (mg/kg)	USP Limit (ppm)
Acephate	None Detected	0.01	0.1
Aiachlor	None Detected	0.01	0.05
Aldrin & Dieldrin	None Detected None Detected	0.01 0.01	0.05
Azinphos-ethyl	None Detected	0.01	0.1
Azinphos-methyl	None Detected	0.01	1.0
Bromine, inorganic	None Detected	0.01	50
Bromophos-ethyl	None Detected	0.01	0.05
Bromophos-methyl	None Detected	0.01	0.05
Bromopropylate	None Detected	0.01	3.0
Chlordane	None Detected	0.01	0.05
Chorfenvinphos	None Detected	0.01	0.5
Chlorpyrifos	None Detected	0.01	0.2
Chlorpyrifos-methyl	None Detected	0.01	0.1
Chlorthal dimethyl	None Detected	0.01	0.1

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GRAS Assessment – MINISTAR International, Inc.
 Sweetn' Up™ - Stevia Reb A ≥ 98%

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Cyfluthrin	None Detected	0.01	1.0
γ-Cyhalothrin	None Detected	0.01	1.0
Cypermethrin	None Detected	0.03	1.0
DDT'S (includes o,p'-DDT, p,p'-DDT, p,p'-DDE, p,p'-DDD)	None Detected	0.01	1.0
Deltamethrin	None Detected	0.05	0.5
Diazinon	None Detected	0.01	0.5
Dichlorfuanid	None Detected	0.01	0.1
Dichlorvos	None Detected	0.01	1.0
Disofol	None Detected	0.01	0.5
Dimethoate & Omethoate	None Detected	0.01	0.1
Dithiocarbamate (as CS2)	None Detected	0.01	1.0
Endosulfans (includes endosulfan I, II, sulfate)	None Detected	0.01	3.0
Endrin	None Detected	0.01	0.05
Ethion	None Detected	0.01	2.0
Etrimphos	None Detected	0.01	0.05
Fenitrothion	None Detected	0.01	0.1
Fenpropathrin	None Detected	0.01	0.03
Fensulfothion	None Detected	0.01	0.05
Fenthion	None Detected	0.01	0.05
Fenvalerate	None Detected	0.03	1.5
Flucytriniate	None Detected	0.01	0.05
r-Fluvalinate	None Detected	0.01	0.05
Fonofos	None Detected	0.01	0.05
Heptachlor	None Detected	0.01	0.05

#2087 P.002/004

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GRAS Assessment – MINISTAR International, Inc.
 Sweetn' Up™ - Stevia Reb A ≥ 98%

Ministar International, Inc.
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 LR 075454

Heptachlor Epoxide	None Detected	0.01	0.05
Hexachlorobenzene	None Detected	0.01	0.1
Hexachlorocyclohexane	None Detected	0.01	0.3
Lindane	None Detected	0.01	0.6
Malathion & Malexon	None Detected	0.01	1.0
Mecarban	None Detected	0.01	0.5
Methacriphos	None Detected	0.01	0.05
Methamidophos	None Detected	0.01	0.05
Methidathion	None Detected	0.01	0.2
Methoxychlor	None Detected	0.01	0.05
Mirex	None Detected	0.01	0.01
Monocrotophos	None Detected	0.01	0.1
Parathion-ethyl & Paraoxon-ethyl	None Detected	0.01	0.5
Parathion-methyl & Paraoxon-methyl	None Detected	0.01	0.2
Pencimethalin	None Detected	0.01	0.1
Pentachloranisol	None Detected	0.01	0.01
Permethrin	None Detected	0.05	1.0
Phosalone	None Detected	0.01	0.1
Phosmet	None Detected	0.01	0.05
Piperonyl butoxide	None Detected	0.01	3.0
Pirimiphos-ethyl	None Detected	0.01	0.05
Pirimiphos-methyl	None Detected	0.01	4.0
Procymidone	None Detected	0.01	0.1
Profenophos	None Detected	0.01	0.1
Prothiophos	None Detected	0.01	0.05

#2087 P.003/004

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GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%

Ministar International, Inc.
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Pyrethrins	None Detected	0.10	3.0
Quinalphos	None Detected	0.01	0.05
Quintozene (PCNB) (includes α -BHC, β -BHC, δ -BHC)	None Detected	0.01	1.0
S-421	None Detected	0.02	0.02
Tecnazene	None Detected	0.05	0.05
Tetradifon	None Detected	0.10	0.3
Vinclozolin	None Detected	0.10	0.4

Analyzed by: (b) (6)
Chemist

Approved by: (b) (6)
Wendi Wang, PhD, President

#2087 P. 004/004

12/05/2011 09:18



NOP Certificate

Certificate N°证书号: 9149

issued by CERES to由CERES颁发至:

Qufu Xiangzhou Stevia Products Co., Ltd.
曲阜香州甜菊制品有限责任公司
No 11, Yulong Road, Qufu City, Shandong Province, PR China
山东省曲阜市裕隆路11号

The following products and activities are certified organic under the US National Organic Program 7 CFR Part 205 下列产品和活动根据美国联邦法典第7卷205部分NOP进行有机认证:

Product 产品	Raw Material 植物	Quantity (Estimate) 估计产量	Label Category 标签类别
Stevia extract 甜菊提取物 (Steviol glycosides 甜菊糖苷, Rebaudioside A 莱鲍迪苷A)	Stevia leaves 甜菊叶	150 T	Made with Organic 有机制造

Scopes 范围:

- **Processing (Handling) by 加工 (处理) 方:** Qufu Xiangzhou Stevia Products Co., Ltd. 曲阜香州甜菊制品有限责任公司
- **Trade (Handling) by 贸易 (处理) 方:** 1) Qufu Fangzheng Trading Co., Ltd.
2) Qufu Focuschem Trading Co., Ltd. 曲阜方正贸易有限公司, 曲阜福克斯贸易有限公司

The operator's certification is valid until being surrendered, suspended, revoked, or replaced by an updated version. For the validity of CERES NOP certificates, see www.ceres-cert.com. In order to assure traceability, we recommend trade partners to request from CERES transaction certificates for each shipment of organic product.

(b) (6)

Happurg, Oct. 14, 2011

Martin Weinschenk-Foerster, CERES GmbH

Date of first NOP certification: Oct. 14, 2011
首次NOP认证日期

Next inspection due date: July 2012
下次检查期限

Note that this certificate applies only to the organic mode of production and not to any other aspect of food quality. CERES authorises the above mentioned operator to use the CERES seal on the organic products specified above. The CERES Seal is property of CERES GmbH, Happurg, Germany. The USDA seal may not be used on products "made with organic ingredients". CERES is accredited by the US Department of Agriculture (USDA) (see www.ams.usda.gov/nop). (4.8.2zh v110320)

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www.ceres-cert.com
Trade Register: HRB 21261



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APPENDIX D:

Identity-Related Analyses for Reb A and Other Steviol Glycosides (Certificate of Analysis and HPLC Chromatograph)

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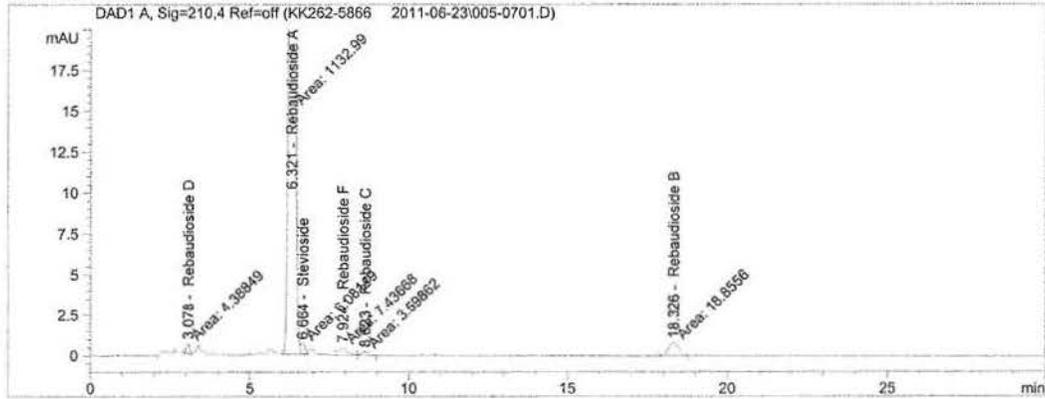
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 Sample Name: 11-5866

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Acq. Instrument : HPLC 17                  Location  : Vial 5
Injection Date  : 6/24/2011 12:23:53 AM     Inj       :    1
                                           Inj Volume: 5.0 µl
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Analysis Method: C:\CHEM32\17\DATA\KK262-5866 2011-06-23\KK262.M
Last changed   : 6/24/2011 8:31:28 AM by Mariel Esguerra
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ECM Operator    : Mariel Esguerra
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ESTD Percent Report

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Dilution:     : 1.0000
Sample Amount: : 1.26850 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs
  
```

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
3.078	MM	4.38849	1.29214e-3	0.447029		Rebaudioside D
6.321	MF	1132.99084	1.11202e-3	99.322937		Rebaudioside A
6.664	MF	6.08149	9.22956e-4	0.442487		Stevioside
7.924	MF	7.43668	1.07063e-3	0.627668		Rebaudioside F
8.623	FM	3.59862	1.08909e-3	0.308966		Rebaudioside C
9.332	-	-	-	-		Dulcoside A
12.808	-	-	-	-		Rubusoside
18.326	MM	18.85561	9.22960e-4	1.371933		Rebaudioside B

HPLC 17 6/24/2011 8:37:25 AM Mariel Esguerra

Page 1 of 2

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Data File C:\CHEM32\17\DATA\KK262-5866 2011-06-23\005-0701.D
Sample Name: 11-5866

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
19.758		-	-	-		Steviolbioside
Totals :				102.521020		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

=====
*** End of Report ***

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Sweetn' Up™ - Stevia Reb A ≥ 98%

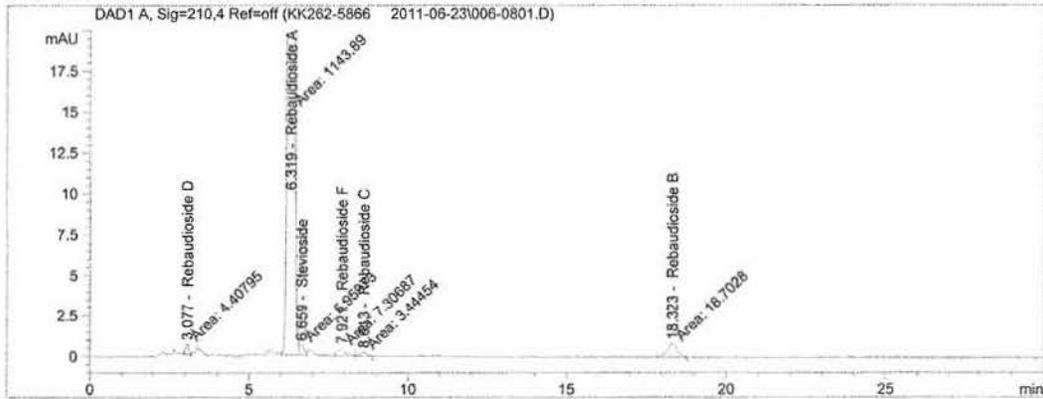
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Injection Date  : 6/24/2011 12:55:31 AM     Inj       :    1
                                           Inj Volume: 5.0 µl
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Analysis Method: C:\CHEM32\17\DATA\KK262-5866 2011-06-23\KK262.M
Last changed   : 6/24/2011 8:31:28 AM by Mariel Esguerra
Method Info    : Steviolglycosides JECFA, 2010
  
```

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ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
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ECM Version     : 13
  
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ESTD Percent Report

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Dilution:      : 1.0000
Sample Amount: : 1.27250 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs
  
```

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
3.077	MM	4.40795	1.29214e-3	0.447599		Rebaudioside D
6.319	MF	1143.89429	1.11202e-3	99.963562		Rebaudioside A
6.659	FM	5.95973	9.22956e-4	0.432265		Stevioside
7.921	MF	7.30687	1.07063e-3	0.614773		Rebaudioside F
8.613	FM	3.44454	1.08909e-3	0.294808		Rebaudioside C
9.332	-	-	-	-		Dulcoside A
12.808	-	-	-	-		Rubusoside
18.323	MM	18.70277	9.22960e-4	1.356535		Rebaudioside B

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Data File C:\CHEM32\17\DATA\KK262-5866 2011-06-23\006-0801.D
Sample Name: 11-5866D

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
19.758	-	-	-	-	-	Steviolbioside
Totals :				103.109542		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found .

*** End of Report ***

**GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%**



Eurofins Scientific Inc., Petaluma
1365 Redwood Way
Petaluma, CA 94954

Tel: +1 707 792 7300
Fax: +1 707 792 7309

June 24, 2011

Nick Huang
MiniStar International, Inc.
21118 Commerce Pointe Drive
Walnut, CA 91789

CERTIFICATE OF ANALYSIS

AR-11-KK-005933-01
Batch#EUCAPE-00018987

Sample Identification:

Sample #: 740-2011-00005866
Description: Stevia Leaf 98% Reb-A PE, Powder, Lot #08506R10616
Condition: White powder in plastic heat sealed pouch received at room temperature.
Date Received: June 21, 2011

Method:

KK262: Steviol Glycosides (HPLC) JECFA (2010)

Date Completed:

June 24, 2011

Results:

Sample #740-2011-00005866

Test	Result	Units
Rebaudioside D	0.447	% (w/w)
Rebaudioside A	99.6	% (w/w)
Stevioside	0.437	% (w/w)
Rebaudioside F	0.621	% (w/w)
Rebaudioside C	0.302	% (w/w)
Dulcoside A	<0.1	% (w/w)
Rebaudioside B	1.36	% (w/w)
Steviolbioside	<0.1	% (w/w)
Total Stevia Glycosides	103	% (w/w)
Rubusoside	<0.1	% (w/w)

Results pertain only to the items tested.

Estimation of uncertainty of measurement is available upon request.

Results shall not be reproduced except in full without written permission from Eurofins Scientific, Inc.

(b) (6)

Jennifer Coulter
Client Services Supervisor

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APPENDIX E:

JECFA 2010 Specifications (Expanded)

STEVIOLE GLYCOSIDES

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

SYNONYMS

INS no. 960

DEFINITION

The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and the product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside which are generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

Chemical name

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

C.A.S. number

Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

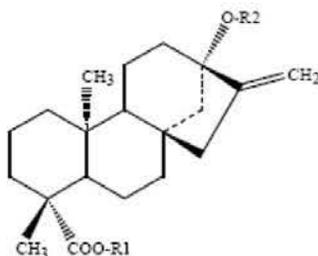
Chemical formula

Stevioside: C₃₈H₆₀O₁₈
Rebaudioside A: C₄₄H₇₀O₂₃

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Structural Formula

The nine named steviol glycosides:



<u>Compound name</u>	<u>R1</u>	<u>R2</u>
Stevioside	β -Glc	β -Glc- β -Glc(2→1)
Rebaudioside A	β -Glc	β -Glc- β -Glc(2→1) β -Glc(3→1)
Rebaudioside B	H	β -Glc- β -Glc(2→1) β -Glc(3→1)
Rebaudioside C	β -Glc	β -Glc- α -Rha(2→1) β -Glc(3→1)
Rebaudioside D	β -Glc- β -Glc(2→1)	β -Glc- β -Glc(2→1) β -Glc(3→1)
Rebaudioside F	β -Glc	β -Glc- β -Xyl(2→1) β -Glc(3→1)
Dulcoside A	β -Glc	β -Glc- α -Rha(2→1)
Rubusoside	β -Glc	β -Glc
Steviolbioside	H	β -Glc- β -Glc(2→1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides. Glc, Rha and Xyl represent, respectively, glucose, rhamnose and xylose sugar moieties.

Formula weight

Stevioside: 804.88
Rebaudioside A: 967.03

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Assay	Not less than 95% of the total of the nine named steviol glycosides on the dried basis.
DESCRIPTION	White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.
FUNCTIONAL USES	Sweetener
CHARACTERISTICS	
IDENTIFICATION	
<u>Solubility</u> (Vol. 4)	Freely soluble in water
<u>Stevioside and rebaudioside A</u>	The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.
<u>pH</u> (Vol. 4)	Between 4.5 and 7.0 (1 in 100 solution)
PURITY	
<u>Total ash</u> (Vol. 4)	Not more than 1%
<u>Loss on drying</u> (Vol. 4)	Not more than 6% (105°, 2h)
<u>Residual solvents</u> (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)
<u>Arsenic</u> (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
<u>Lead</u> (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities").
METHOD OF ASSAY	Determine the percentages of the individual steviol glycosides by HPLC (Vol. 4) under the following conditions.
	<u>Reagents</u> Acetonitrile: more than 95% transmittance at 210 nm.
	<u>Standards</u> Stevioside: more than 99.0% purity on the dried basis. Rebaudioside A: more than 99.0% purity on the dried basis. Mixture of nine steviol glycosides standard solution: Containing stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and

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steviolbioside. This solution is diluted with water-acetonitrile (7:3) accordingly and is used for the confirmation of retention times. Standards are available from Wako Pure Chemical Industries, Ltd. Japan and ChromaDex, USA.

Standard solution

Accurately weigh 50 mg of stevioside and rebaudioside A standard into each of two 50-ml volumetric flasks. Dissolve and make up to volume with water-acetonitrile (7:3).

Sample solution

Accurately weigh 50-100 mg of sample into a 50-ml volumetric flask. Dissolve and make up to volume with water-acetonitrile (7:3).

Procedure

Inject 5 µl of sample solution under the following conditions.
Column: Capcell pak C₁₈ MG II (Shiseido Co.Ltd) or Luna 5µ C18(2) 100A (Phenomenex) or equivalent (length: 250 mm; inner diameter: 4.6 mm, particle size: 5µm)
Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L sodium phosphate buffer (pH 2.6)
Flow rate: 1.0 ml/min
Detector: UV at 210 nm
Column temperature: 40°
Record the chromatogram for about 30 min.

Identification of the peaks and Calculation

Identify the peaks from the sample solution by comparing the retention time with the peaks from the mixture of nine steviol glycosides standard solution (see under figure). Measure the peak areas for the nine steviol glycosides from the sample solution. Measure the peak area for stevioside and rebaudioside A from their standard solutions. Calculate the percentage of each of the eight steviol glycosides except rebaudioside A in the sample from the formula:

$$\%X = [W_S/W] \times [f \times A_X/A_S] \times 100$$

Calculate the percentage of rebaudioside A in the sample from the formula:

$$\%Rebaudioside\ A = [W_R/W] \times [A_X/A_R] \times 100$$

where

- X is each steviol glycoside;
- W_S is the amount (mg) calculated on the dried basis of stevioside in the standard solution;
- W_R is the amount (mg) calculated on the dried basis of rebaudioside A in the standard solution;
- W is the amount (mg) calculated on the dried basis of sample in the sample solution;
- A_S is the peak area for stevioside from the standard solution;
- A_R is the peak area for rebaudioside from the standard solution;

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A_X is the peak area of X for the sample solution; and f_X is the ratio of the formula weight of X to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

Calculate the percentage of total steviol glycosides (sum the nine percentages).

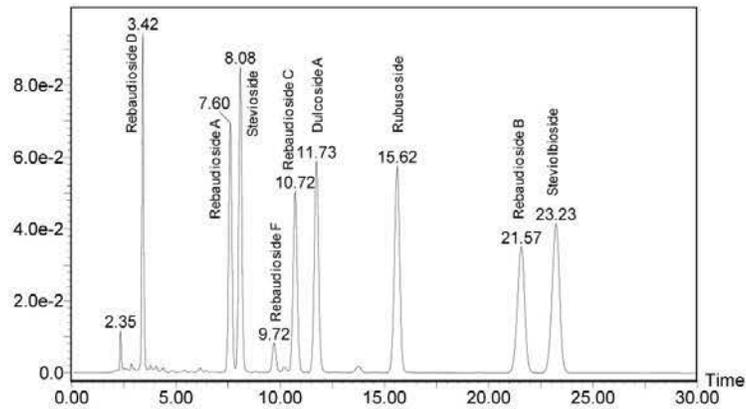


Figure. Chromatogram of mixture of nine steviol glycosides standard solution

Column: Capcell pak C₁₈ MG II

Concentration: 0.5 mg/ml each except rebaudioside F (about 0.1 mg/ml)

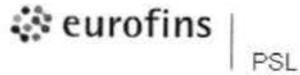
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Sweetn' Up™ - Stevia Reb A ≥ 98%

APPENDIX F:

Toxicology Reports of MiniStar's Sweetn' Up™

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APPENDIX F.1:
Acute Oral Toxicology Report



PRODUCT

Stevia Leaf 98% Reb-A Powder Extract

STUDY TITLE

Acute Oral Toxicity Up And Down Procedure In Rats

DATA REQUIREMENT

OECD Guidelines for Testing of Chemicals, Test No. 425

AUTHOR

Aija McKenzie, B.A., L.A.T.G.

STUDY COMPLETED ON

December 13, 2011

PERFORMING LABORATORY

Eurofins PSL

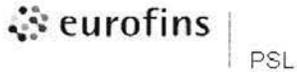
LABORATORY STUDY NUMBER

32663

Page 1 of 15

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www.productsafetylabs.com



GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Stevia Leaf 98% Reb-A Powder Extract

This study meets the requirements of U.S. FDA: 21 CFR 58, 1987 and 11-Nousan-No. 6283, 1 October, 1999; JMAFF. Specific information related to the characterization of the test substance as received and tested is the responsibility of the study Sponsor (see Test Substance section).

Study Director: (b) (6)
Aija McKenzie, B.A., L.A.T.G.
Eurofins PSL

December 13, 2011
Date

Submitter: _____
Signature

Date

Sponsor: _____
Signature

Date

QUALITY ASSURANCE STATEMENT

The Eurofins PSL Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	June 23, 2010 ¹ ; Oct 12, 2011	June 23, 2010; Oct 12, 2011
In-process inspection: <i>Day 2 in-life observations for Animal #3101</i>	Aug 18, 2011	Oct 12, 2011
Raw data audit	Oct 12, 2011	Oct 12, 2011
Draft report review	Oct 12, 2011	Oct 12, 2011

Final report reviewed by:

(b) (6)


Ilya Stolyar
Quality Assurance Auditor
Eurofins PSL

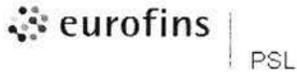
Date

12/12/11

¹ EP SL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

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QUALITY ASSURANCE STATEMENT	3
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ACUTE ORAL TOXICITY UP AND DOWN PROCEDURE IN RATS

PROTOCOL NO.:	P320 UDP
AGENCY:	FDA and JMAFF
STUDY NUMBER:	32663
SPONSOR:	MINISTAR INTERNATIONAL INC. 21118 Commerce Pointe Drive Walnut, CA 91789
TEST SUBSTANCE IDENTIFICATION:	Stevia Leaf 98% Reb-A Powder Extract Batch #: 20100910
DATE RECEIVED:	June 17, 2011
EPSL REFERENCE NO.:	110617-1D
STUDY INITIATION DATE:	July 8, 2011
DATES OF TEST:	August 16 – October 5, 2011
NOTEBOOK NO.:	11-183: pages 45, 45A, 46-58, 58A-58D, 59-61

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to Stevia Leaf 98% Reb-A Powder Extract by the oral route.

2. SUMMARY

An acute oral toxicity test (Up and Down Procedure) was conducted with rats to determine the potential for Stevia Leaf 98% Reb-A Powder Extract to produce toxicity from a single dose via the oral route. Under the conditions of this study, the acute oral LD₅₀ of the test substance is greater than 5,000 mg/kg of body weight in female rats.

A Main Test was conducted using a default starting dose level of 175 mg/kg which was administered to one healthy female rat by oral gavage. Following the Up and Down procedure, six additional animals were dosed at levels of 233, 781, 2231 or 5,000 mg/kg. Females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males. All animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days after dosing. Body weights were recorded prior to administration and again on Days 7 and 14 (termination) following dosing. Necropsies were performed on all animals at terminal sacrifice.

3. MATERIALS

A. Test Substance

The test substance, identified as Stevia Leaf 98% Reb-A Powder Extract, Batch #: 20100910, was received on June 17, 2011 and was further identified with EPSL Reference Number 110617-1D. The test substance was stored at room temperature. The sample was administered as a 50% w/w mixture in distilled water. Preliminary solubility testing conducted by EPSL indicated mixtures in excess of 50% (60%-80%) were too viscous to be administered properly. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The following information related to the characterization of the test substance was provided by the Sponsor:

Composition: Greater than equal to 98% Rebaudioside A; greater than equal to 98% stevioside; 2% other ingredients

Physical Description: White + solid (powder form)

Solubility: Soluble in water.

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: Not applicable.

B. Animals

3.B.1 Number of Animals: 7

3.B.2 Sex: Female. All animals assigned to test were nulliparous and non-pregnant.

3.B.3 Species/Strain: Rat/Sprague-Dawley derived, albino.

3.B.4 Age/Body weight: Young adult (9-10 weeks)/168-180 grams at experimental start.

3.B.5 Source: Received from Harlan Laboratories, Inc. on August 3 and 17, 2011.

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors, which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room Temperature and Relative Humidity Ranges: 19-23°C and 55-85%, respectively. The humidity was above the targeted upper limit for twenty days during the study. A portable dehumidifier was used to lower the humidity levels during this time.

4.A.3 Animal Room Air Changes/Hour: 15. Airflow measurements are evaluated regularly and the records are kept on file at Product Safety Labs.

- 4.A.4 Photoperiod: 12-hour light/dark cycle
- 4.A.5 Acclimation Period: 6-19 days
- 4.A.6 Food: Harlan Teklad Global 16% Protein Rodent Diet[®] 2016. The diet was available *ad libitum*, except during fasting.
- 4.A.7 Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system.
- 4.A.8 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at Eurofins PSL.

B. Identification:

- 4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number, dose level, identification, and sex of the animal.
- 4.B.2 Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the rat. This number, together with a sequential animal number assigned to study number 32663, constituted unique identification.

5. PROCEDURE

A. Selection of Animals

Prior to each dosing, experimentally naive rats (not previously tested) were fasted overnight by removing the feed from their cages. During the fasting period, the rats were examined for health and weighed (initial). Seven healthy naive female rats were selected for test.

B. Dose Calculations

Individual doses were calculated based on the initial body weights, taking into account the specific gravity (determined by EPSL) and concentration of the test mixture.

C. Dosing

The test substance was administered as a 50% w/w mixture in distilled water to the stomach using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Following administration, each animal was returned to its designated cage. Feed was replaced approximately 3-4 hours after dosing.

Individual animals were dosed as follows:

Main Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	175	S	S
2	3102	233	S	S
3	3103	781	S	S
4	3104	2231	S	S
5	3107	5000	S	S
6	3108	5000	S	S
7	3109	5000	S	S

S – Survival

D. Body Weights

Individual body weights of the animals were recorded prior to test substance administration (initial) and again on Days 7 and 14 (termination) following dosing.

E. Cage-Side Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, and coma.

F. Necropsy

All rats were euthanized via CO₂ inhalation at the end of the 14-day observation period. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined.

6. STUDY CONDUCT

This study was conducted at Eurofins Product Safety Labs' (Eurofins PSL) test facility at 725 Cranbury Road, East Brunswick, New Jersey, 08816. The Study Director for this study was Aija McKenzie, B.A., L.A.T.G. The primary scientist for this study was Maryann Zakrzewski with contributions from Jessica Beyenhof, B.S., Cynthia Bodnar, Kristin Booth, Arnold Gonzalez, B.S., Harry Maselli, L.A.T., Carolyn Montefusco, B.S. and Matthew Sorber, B.S. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- U.S. FDA GLP: 21 CFR 58, 1987
- JMAFF GLP: 11-Nousan-No. 6283, 1 October, 1999

and based on the following testing guideline:

- OECD Guidelines for Testing of Chemicals, Test No. 425

7. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Eurofins PSL Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

8. DEVIATION FROM THE PROTOCOL

Due to a transcription error, the actual dose was calculated incorrectly for animals 3102 through 3106, because the wrong specific gravity (1.803 g/ml instead of .803 g/ml) was used. These five test animals were administered a lower dose than required. The results from animals 3101, 3102, 3103, and 3104 will be entered into the AOT 425 Statistical Program and dosing will continue until stopping criteria is met to estimate an LD50. The data from animals 3105 and 3106 are reported in Appendix A. Although the dose levels did not reach the intended 5,000 mg/kg and additional testing is required to establish an estimated LD50, this deviation does not adversely impact the interpretation of the study.

9. FINAL REPORT AND RECORDS TO BE MAINTAINED

The original, signed final report will be forwarded to the Sponsor. A copy of this signed report, together with the protocol and all raw data generated at Eurofins PSL, is maintained in the Eurofins PSL Archives. EPSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or may request continued archiving by EPSL.

10. RESULTS

Individual body weights and doses are presented in Table 1. Individual cage-side and necropsy observations are presented in Tables 2 and 3, respectively. The individual raw data for animal #3105 and 3106 is presented in Appendix A.

All animals survived test substance administration, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

175 mg/kg Dose Level (1 animal)

The animal survived exposure to the test substance, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for the animal when necropsied at the conclusion of the 14-day observation period.

233 mg/kg Dose Level (1 animal)

The animal survived exposure to the test substance, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for the animal when necropsied at the conclusion of the 14-day observation period.

781 mg/kg Dose Level (1 animal)

The animal survived exposure to the test substance, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for the animal when necropsied at the conclusion of the 14-day observation period.

2231 mg/kg Dose Level (1 animal)

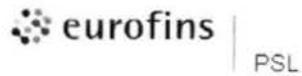
The animal survived exposure to the test substance, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for the animal when necropsied at the conclusion of the 14-day observation period.

5000 mg/kg Dose Level (3 animals)

All animals survived exposure to the test substance, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for these animals when necropsied at the conclusion of the 14-day observation period.

11. CONCLUSION

Under the conditions of this study, the acute oral LD₅₀ of Stevia Leaf 98% Reb-A Powder Extract is greater than 5,000 milligrams per kilogram of body weight in female rats.



SIGNATURE

Stevia Leaf 98% Reb-A Powder Extract

I, the undersigned, declare that the methods, results, and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

(b) (6)

Aija McKenzie, B.A./L.A.T.G.
Study Director
Eurofins PSL

December 13, 2011
Date

TABLE 1: INDIVIDUAL BODY WEIGHTS AND DOSES

Animal No.	Sex	Dose Level (mg/kg)	Body Weight (g)			Dose ¹
			Initial	Day 7	Day 14	mL
3101	F	175	177	200	210	0.078
3102	F	233	172	191	204	0.10
3103	F	781	180	201	207	0.35
3104	F	2231	171	192	194	0.95
3107	F	5000	168	191	206	2.1
3108	F		173	197	211	2.2
3109	F		168	194	200	2.1

¹ The test substance was administered as a 50% w/w mixture in distilled water. Specific Gravity –0.803 g/mL.

TABLE 2: INDIVIDUAL CAGE-SIDE OBSERVATIONS

<u>Animal Number</u>	<u>Findings</u>	<u>Day of Occurrence</u>
175 mg/kg Dose Level		
3101	Active and healthy	0-14
233 mg/kg Dose Level		
3102	Active and healthy	0-14
781 mg/kg Dose Level		
3103	Active and healthy	0-14
2231 mg/kg Dose Level		
3104	Active and healthy	0-14
5,000 mg/kg Dose Level		
3107 -3109	Active and healthy	0-14

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

<u>Animal Number</u>	<u>Tissue</u>	<u>Findings</u>
175 mg/kg Dose Level		
3101	All tissues and organs	No gross abnormalities
233 mg/kg Dose Level		
3102	All tissues and organs	No gross abnormalities
781 mg/kg Dose Level		
3103	All tissues and organs	No gross abnormalities
2231 mg/kg Dose Level		
3104 – 3106	All tissues and organs	No gross abnormalities
5000 mg/kg Dose Level		
3107 – 3109	All tissues and organs	No gross abnormalities

APPENDIX A: INDIVIDUAL ANIMAL RAW DATA FOR ANIMAL #3105 AND #3106

A. Animals (see Section 8)

- 1.A.1 Number of Animals: 2
- 1.A.2 Sex: Female, nulliparous and non-pregnant.
- 1.A.3 Species/Strain: Rat/ Sprague-Dawley derived, albino.
- 1.A.4 Age/Body weight: Young adult (9-10 weeks)/ 169 and 198 grams at experimental start.
- 1.A.5 Source: Received from Harlan Laboratories, Inc. on August 3 and 17, 2011.

B. Husbandry

- 1.B.1 Acclimation Period: 6 or 19 days

TABLE 1: INDIVIDUAL BODY WEIGHTS, DOSES, AND MORTALITIES

Animal No.	Sex	Dose Level (mg/kg)	Body Weight (g)			Dose ¹
			Initial	Day 7	Day 14	mL
3105	F	2231	198	230	238	1.1
3106	F		169	196	208	0.94

TABLE 2: INDIVIDUAL CAGE-SIDE OBSERVATIONS

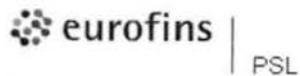
<u>Animal Number</u>	<u>Findings</u>	<u>Day of Occurrence</u>
2231 mg/kg Dose Level		
3105, 3106	Active and healthy	0-14

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

<u>Animal Number</u>	<u>Tissue</u>	<u>Findings</u>
2231 mg/kg Dose Level		
3105, 3106	All tissues and organs	No gross abnormalities

¹ The test substance was administered as received. Specific Gravity – 0.803 g/mL.

APPENDIX F.2:
Acute Dermal Toxicology Report



PRODUCT

Stevia Leaf 98% Reb-A Powder Extract

STUDY TITLE

Acute Dermal Toxicity Study in Rats - Limit Test

DATA REQUIREMENT

OECD Guidelines for Testing of Chemicals, Test No. 402
JMAFF 12-Nousan-8147

AUTHOR

Aija McKenzie, B.A., L.A.T.G.

STUDY COMPLETED ON

December 13, 2011

PERFORMING LABORATORY

Eurofins PSL

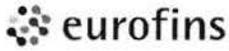
LABORATORY STUDY NUMBER

32664

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Product Safety Laboratories

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Stevia Leaf 98% Reb-A Powder Extract

This study meets the requirements of U.S. FDA GLP: 21 CFR 58, 1987 and 11-Nousan-No. 6283, 1 October, 1999: JMAFF. Specific information related to the characterization of the test substance as received and tested is the responsibility of the study Sponsor (see Test Substance section).

Study Director: (b) (6)
Aijj McKenzie, B.A., I.A.T.G.
Eurofins PSL
December 13, 2011
Date

Submitter: _____
Signature

Date

Sponsor: _____
Signature

Date



QUALITY ASSURANCE STATEMENT

The Eurofins PSL Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	June 23, 2010 ¹ ; Sept 26, 2011	June 23, 2010; Sept 29, 2011
In-process inspection: <i>Test Substance preparation</i>	Aug 4, 2011	Sept 29, 2011
Raw data audit	Sept 26, 2011	Sept 29, 2011
Draft report review	Sept 26, 2011	Sept 29, 2011

(b) (6)

Rhonda S. Krick, B.S.
Quality Assurance Director
Eurofins PSL

Dec 12, 2011
Date

¹ EPSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.



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ACUTE DERMAL TOXICITY STUDY IN RATS - LIMIT TEST

PROTOCOL NO.: P322.RAT
AGENCY: FDA and JMAFF
STUDY NUMBER: 32664
SPONSOR: MiniStar International Inc.
21118 Commerce Pointe Drive
Walnut, CA 91789
TEST SUBSTANCE IDENTIFICATION: Stevia Leaf 98% Reb-A Powder Extract
Batch #: 20100910
DATE RECEIVED: June 17, 2011
EPSL REFERENCE NO.: 110617-1D
STUDY INITIATION DATE: July 8, 2011
DATES OF TEST: August 4 - 18, 2011
NOTEBOOK NO.: 11-168: pages 281-290

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to Stevia Leaf 98% Reb-A Powder Extract by the dermal route.

2. SUMMARY

An acute dermal toxicity test was conducted with rats to determine the potential for Stevia Leaf 98% Reb-A Powder Extract to produce toxicity from a single topical application. Under the conditions of this study, the single dose acute dermal LD₅₀ of the test substance is greater than 2,000 mg/kg of body weight in male and female rats.

Two thousand milligrams of the test substance per kilogram of body weight was applied to a patch and then applied to the skin of ten healthy rats for 24 hours. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Body weights were recorded prior to application and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

All animals survived exposure to the test substance and appeared active and healthy during the study. Although three females lost weight through Day 7, all animals gained body weight over the 14-day observation period. There were no other signs of gross toxicity, dermal irritation, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

3. MATERIALS

A. Test Substance

The test substance, identified as Stevia Leaf 98% Reb-A Powder Extract, Batch #: 20100910, was received on June 17, 2010 and was further identified with EPSL Reference Number 110617-1D. The test substance was stored at room temperature. In order to ensure adequate contact with the skin, the sample was applied as a dry paste (80% w/w mixture in distilled water). Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The following information related to the characterization of the test substance was provided by the Sponsor:

Composition: Greater than equal to 98% Rebaudioside A; greater than equal to 98% stevioside; 2% other ingredients

Physical Description: White + solid (powder form)

Solubility: Soluble in water.

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: Not applicable.

B. Animals

3.B.1 Number of Animals: 10

3.B.2 Sex: 5 Males and 5 Females. Females assigned to test were nulliparous and non-pregnant.

3.B.3 Species/Strain: Rats/Sprague-Dawley derived, albino

3.B.4 Age/Body weight: Young adult (9-10 weeks)/males 238-263 grams and females 195-219 grams at experimental start.

3.B.5 Source: Received from Harlan Laboratories, Inc. on July 27, 2011.

4. METHODS

A. Husbandry

- 4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). Litter paper was placed beneath the cage and was changed at least three times per week.
- 4.A.2 Animal Room Temperature and Relative Humidity Ranges: 19-25°C and 58-76%, respectively. The humidity was above the targeted upper limit for nine days during the study. A portable dehumidifier was used to lower the humidity levels during this time.
- 4.A.3 Animal Room Air Changes/Hour: 12 and 13. Airflow measurements are evaluated regularly and the records are kept on file at Eurofins PSL.
- 4.A.4 Photoperiod: 12-hour light/dark cycle
- 4.A.5 Acclimation Period: 8 days
- 4.A.6 Food: Harlan Teklad Global 16% Protein Rodent Diet 2016
- 4.A.7 Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system.
- 4.A.8 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at Eurofins PSL.

B. Identification

- 4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.
- 4.B.2 Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the animal. This number, together with a sequential animal number assigned to study 32664, constituted unique identification.

5. PROCEDURE

A. Preparation and Selection of Animals

On the day prior to application, a group of animals was prepared by clipping the dorsal area and the trunk. After clipping and prior to application, the animals were examined for health, weighed (initial) and the skin checked for any abnormalities. Ten healthy rats (five males and five females; not previously tested) were selected for test.

B. Dose Calculations

Individual doses were calculated based on the initial body weights and the concentration of the test substance.

C. Application of Test Substance

Two thousand milligrams of test substance per kilogram of body weight was applied to a 2-inch x 3-inch, 4-ply gauze pad and placed on a dose area of approximately 2 inches x 3 inches (approximately 10% of the body surface). The gauze pad and entire trunk of each animal were then wrapped with 3-inch Durapore tape to avoid dislocation of the pad and to minimize loss of the test substance. The rats were then returned to their designated cages. The day of application was considered Day 0 of the study.

After 24 hours of exposure to the test substance, the pads were removed and the test sites were gently cleansed of any residual test substance.

D. Body Weights

Individual body weights of the animals were recorded prior to test substance application (initial) and again on Days 7 and 14 (termination).

E. Cage-Side Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes during the first several hours after application and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, and coma.

F. Necropsy

All rats were euthanized via CO₂ inhalation on Day 14. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined.

6. STUDY CONDUCT

This study was conducted at Eurofins Product Safety Labs' (Eurofins PSL) test facility at 2394 US Highway 130, Dayton, New Jersey 08810. The Study Director for this study was Aija McKenzie, B.A., L.A.T.G. The primary scientist for this study was Cindy Bodnar with contributions from Matthew Sorber, B.S., and Maryann Zakrzewski. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- 21 CFR 58: U.S. FDA GLP Standards, 1987
- JMAFF GLP: 11-Nousan-No. 6283, 1 October, 1999

and based on the following testing guidelines:

- OECD Guidelines for the Testing of Chemicals, Test No. 402
- JMAFF 12-Nousan-8147



7. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Eurofins PSL Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

8. DEVIATIONS FROM THE PROTOCOL

None

9. FINAL REPORT AND RECORDS TO BE MAINTAINED

The original, signed final report will be forwarded to the Sponsor. A copy of this signed report, together with the protocol and all raw data generated at Eurofins PSL, is maintained in the Eurofins PSL Archives. EPSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or may request continued archiving by EPSL.

10. RESULTS

Individual body weights and doses are presented in Table 1. Individual cage-side and necropsy observations are presented in Tables 2 and 3, respectively.

All animals survived exposure to the test substance and appeared active and healthy during the study. Although three females lost weight through Day 7, all animals gained body weight over the 14-day observation period. There were no other signs of gross toxicity, dermal irritation, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

11. CONCLUSION

Under the conditions of this study, the single dose acute dermal LD₅₀ of Stevia Leaf 98% Reb-A Powder Extract is greater than 2,000 mg/kg of body weight in male and female rats.



Product Safety Laboratories

SIGNATURE

Stevia Leaf 98% Reb-A Powder Extract

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

(b) (6)



Aija McKenzie, B.A., M.A.T.G.
Study Director
Eurofins PSL

December 13, 2011

Date

TABLE 1: INDIVIDUAL BODY WEIGHTS AND DOSES

Animal No.	Sex	Body Weight (g)			Dose ¹ g
		Initial	Day 7	Day 14	
3201	M	238	266	301	0.60
3202	M	253	274	303	0.63
3203	M	263	282	316	0.66
3204	M	251	273	317	0.63
3205	M	258	290	325	0.65
3206	F	215	221	247	0.54
3207	F	213	200	206	0.53
3208	F	219	213	231	0.55
3209	F	199	206	222	0.50
3210	F	195	193	204	0.49

¹ The test substance was applied as an 80% w/w mixture in distilled water.

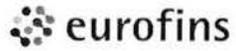


TABLE 2: INDIVIDUAL CAGE-SIDE OBSERVATIONS

<u>Animal Number</u>	<u>Findings</u>	<u>Day of Occurrence</u>
<u>MALES</u>		
3201-3205	Active and healthy	0-14
<u>FEMALES</u>		
3206, 3208	Active and healthy	0-14
3207, 3210	Active and healthy Mechanical damage around dose site due to unwrapping	0-14 1
3209	Active and healthy Mechanical damage around dose site due to unwrapping	0-14 1-4

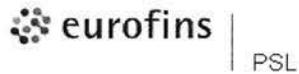


TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

<u>Animal Number</u>	<u>Tissue</u>	<u>Findings</u>
<u>MALES</u>		
3201 - 3205	All tissues and organs	No gross abnormalities
<u>FEMALES</u>		
3206 - 3210	All tissues and organs	No gross abnormalities

GRAS Assessment – MINISTAR International, Inc.
Sweetn' Up™ - Stevia Reb A ≥ 98%

APPENDIX F.3:
Primary Skin Toxicology Report



PRODUCT

Stevia Leaf 98% Reb-A Powder Extract

STUDY TITLE

Primary Skin Irritation Study In Rabbits

DATA REQUIREMENTS

OECD Guidelines for Testing of Chemicals, Test No. 404
JMAFF 12-Nousan-8147

AUTHOR

Aija McKenzie, B.A., L.A.T.G.

STUDY COMPLETED ON

December 13, 2011

PERFORMING LABORATORY

Eurofins PSL

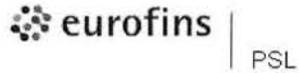
LABORATORY STUDY NUMBER

32666

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Stevia Leaf 98% Reb-A Powder Extract

This study meets the requirements U.S. FDA: 21 CFR 58, 1987 and 11-Nousan-No. 6283, 1 October, 1999: JMAFF. Specific information related to the characterization of the test substance as received and tested is the responsibility of the study Sponsor (see Test Substance section).

Study Director: (b) (6)
Aija McKenzie, B.A., L.A.T.G.
Eurofins PSL
December 13, 2011
Date

Submitter: _____
Signature

Date

Sponsor: _____
Signature

Date

QUALITY ASSURANCE STATEMENT

The Eurofins PSL Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

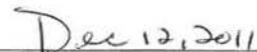
QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	June 23, 2010 ¹ ; Sept 26, 2011	June 23, 2010; Sept 26, 2011
In-process inspection: <i>Initiation of exposure</i>	July 26, 2011	Sept 26, 2011
Raw data audit	Sept 26, 2011	Sept 26, 2011
Draft report review	Sept 26, 2011	Sept 26, 2011

Final report reviewed by:

(b) (6)

Rhonda S. Krick, B.S.
Quality Assurance Director
Eurofins PSL


Date

¹ EPSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

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PRIMARY SKIN IRRITATION STUDY IN RABBITS

PROTOCOL NO.:	P326
AGENCY:	FDA and JMAFF
STUDY NUMBER:	32666
SPONSOR:	MiniStar International Inc. 21118 Commerce Pointe Drive Walnut, CA 91789
TEST SUBSTANCE IDENTIFICATION:	Stevia Leaf 98% Reb-A Powder Extract Batch #: 20100910
DATE RECEIVED:	June 17, 2011
EPSL REFERENCE NO.:	110617-1D
STUDY INITIATION DATE:	July 8, 2011
DATES OF TEST:	July 26-29, 2011
NOTEBOOK NO.:	11-163: pages 292-299

1. PURPOSE

To provide information on the potential for skin irritation to arise from a single topical exposure to Stevia Leaf 98% Reb-A Powder Extract.

2. SUMMARY

A primary skin irritation test was conducted with rabbits to determine the potential for Stevia Leaf 98% Reb-A Powder Extract to produce irritation after a single topical application. Under the conditions of this study, the test substance is classified as non-irritating to the skin.

Five-tenths of a gram of the test substance was moistened with distilled water and then applied to the skin of three healthy rabbits for 4 hours. Following exposure, dermal irritation was evaluated by the method of Draize *et al.*¹ (see Table 3).

There was no dermal irritation observed at any treated dose site during the study.

¹ Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 2764; 82:377-390.

The incidence and severity of irritation are detailed below:

Time After Patch Removal	Incidence of Irritation	
	Erythema	Edema
30-60 minutes	0/3	0/3
24 hours	0/3	0/3
48 hours	0/3	0/3
72 hours	0/3	0/3

Time After Patch Removal	Severity of Irritation – Mean Score
30-60 minutes	0.0
24 hours	0.0
48 hours	0.0
72 hours	0.0

The Primary Dermal Irritation Index (PDII) calculated for this test substance was 0.0.

3. MATERIAL

A. Test Substance

The test substance, identified as Stevia Leaf 98% Reb-A Powder Extract, Batch #: 20100910, was received on June 17, 2011 and was further identified with EPSL Reference Number 110617-1D. The test substance was stored at room temperature. In order to ensure adequate contact with the skin, the sample was applied as a dry paste (80% w/w mixture in distilled water). Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by Sponsor.

The following information related to the characterization of the test substance was provided by the Sponsor:

Composition: Greater than equal to 98% Rebaudioside A; greater than equal to 98% stevioside; 2% other ingredients

Physical Description: White + solid (powder form)

Solubility: Soluble in water.

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: Not applicable

B. Animals

3.B.1 Number of Animals: 3

3.B.2 Sex: Female, nulliparous and non-pregnant.

3.B.3 Species/Strain: Rabbit/New Zealand albino.

3.B.4 Age/Body weight: Young adult

3.B.5 Source: Received from Robinson Services, Inc. on July 20, 2011.

4. METHODS

A. Husbandry

- 4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). Litter paper was placed beneath the cage and was changed at least three times per week.
- 4.A.2 Animal Room Temperature and Relative Humidity Ranges: 20-23°C and 59-86%, respectively. The humidity was above the targeted upper limit for one day during the study. A portable dehumidifier was used to lower the humidity levels during this time.
- 4.A.3 Animal Room Air Changes/Hour: 12; Airflow measurements are evaluated regularly and the records are kept on file at Eurofins PSL.
- 4.A.4 Photoperiod: 12-hour light/dark cycle
- 4.A.5 Acclimation Period: 6 days
- 4.A.6 Feed: Pelleted Harlan Teklad Global High Fiber Rabbit Diet 2031 was supplied *ad libitum*.
- 4.A.7 Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system.
- 4.A.8 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at Eurofins PSL.

B. Identification

- 4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.
- 4.B.2 Animal: A number was allocated to each rabbit on receipt and a stainless steel ear tag bearing this number was attached to the animal. This number, together with a sequential animal number assigned to study 32666, constituted unique identification.

5. PROCEDURE

A. Preparation and Selection of Animals

On the day before application, a group of animals were prepared by clipping the dorsal area and the trunk. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. Three healthy naive animals (not previously tested) without pre-existing skin irritation were selected for test.

B. Application of Test Substance

Prior to application, the test substance was moistened with distilled water to achieve a dry paste by preparing an 80% w/w mixture. Five-tenths of a gram of the test substance (0.63 g of test mixture) was then applied to a 1-inch x 1-inch, 4-ply gauze pad and placed on one 6-cm² intact dose site on each animal. The pad and entire trunk of each animal were then wrapped with semi-occlusive 3-inch Micropore tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.

After 4 hours of exposure to the test substance, the pads and the collars were removed, and the test sites were gently cleansed to remove any residual test substance.

C. Evaluation of Test Sites

Individual dose sites were scored according to the Draize scoring system¹ (see Table 3) at approximately 30-60 minutes, 24, 48, and 72 hours after patch removal.

The classification of irritancy was obtained by adding the average erythema and edema scores for the 30-60 minute, 24, 48, and 72-hour scoring intervals and dividing by the number of evaluation intervals (4).

The resulting Primary Dermal Irritation Index (PDII) was classified as follows:

<u>PDII</u>	<u>Classification</u>
0	Non-irritating
> 0 - 2.0	Slightly irritating
2.1 - 5.0	Moderately irritating
> 5.0	Severely irritating

D. Cage-Side Observations

The animals were observed for signs of gross toxicity and behavioral changes at least once daily during the test period.

6. STUDY CONDUCT

This study was conducted at Eurofins Product Safety Labs' (Eurofins PSL) test facility at 725 Cranbury Road, East Brunswick, New Jersey 08816. The study director for this study was Aija McKenzie, B.A., L.A.T.G. The primary scientist for this study was Kristin Booth. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- U.S. FDA GLP: 21 CFR 58, 1987
- JMAFF GLP: 11-Nousan-No. 6283, 1 October, 1999

and based on the following testing guidelines:

- OECD Guidelines for Testing of Chemicals, Test No. 404
- JMAFF 12-Nousan-8147

7. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Eurofins PSL Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

¹ Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 2764; 82:377-390.

8. DEVIATIONS FROM THE PROTOCOL

None.

9. FINAL REPORT AND RECORDS TO BE MAINTAINED

The original, signed final report will be forwarded to the Sponsor. A copy of this signed report, together with the protocol and all raw data generated at Eurofins PSL, is maintained in the Eurofins PSL Archives. Eurofins PSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or may request continued archiving by Eurofins PSL.

10. RESULTS

Individual skin irritation scores are presented in Table 1. A summary of primary skin irritation scores is presented in Table 2. The Draize Primary Skin Irritation Scoring System is presented in Table 3.

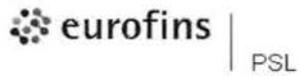
All animals appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

There was no dermal irritation observed at any treated dose site during the study.

The Primary Dermal Irritation Index for Stevia Leaf 98% Reb-A Powder Extract is 0.0.

11. CONCLUSION

Under the conditions of this study, Stevia Leaf 98% Reb-A Powder Extract is classified as non-irritating to the skin.



SIGNATURE

Stevia Leaf 98% Reb-A Powder Extract

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

(b) (6) 

Aija McKenzie, B.A., L.A.T.G.
Study Director
Eurofins PSL

December 13, 2011
Date

TABLE 1: INDIVIDUAL SKIN IRRITATION SCORES

ERYTHEMA/EDEMA

Animal No.	Sex	Time After Patch Removal			
		30-60 min	24 hrs	48 hrs	72 hrs
3501	F	0/0	0/0	0/0	0/0
3502	F	0/0	0/0	0/0	0/0
3503	F	0/0	0/0	0/0	0/0
Total		0/0	0/0	0/0	0/0
Mean		0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0

TABLE 2: SUMMARY OF PRIMARY SKIN IRRITATION SCORES¹

	Time After Patch Removal			
	30-60 min	24 hrs	48 hrs	72 hrs
Erythema	0.0	0.0	0.0	0.0
Edema	0.0	0.0	0.0	0.0
TOTAL (PDI) ²	0.0	0.0	0.0	0.0

Primary Dermal Irritation Index (PDII): $\frac{\text{PDI for 30-60 minutes, 24, 48 and 72 hours}}{4} = 0.0$

Classification: Non-irritating

CLASSIFICATION SYSTEM³

<u>PDII</u>	<u>Classification</u>
0	Non-irritating
> 0 - 2.0	Slightly irritating
2.1 - 5.0	Moderately irritating
> 5.0	Severely irritating

¹ Average values for three rabbits.

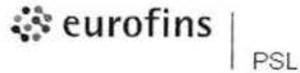
² PDI = Average Erythema + Average Edema

³ U.S. EPA Addendum 3 on data reporting to pesticide assessment guidelines; Dermal Irritation, January 1988.

TABLE 3: PRIMARY SKIN IRRITATION SCORING SYSTEM

<u>Evaluation of Skin Reactions</u>	<u>Value</u>
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible).....	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth).....	4
Edema formation:	
No edema.....	0
Very slight edema (barely perceptible).....	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)..	4

APPENDIX F.4:
Primary Eye Toxicology Report



PRODUCT

Stevia Leaf 98% Reb-A Powder Extract

STUDY TITLE

Primary Eye Irritation Study in Rabbits

DATA REQUIREMENT

OECD Guidelines for Testing of Chemicals, Test No. 405

JMAFF 12-Nousan-8147, November 2000

AUTHOR

Aija McKenzie, B.A., L.A.T.G.

STUDY COMPLETED ON

December 13, 2011

PERFORMING LABORATORY

Eurofins PSL

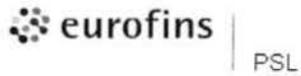
LABORATORY STUDY NUMBER

32665

Page 1 of 14

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Stevia Leaf 98% Reb-A Powder Extract

This study meets the requirements of U.S. FDA: 21 CFR 58, 1987 and 11-Nousan-No. 6283, 1 October, 1999: JMAFF. Specific information related to the characterization of the test substance as received and tested is the responsibility of the study Sponsor (see Test Substance section).

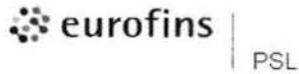
Study Director: (b) (6)
Aija McKenzie, B.A., L.A.T.G.
Eurofins PSL
December 13, 2011
Date

Submitter: _____
Signature

Date

Sponsor: _____
Signature

Date



QUALITY ASSURANCE STATEMENT

The Eurofins PSL Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	June 23, 2010 ¹ ; Sept 26, 2011	June 23, 2010; Sept 29, 2011
In-process inspection: <i>48-hour scoring</i>	Aug 10, 2011	Sept 29, 2011
Raw data audit	Sept 26, 2011	Sept 29, 2011
Draft report review	Sept 26, 2011	Sept 29, 2011

Final report reviewed by:

(b) (6)

Rhonda S. Krick, B.S.
Quality Assurance Director
Eurofins PSL

Dec 12, 2011
Date

¹ EPSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

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PRIMARY EYE IRRITATION STUDY IN RABBITS

PROTOCOL NO.:	P324
AGENCY:	FDA and JMAFF
STUDY NUMBER:	32665
SPONSOR:	MINISTAR INTERNATIONAL INC. 21118 Commerce Pointe Drive Walnut, CA 91789
TEST SUBSTANCE IDENTIFICATION:	Stevia Leaf 98% Reb-A Powder Extract Batch #: 20100910
DATE RECEIVED:	June 17, 2011
EPSL REFERENCE NO.:	110617-1D
STUDY INITIATION DATE:	July 8, 2011
DATES OF TEST:	August 8-11, 2011
NOTEBOOK NO.:	11-168: pages 291-303

1. PURPOSE

To provide information on the irritation likely to arise from a single instillation of Stevia Leaf 98% Reb-A Powder Extract into the eye.

2. SUMMARY

A primary eye irritation test was conducted with rabbits to determine the potential for Stevia Leaf 98% Reb-A Powder Extract to produce irritation from a single instillation via the ocular route. Under the conditions of this study, the test substance is classified as minimally irritating to the eye.

One-tenth of a milliliter (0.04 grams) of the test substance was instilled into the right eye of three healthy rabbits. The left eye remained untreated and served as a control. Ocular irritation was evaluated by the method of Draize *et al.*¹ (see Table 2).

¹ Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

There was no corneal opacity or iritis observed in any treated eye during this study. One hour after test substance instillation, two animals exhibited positive conjunctivitis. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by 48 hours.

The incidence of positive effects, severity and reversibility of irritation are detailed below:

Time Post Instillation	Incidence of Positive Effects		
	Corneal Opacity	Iritis	Conjunctivitis
1 hour	0/3	0/3	2/3
24 hours	0/3	0/3	0/3
48 hours	0/3	0/3	0/3
72 hours	0/3	0/3	0/3

Time Post Instillation	Severity of Irritation – Mean Score
1 hour	9.3
24 hours	2.0
48 hours	0.0
72 hours	0.0

3. MATERIALS

A. Test Substance

The test substance, identified as Stevia Leaf 98% Reb-A Powder Extract, Batch #: 20100910, was received on June 17, 2011 and was further identified with EPLS Reference Number 110617-1D. The test substance was stored at room temperature. The sample was instilled as received. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The following information related to the characterization of the test substance was provided by the Sponsor:

Composition: Greater than equal to 98% Rebaudioside A; greater than equal to 98% stevioside; 2% other ingredients

Physical Description: White + solid (powder form)

Solubility: Soluble in water.

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: Not applicable

B. Animals

- 3.B.1 Number of Animals: 3
- 3.B.2 Sex: 2 Males and 1 Female. Females assigned to test were nulliparous and non-pregnant.
- 3.B.3 Species/Strain: Rabbit/New Zealand albino.
- 3.B.4 Age: Young adult.
- 3.B.5 Source: Received from Robinson Services, Inc. on July 20 and 27, 2011.

4. METHODS

A. Husbandry

- 4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors, which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Nat. Res. Council, 2011). Litter paper was placed beneath the cage and was changed at least three times per week.
- 4.A.2 Animal Room Temperature and Relative Humidity Ranges: 19-22°C and 62-82%, respectively. The humidity was above the targeted upper limit for two days during the study. A portable dehumidifier was used to lower the humidity levels during this time.
- 4.A.3 Animal Room Air Changes/Hour: 12. Airflow measurements are evaluated regularly and the records are kept on file at Product Safety Labs.
- 4.A.4 Photoperiod: 12-hour light/dark cycle
- 4.A.5 Acclimation Period: 12 or 19 days
- 4.A.6 Food: Harlan Teklad Global High Fiber Rabbit Diet 2031
- 4.A.7 Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system.
- 4.A.8 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at Eurofins PSL.

B. Identification

- 4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.
- 4.B.2 Animal: A number was allocated to each rabbit on receipt and a stainless steel ear tag bearing this number was attached to the animal. This number, together with a sequential animal number assigned to study 32665, constituted unique identification.

5. PROCEDURE

A. Preparation and Selection of Animals

Prior to test initiation, both eyes of a group of animals were examined using a white light source and a fluorescein dye procedure. One drop of 2% ophthalmic fluorescein sodium was instilled into both eyes of each rabbit. The eyes were rinsed with physiological saline (0.9% NaCl) after instillation of the fluorescein and then evaluated for corneal damage using an ultraviolet light source. Prior to test substance instillation, the eyes were re-examined and scored for abnormalities according to the "Scale for Scoring Ocular Lesions" (see Table 2). Three healthy animals (not previously tested) without pre-existing ocular irritation were selected for test.

B. Instillation

Prior to instillation, 2-3 drops of ocular anesthetic (Tetracaine Hydrochloride Ophthalmic Solution, 0.5%) were placed into both the treated and control eye of each animal. One-tenth of a milliliter (0.04 grams) of the test substance was then instilled into the conjunctival sac of the right eye of each rabbit by pulling the lower lid away from the eyeball. The upper and lower lids were then gently held together for about one second before releasing to minimize loss of the test substance. The other eye of each rabbit remained untreated with the test substance and served as a control. The rabbits were then returned to their designated cages.

C. Ocular Scoring

Ocular irritation was evaluated using a high-intensity white light (Mag Lite) in accordance with Draize *et al.*¹ (see Table 2) at 1, 24, 48, and 72 hours post-instillation. The fluorescein dye evaluation procedure described in Section 5.A. was used in the treated eye at 24 hours to verify the absence of corneal damage. Individual scores were recorded for each animal. In addition to observations of the cornea, iris and conjunctivae, any other observed lesions were noted. The average score for all rabbits at each scoring period was calculated to aid in data interpretation.

D. Classification of Eye Scores

The time interval with the highest mean score (Maximum Mean Total Score - MMTS) for all rabbits was used to classify the test substance by the system of Kay and Calandra².

E. Cage-Side Observations

The animals were observed for signs of gross toxicity and behavioral changes at least once daily during the test period.

¹ Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

² Kay, J.H. and Calandra, J.C. Interpretation of eye irritation tests. *J. Soc. Cos. Chem.* 1962; 13:281-289.

6. STUDY CONDUCT

This study was conducted at Eurofins Product Safety Labs' (Eurofins PSL) test facility at 725 Cranbury Road, East Brunswick, New Jersey, 08816. The Study Director for this study was Aija McKenzie, B.A., L.A.T.G. The primary scientist for this study was Kristin Booth. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- 21 CFR 58: U.S. FDA GLP Standards 1987
- JMAFF GLP: 11-Nousan-No. 6283, 1 October, 1999

and based on the following testing guidelines:

- OECD Guidelines for Testing of Chemicals, Test No. 405
- JMAFF 12-Nousan-8147

7. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Eurofins PSL Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

8. DEVIATIONS FROM THE PROTOCOL

None

9. FINAL REPORT AND RECORDS TO BE MAINTAINED

The original, signed final report will be forwarded to the Sponsor. A copy of this signed report, together with the protocol and all raw data generated at Eurofins PSL, is maintained in the Eurofins PSL Archives. EPSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or may request continued archiving by EPSL.

10. RESULTS

Individual eye irritation scores are presented in Table 1. The Draize Scale for Scoring Eye Lesions is presented in Table 2. The Kay and Calandra scheme for classifying eye irritants is presented in Table 3.

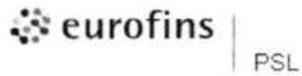
All animals appeared active and healthy during the study. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

There was no corneal opacity or iritis observed in any treated eye during this study. One hour after test substance instillation, two animals exhibited positive conjunctivitis. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by 48 hours.

The Maximum Mean Total Score of Stevia Leaf 98% Reb-A Powder Extract is 9.3.

11. CONCLUSION

Under the conditions of this study, Stevia Leaf 98% Reb-A Powder Extract is classified as minimally irritating to the eye.



SIGNATURE

Stevia Leaf 98% Reb-A Powder Extract

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

(b) (6) 

Aija McKenzie, B.A., L.A., V.G.
Study Director
Eurofins PSL

December 13, 2011
Date

TABLE 1: INDIVIDUAL SCORES FOR OCULAR IRRITATION

	Rabbit No.: 3401 (Male)				Rabbit No.: 3402 (Male)				Rabbit No.: 3403 (Female)			
	Hour				Hour				Hour			
	1	24	48	72	1	24	48	72	1	24	48	72
I. Cornea												
A. Opacity	0	0 ¹	0	0	0	0 ¹	0	0	0	0 ¹	0	0
B. Area	4	4	4	4	4	4	4	4	4	4	4	4
(AxB)x5	0	0	0	0	0	0	0	0	0	0	0	0
II. Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
Ax5	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	1	0	0	0	2	0	0	0	2	0	0	0
B. Chemosis	1	1	0	0	1	1	0	0	1	1	0	0
C. Discharge	2	0	0	0	2	0	0	0	2	0	0	0
(A+B+C)x2	8	2	0	0	10	2	0	0	10	2	0	0
Total	8	2	0	0	10	2	0	0	10	2	0	0

¹ 2% ophthalmic fluorescein sodium used to verify the absence of corneal opacity.

TABLE 2: SCALE FOR SCORING OCULAR LESIONS¹

1. Cornea	
A. Opacity-degree of density (area most dense taken for reading)	
No Opacity.....	0
Scattered or diffuse area, details of iris clearly visible.....	1 ²
Easily discernible translucent areas, details of iris slightly obscured.....	2 ²
Opalescent areas, no details of iris visible, size of pupil barely discernible.....	3 ²
Opaque, iris invisible.....	4 ²
B. Area of cornea involved	
One quarter (or less) but not zero.....	1
Greater than one quarter, but less than half.....	2
Greater than half, but less than three quarters.....	3
Greater than three quarters, up to whole area.....	4
A X B X 5	Total Maximum = 80
2. Iris	
A. Values	
Normal.....	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive).....	1 ²
No reaction to light, hemorrhage, gross destruction (any or all of these).....	2 ²
A X 5	Total Maximum = 10
3. Conjunctivae	
A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal.....	0
Vessels definitely injected above normal.....	1
More diffuse, deeper crimson red, individual vessels not easily discernible.....	2 ²
Diffuse beefy red.....	3 ²
B. Chemosis	
No swelling.....	0
Any swelling above normal (includes nictitating membrane).....	1
Obvious swelling with partial eversion of lids.....	2 ²
Swelling with lids about half-closed.....	3 ²
Swelling with lids about half-closed to completely closed.....	4 ²
C. Discharge	
No discharge.....	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals).....	1
Discharge with moistening of the lids and hairs just adjacent to lids.....	2
Discharge with moistening of the lids and hairs, and considerable area around the eye.....	3
Score (A + B + C) X 2	Total Maximum = 20

Total Maximum Score: 110 represents the sum of all scores obtained for the cornea, iris and conjunctivae.

¹ Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 1944; 82:377-390.

² These scores represent a positive response.

TABLE 3: CLASSIFICATION OF EYE IRRITATION SCORES

MMTS	Irritation Classification	Requirement For Maintenance of Classification ¹
0.0 - 0.5	non	Up to 0.5 at 1 hour with zeros at 24 hours; otherwise, increase one level
0.6 - 2.5	practically non	with zeros at 24 hours; otherwise, increase one level
2.6 - 15.0	minimally	with zeros at 48 hours; otherwise, increase one level
15.1 - 25.0	mildly	with zeros at 96 hours; otherwise, increase one level
25.1 - 50.0	moderately	with 7 day mean ≤ 20 and individual total scores ≤ 10 in at least 60% of the rabbits with no total score >30; otherwise, increase one level
50.1 - 80.0	severely	with 7 day mean ≤ 40 and individual total scores ≤ 30 in at least 60% of the rabbits with no total score > 60; otherwise, increase one level
80.1 - 100.0	extremely	with 7 day mean ≤ 80 and individual total scores ≤ 60 in at least 60% of the rabbits with no total score >100; otherwise, increase one level
100.1 - 110	maximally	with 7 day mean > 80 and individual total scores > 60 in at least 60% of the rabbits; otherwise, decrease one level

¹ Kay, J.H, and Calandra, J.C. Interpretation of eye irritation tests. *J. Soc. Cos. Chem.* 1962; 13:281-289.

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