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GRAS Notice (GRN) No. 369
http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/default.htm

ORIGINAL SUBMISSION

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Division of
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GRAS Notice Review

November 22, 2010

Office of Food Additive Safety (HFS-225)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Pkwy
College Park, MD 20740

RE: **GRAS EXEMPTION CLAIM: REBAUDIOSIDE A DERIVED FROM *STEVIA REBAUDIANA***

Dear Sir or Madam,

Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. are pleased to submit the attached GRAS Exemption claim for rebaudioside A derived from *Stevia rebaudiana* for use as a general purpose non-nutritive sweetener. Rebaudioside A derived from *Stevia rebaudiana* has been determined by Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination is based on scientific procedures and has been evaluated by experts qualified by scientific training and experience to assess the safety of rebaudioside A derived from *Stevia rebaudiana* under the conditions of its intended use in food. Therefore, the use of rebaudioside A derived from *Stevia rebaudiana* in food as described in this GRAS Exemption Claim is exempt from the requirement of premarket approval.

The attached GRAS Notification provides a review of the information related to intended uses, manufacturing and safety of Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd.'s rebaudioside A derived from *Stevia rebaudiana.* Included with this GRAS Notification submission you'll find three *(3)* hard copies of the GRAS Notification dossier which include the signed Expert Panel Consensus Statement. The data and information that serve as the basis for the GRAS determination, as well as the information that has become available since the GRAS determination, will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times as arranged at Shanghai Freemen Americas, LLC.

Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. look forward to the Agency's review of the accompanying dossier and is prepared to respond to any questions that arise in the review process. We are confident in our safety determination of rebaudioside A derived from *Stevia rebaudiana* for use as a general purpose non-nutritive sweetener and trust that the Agency will concur with this conclusion.

Respectfully submitted,

(b) (6)

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## I. GRAS EXEMPTION CLAIM

### A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

As defined herein, rebaudioside A derived from *Stevia rebaudiana* has been determined by Zhucheng Haotian Pharm Co., Ltd and Shanghai Freemen Lifescience Co., Ltd. to be Generally Recognized As Safe (GRAS), consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food. Therefore, the use of rebaudioside A derived from *Stevia rebaudiana* in food as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

11/25/2010

Liping Zhu
Zhecheng Haotian Pharm Co., Ltd.
Xinxing Town, Zhucheng City
Shandong Province, China

Date

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Date

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12/6/10

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Date

## B. Name and Address of Notifier

Tom Tchang
Shanghai Freemen Americas, LLC
377 Hoes Lane, Suite 306
Piscataway, NJ 08854

## C. Common Name of the Notified Substance

Rebaudioside A derived from *Stevia rebaudiana*

## D. Conditions of Intended Use in Food

Rebaudioside A derived from *Stevia rebaudiana* is intended for use as a general purpose non-nutritive sweetener in a variety of food products. Rebaudioside A is intended for use at levels determined by current good manufacturing practices (cGMPs) such that intakes are consistent with the acceptable daily intake (ADI) of 0 to 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, rebaudioside A derived from *Stevia rebaudiana* intended for use in foods by Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. as defined herein has been determined to be GRAS based on scientific procedures. This determination is based on data that is generally available in the public domain, pertaining to the safety of rebaudioside A derived from *Stevia rebaudiana* and is supported by a consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of rebaudioside A derived from *Stevia rebaudiana* as a component of food[1] [see Appendix 1, entitled, "**GRAS EXPERT EVALUATION OF REBAUDIOSIDE A DERIVED FROM STEVIA REBAUDIANA FOR USE AS A GENERAL PURPOSE NON-NUTRITIVE SWEETENER**"].

## F. Availability of Information

The data and information that serve as the basis for the GRAS determination, as well as the information that has become available since the GRAS determination, will be sent to the FDA

[1] The Panel consisted of Robert Nicolosi, Ph.D., John A Thomas, Ph.D., and David Bechtel, Ph.D., DABT.

upon request, or are available for the FDA's review and copying at reasonable times as arranged at Shanghai Freemen Americas, LLC, 377 Hoes Lane, Suite 306, Piscataway, NJ 08854: contact Tom Tchang [ttchang@shanghaifreemen.com]

## II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

### A. Identity

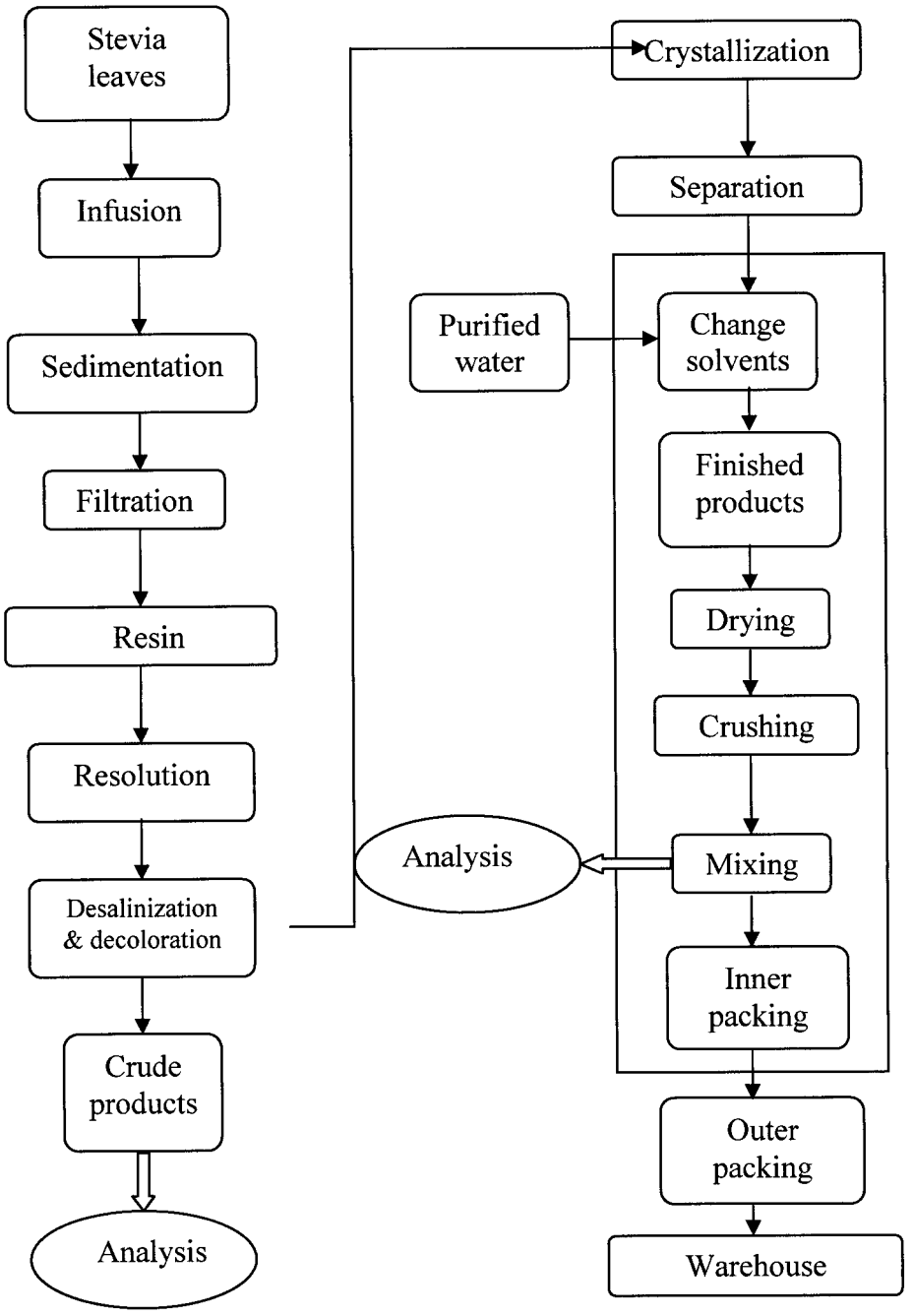
Rebaudioside A (19-O-beta-glucopyranosyl-13-O-(beta-glucopyranosyl(1-2)-beta-glucopyranosyl(1-3))-beta-glucopyranosyl-13-hydroxykaur-16-en-19-oic acid) is a glycoside from the stevia plant (*Stevia rebaudiana* Bertoni), a member of the Compositae (Asteraceae) family. Extracts of the plant, consisting of various combinations of its glycosides, have been consumed for hundreds of years by humans, and are used as sweeteners in South America, Japan, and China (Geuns *et al.*, 2003a; Brusick, 2008).

### B. Method of Manufacture

#### 1. Overview

An overview of the manufacturing process for Rebaudioside A derived from *Stevia rebaudiana* is shown below.

**Figure 1 Process used to isolate rebaudioside A from the stevia plant**




## 2. Product Characterization

### a. Raw Material Specifications

Product specifications for rebaudioside A derived from *Stevia rebaudiana* manufactured by Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. are shown below.

| Test Item | Specification |
|---|---|
| Appearance | White powder |
| Identification | The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the reference standard |
| Rebaudioside A, %(wt/wt) on anhydrous basis | ≥ 97.0 |
| Related steviol glycosides, % | ≤ 5 |
| Arsenic, ppm | ≤ 1 |
| Lead, ppm | ≤ 1 |
| Residual ethanol, % | ≤ 0.50 |
| Residual methanol, % | ≤ 0.020 |
| pH | 4.5-7.0 |
| Residue on Ignition, % | ≤ 1 |
| Water, % | ≤6 |
| Total plate count, CFU/g | ≤1000 |
| Mould and yeast, CFU/g | ≤100 |
| Escherichia coli, CFU/g | Absent |

Analysis of three non-consecutive batches is shown in Appendix 2. These data demonstrate that the manufacturing process described herein results in a product that consistently meets product specifications.

As shown in Table 1, Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. have adopted product specifications for its rebaudioside A product that meet or exceed JECFA recommendations and are substantially equivalent to other products determined to be GRAS.

**Table 1 Product Comparison**

| Test Item | JECFA Specification | Manufacturer | | | | |
|---|---|---|---|---|---|---|
| | | Zhucheng Haotian Pharm Co. | Whole Earth Sweetener Company LLC [1] | Cargill, Inc.[2] | Blue California[3] | Sweet Green Fields LLC[4] |
| Appearance | White to light yellow powder | White powder | White powder | White, loose powder or crystals | White powder | White to off-white powder |
| Solubility | Freely soluble in water and ethanol | NS | NS | Freely soluble in water | Soluble in water and alcohol | Up to 40% (w/v) in water at 25°C |
| pH | 4.5-7.0 | 4.5-7.0 | NS | 4.5-7.0 | 4.5-7.0 | 4.5-7.0 |
| Ash | < 1% | NS | < 0.2% | < 1.0% | < 1% | < 0.2% |
| Loss on Drying | < 6% | ≤ 6.0% | ≤ 5.0% | < 6.0% | ≤ 5.0% | < 6.0% |
| Residual Solvents | < 200 ppm methanol | < 200 ppm methanol<br>< 500 ppm ethanol | NS | < 200 ppm methanol<br>< 500 ppm ethanol | NA | ≤ 0.5% (w/w) ethanol<br>≤ 0.02%(w/w) methanol |
| Arsenic | < 1 ppm | < 1.0 ppm | < 0.1 ppm | NS | < 0.5 ppm | ≤ 1 ppm |
| Lead | < 1 ppm | < 1.0 ppm | < 1.0 ppm | < 1 ppm | < 0.5 ppm | ≤ 1 ppm |
| Specific optical rotation | NS | NS | -29 – -33 ° | -29 – -31 ° | NS | NS |
| Specific Absorbency | NS | NS | NS | NS | NS | NS |
| Total plate count | NS | ≤ 1000 cfu | < 1000 cfu | < 1000 cfu | < 3000 cfu/g | < $10^4$ cfu/g |
| Yeast/Mold | NS | < 100 cfu/g | < 100 cfu/g | < 100 cfu | < 100 CFU/g | <1000 cfu |
| Salmonella sp. | NS | NS | Negative/25 g | Negative/25 g | Negative | Negative |
| Coliforms | NS | NS | < 3 cfu/g | < 3 cfu | Negative | Negative |
| Escherichia coli | NS | Absent | NS | NS | NS | Negative |
| Rebaudioside A content | > 95% | ≥ 97.0% | > 97.0% | 97.0%-102.0% | ≥ 97.0% | > 97% |

NS = Not specified



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## III. BASIS FOR GRAS DETERMINATION

### A. Documentation to Support the Safety of Rebaudioside A Derived from *Stevia rebaudiana*

The determination that rebaudioside A derived from *Stevia rebaudiana* is GRAS is on the basis of scientific procedures. The information supporting the general recognition of the safe use of rebaudioside A derived from *Stevia rebaudiana* includes:

- Preclinical safety data available in the published scientific literature demonstrate rebaudioside A derived from *Stevia rebaudiana* exhibits low toxicity at levels well-above the estimated intakes from the intended uses specified herein;
- Supporting evidence for the safety of this ingredient in humans from clinical investigations of its use;
- Batch analysis data demonstrating that the manufacturing process results in a product that reproducibly meets specifications, and whose composition is substantially equivalent to rebaudioside A products tested in the published literature and determined to be GRAS;
- An estimate of rebaudioside A intake from the proposed uses is provided. Estimated intake is in the range considered safe in the scientific literature and is consistent with the acceptable daily intake (ADI) of 0 to 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).
- Accepted GRAS status of similar rebaudioside A products.

A summary of these data is presented herein. Moreover, these data were reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of rebaudioside A derived from *Stevia rebaudiana* as a component of food, who concluded that the proposed use of rebaudioside A derived from *Stevia rebaudiana* is safe and suitable and would be GRAS based on scientific procedures [see Appendix 1, entitled, "**GRAS EXPERT EVALUATION OF REBAUDIOSIDE A DERIVED FROM STEVIA REBAUDIANA FOR USE AS A GENERAL PURPOSE NON-NUTRITIVE SWEETENER"].**

## B. Estimated Intake of EPA-rich triglyceride oil

Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. are interested in establishing that rebaudioside A derived from *Stevia rebaudiana* is generally recognized as safe (GRAS) when used as a general purpose non-nutritive sweetener in a variety of food products in the United States. Rebaudioside A is intended for use at levels determined by current good manufacturing practices (GMPs) such that intakes are consistent with the ADI of 0 to 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).

Using published dietary exposure data for other high intensity sweeteners (*e.g.*, aspartame) with adjustment for their relative sweetness intensities, Renwick (2008) estimated rebaudioside A exposure in various population subgroups. Rebaudioside A was assumed to have relative sweetness 200 times that of sucrose. Table 2 shows the projected intakes by average and high consumers in different population groups.

**Table 2 Estimated Intake of Rebaudioside A by Population Group (mg/kg body weight/day)**

| Population group | Intake of intense sweeteners[1] | | Projected intakes of rebaudioside A | | Steviol equivalents[2] | |
|---|---|---|---|---|---|---|
| | Average Consumer | High Consumer | Average Consumer | High Consumer | Average Consumer | High Consumer |
| **Non-diabetic adults** | 255 | 675 | 1.3 | 3.4 | 0.4 | 1.1 |
| **Diabetic adults** | 280 | 897 | 1.4 | 4.5 | 0.5 | 1.5 |
| **Non-diabetic children** | 425 | 990 | 2.1 | 5.0 | 0.7 | 1.7 |
| **Diabetic children** | 672 | 908 | 3.4 | 4.5 | 1.1 | 1.5 |

[1] Expressed as sucrose equivalents.
[2] Values are estimated as one-third of rebaudioside A values since the molecular weight of rebaudioside A is about three times that of steviol (*i.e.*, 967 *vs.* 318).

As shown in the table above, the predicted rebaudioside A exposure is 1.3 mg/kg body weight/day for average consumers and 3.4 mg/kg body weight/day for high consumers. These intakes are equivalent to 0.4 and 1.1 mg/kg as steviol. Children and individuals with diabetes would be expected to have the highest exposures. These intakes are below the ADI established by JECFA for steviol glycosides expressed as steviol equivalents (JECFA, 2008).

Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. will notify each food manufacturer purchasing rebaudioside A that the use of this ingredient in foods is generally recognized as safe (GRAS), provided consumption of such foods does not result in daily intakes exceeding 4 mg steviol equivalents per kg body weight per person, as established by JECFA (2008).

As shown in Table 3, the intended uses and use levels for Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. rebaudioside A are similar to those for similar products considered to be GRAS.

**Table 3 Comparison of substantially equivalent products determined as GRAS**

| Manufacturer | Intended Uses | Use Levels | Estimated Exposure |
|---|---|---|---|
| Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. | as a general-purpose, non-nutritive sweetener in a variety of foods | at levels determined by current good manufacturing practices (GMPs) | Predicted rebaudioside A exposure is 1.3 mg/kg body weight/day for average consumers and 3.4 mg/kg body weight/day for high consumers |
| Whole Earth Sweetener Company LLC [1] | 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 of finished product | Estimated intake from all proposed uses is less than or equal to 2 mg/kg bw/day at the mean and 5 mg/kg bw/day for 90th percentile users |
| Cargill, Inc.[2] | as a general-purpose sweetener in foods, excluding meat and poultry products, provided that food standards of identity do not preclude such use | at levels determined by current good manufacturing practices | Mean intakes predicted to range from 1.3 mg/kg body weight/day for non-diabetic adults to 3.4 mg/kg body weight/day for children with diabetes. Predicted intakes for heavy intake consumers ranged from 3.4 mg/kg body weight/day for non-diabetic adults to 5.0 mg/kg body weight/day for non-diabetic children. |
| Blue California[3] | 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, a table top sweetener, and medical foods | at levels determined by good manufacturing practices | Total daily consumption of Reb A for defined food categories and as a general purpose sweetener is expected to be 5 mg/kg bw/day or less |
| Sweet Green Fields LLC[4] | as a general-purpose sweetener in foods, excluding meat and poultry products, provided that food standards of identity do not preclude such use | at levels determined by current good manufacturing practices | Predicted rebaudioside A exposure is 1.3 mg/kg body weight/day for average consumers and 3.4 mg/kg body weight/day for high consumers |

**Table 3 Comparison of substantially equivalent products determined as GRAS (continued)**

| Manufacturer | Intended Uses | Use Levels | Estimated Exposure |
|---|---|---|---|
| Sunwin USA LLC and WILD Flavors[5] | general-purpose sweetener in foods, excluding meat and poultry products | at levels determined by current good manufacturing practice | Estimated daily intakes (EDI) for rebaudioside A in the range of 0.4 - 1.6 mg/kg body weight per day (mg/kg bw/d), expressed as steviol equivalents. If adjustments are made for the increased sweetness of rebaudioside A alone compared to the mixed steviol glycosides, the EDI of rebaudioside A, would likely be about 0.5 to 0.8 mg/kg bw/d (expressed as steviol). |
| Pyure Brands LLC[6] | general-purpose sweetener in foods, excluding meat and poultry products, a table top sweetener and infant formulas | at levels determined by current good manufacturing practice | Estimated daily intakes (EDI) for rebaudioside A in the range of 0.4 - 1.6 mg/kg body weight per day (mg/kg bw/d), expressed as steviol equivalents. If adjustments are made for the increased sweetness of rebaudioside A alone compared to the mixed steviol glycosides, the EDI of rebaudioside A, would likely be about 0.5 to 0.8 mg/kg bw/d (expressed as steviol). |

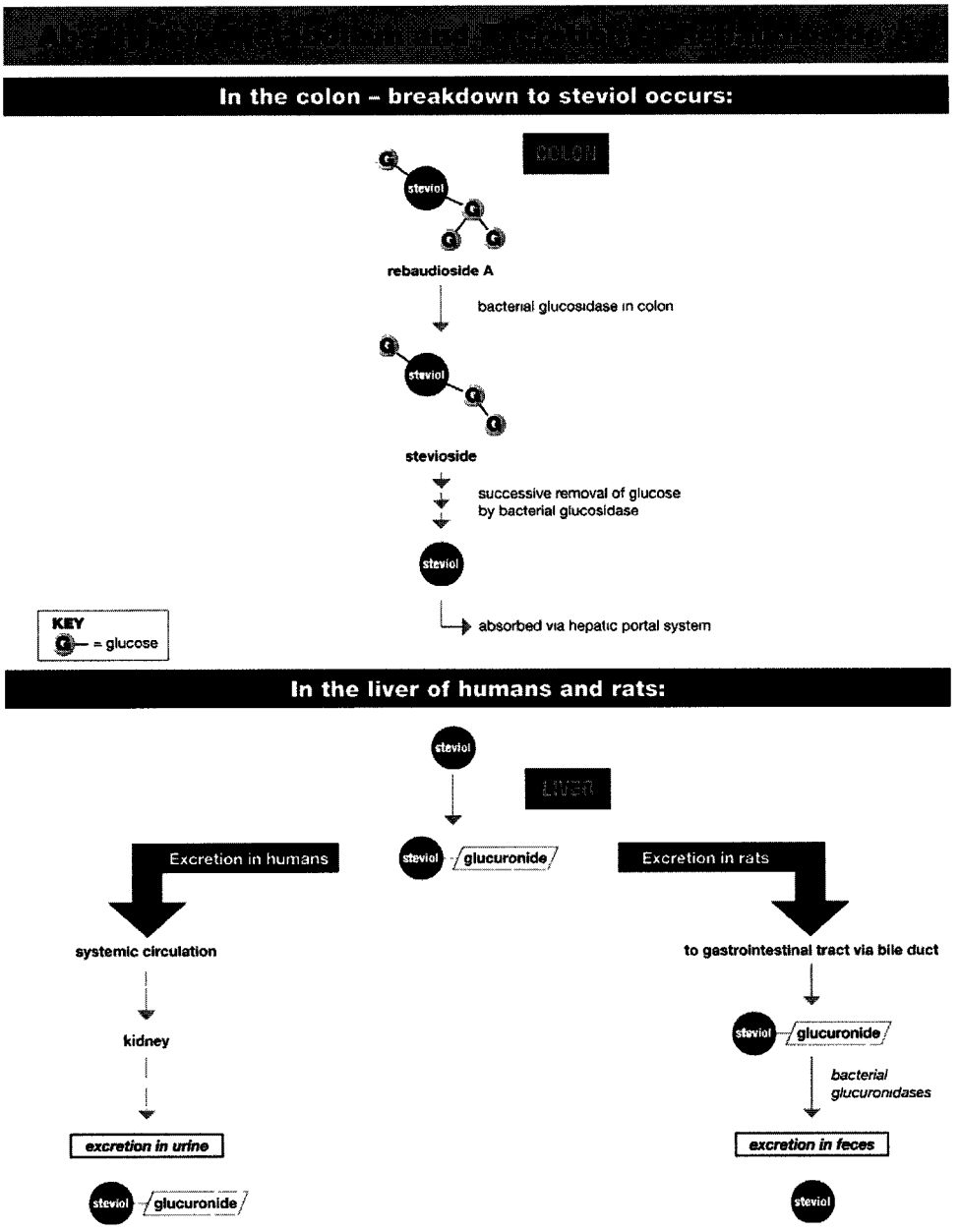
[1]Adapated from GRAS Notice 252
[2]Adapted from GRAS Notice 253
[3]Adapted from GRAS Notice 278
[4]Adapted from GRAS Notice 282
[5]Adapted from GRAS Notice 303
[6]Adapted from GRAS Notice 318

## C. Absorption, Distribution, Metabolism, and Excretion of Rebaudioside A

Like all steviol glycosides, rebaudioside A is resistant to degradation by digestive enzymes of the mouth, stomach, and small intestine (Koyama *et al.*, 2003b; Hutapea *et al.*, 1997). Instead, steviol glycosides are hydrolyzed to the aglycone steviol by colonic microflora *via* successive removal of glucose units (Gardana *et al.*, 2003; Geuns *et al.*, 2003a,b, 2007); this hydrolysis, which is necessary for absorption of steviol derived from rebaudioside, has been observed following incubation with intestinal microflora from the rat cecum (Wingard *et al.*, 1980) and human fecal bacteria (Gardana *et al.*, 2003; Koyama *et al.*, 2003b), with the bacteroides being the most efficient at this hydrolysis. Hydrolysis of rebaudioside A proceeds *via* initial formation of either stevioside (major pathway) or rebaudioside B (minor pathway), which are further degraded to steviol. Stevioside is further degraded to steviolbioside, steviolmonosides, and finally steviol, with glucose released with each sequential hydrolysis (Nakayama *et al.,* 1986; Koyama *et al., 2003a*). The rate of hydrolysis of stevioside is greater than that of rebaudioside A, due partly to the presence of 1 additional glucose moiety in the latter and to differences in structural complexities (Wingard *et al.*, 1980; Koyama *et al.*, 2003b).

Studies with intact steviol glycosides demonstrate that the degree of transport across the intestinal epithelium is very low for steviol glycosides, whereas *in vitro* and *ex vivo* studies confirmed that steviol is rapidly absorbed from the gastrointestinal tract (Geuns *et al.,* 2003b; Koyama *et al.,* 2003b). Following absorption, steviol is transported to the liver by portal blood. Steviol glucuronide is formed in the liver, then is released into the blood (Koyama *et al.,* 2003b). In rats, steviol, administered as steviol or available following cleavage of glycosides in the gut, has been shown to be primarily excreted in the feces *via* the bile (generally within 48 hours), with smaller amounts in the urine (less than 3%) (Wingard *et al.,* 1980; Nakayama *et al.,* 1986; Sung, 2002; Roberts and Renwick, 2008). In contrast, urinary excretion of steviol glucuronide predominates in humans (Kraemer and Maurer, 1994; Geuns and Pietta, 2004; Geuns *et al.,* 2006, 2007; Wheeler *et al.,* 2008). This inter-species difference in the route of elimination of systemically absorbed steviol as steviol glucuronide *(via* the bile in rats and in the urine in humans) is due to the lower molecular weight threshold for biliary excretion in rats (325 Da) compared to humans (500 to 600 Da; molecular weight of steviol glucuronide is 495 Da) (Renwick, 2008).

**Figure 2** **Metabolism of rebaudioside A in rats and humans (From Carakostas *et al.*, 2008)**




## D. Preclinical Studies Pertaining to the Safe Consumption of Rebaudioside A

Considerations and information related to the preclinical safety of rebaudioside A derived from *Stevia rebaudiana* are presented in Sections D.1 through D.4 and provides a summary of the acute, subacute, subchronic or chronic toxicity, genotoxicity, mutagenicity, reproductive or developmental toxicity, and carcinogenicity of rebaudioside A and related compounds. The composition of Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd.

rebaudioside A product currently being considered for GRAS determination is substantially equivalent to the test substances in these studies, thus the data generated in these studies support its safety. In addition, due to the similarities in the metabolic pathway, an overview of safety data for stevioside and steviol are also presented herein (Section D5) to support the safety of rebaudioside A.

## 1. Genotoxicity

Several studies examining the potential genotoxicity of rebaudisoide A were reviewed by Williams and Burdock (2009). Rebaudioside A (Reb A) was evaluated for mutagenic activity in the bacterial reverse mutation test, according to the OECD Test guideline 471. *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537 and *Echerichia coli* WP2uvrA were used. Cells were incubated with Reb A at concentrations of 31.6, 100, 316, 1000, 2500, and 5000 µg/plate in the presence and absence of liver microsomal activation. There were no statistically significant increases in the number of revertant colonies or cytotoxicity in any *S. typhimurium* or *E. coli* strain exposed to Reb A, with or without metabolic activation and Reb A was considered non-mutagenic (Williams and Burdock, 2009).

A chromosome aberration test was also conducted in compliance with OECD guideline 473. Potential mutagenicity of Reb A was evaluated in cells exposed to Reb A for 4 h at concentrations of 1000, 2500, and 5000 µg/mL and 20 h at concentrations of 1000, 2500, and 5000 µg/mL, in the presence and absence of liver microsomal activation. There was no statistically significant increase in the incidence of chromosomal aberrations or polyploidy in cultured Chinese Hamster V79 cells (Williams and Burdock, 2009).

Adult NMRI mice were used in the mammalian erythrocyte micronucleus test (OECD guideline 474). Treatment groups (5 animals/sex) received intraperitoneal injections of Reb A at doses of 150, 375, or 750 mg/kg bw. Samples of peripheral blood were collected from the tail vein 44 or 68 h after treatment. Results showed animals in the highest dose group exhibited signs of toxicity in the form of reduction of spontaneous activity, rough fur, prone position, and cramps. Reb A did not induce any statistically significant increases in the incidence of polychromatic immature erythrocytes or micronucleated immature erythrocytes and was non-mutagenic and non-cytotoxic at doses 150, 375, or 750 mg/kg bw (Williams and Burdock, 2009).

An *in vitro* mammalian cell gene mutation test was also used to evaluate the mutagenicity of Reb A in mouse lymphoma L5178Y +/- cells (OECD guideline 476). Cells were exposed to Reb A for 4 h at concentrations of 10, 39, 156, 313, 625, 1250, 2500, and 5000 µg/mL with and without S9. Cells were also exposed for 24 h at concentrations of 100, 200, 400, 800, 1500, 2500, 3750, and 5000 µg/mL with S9 and 20, 39,156, 625, 1250, 4000, 4500, and 5000 µg/mL without S9. Results indicated Reb A was non-mutagenic in mouse lymphoma L5178Y +/- cells. The unscheduled DNA synthesis test (OECD guideline 486) assessed genotoxicity in male Wistar rats. Groups (4 *per* group) were treated *via* gavage with a single dose of Reb A at 2000

mg/kg bw. Following exposure, rats were anaesthetized and hepatocytes were isolated from the liver. Results showed no treatment-related signs of toxicity in the rats when observed for 2 and 16h post-dose. There were no UDS induction in the hepatocytes of the animals and Reb A was considered non-genotoxic (Williams and Burdock, 2009).

Additional studies of the genotoxicity of rebaudioside A derived from *Stevia rebaudiana* identified in the published literature are summarized in Table 4. The results of these studies indicate that rebaudioside A is not mutagenic or clastogenic.

**Table 4 Genotoxicity/mutagenicity studies of rebaudioside A derived from *Stevia rebaudiana***

| Reference | Study Design | Results |
|---|---|---|
| Nakajima (2000a) | Rebaudioside A (at concentrations ranging from 1.2-55 mg/mL) was examined for its ability to induce chromosome aberrations in CHL/IU Chinese hamster lung fibroblasts both in the presence and absence metabolic activation. | Negative |
| Nakajima (2000b) | In an *in vivo* bone marrow micronucleus test, BDF1 mice were administered rebaudioside A at doses of 500 to 2000 mg/kg body weight (bw) per day for 2 days. Animals were killed 30 h after second administration. Bone marrow samples were analyzed for micronuclei. | Negative |
| Pezzuto *et al.*, 1985a | The mutagenicity of rebaudioside A (purity not specified) was examined in a forward mutation assay (maximum concentration 10.0 mg/mL) with *Salmonella typhimurium* TM677 in the presence and absence of metabolic activation. | Negative |
| Medon *et al.*, 1982 | The mutagenicity of rebaudioside A (purity not specified) was examined in a forward mutation assay (test concentrations not reported) with *Salmonella typhimurium* TM677 in the presence and absence of metabolic activation. | Negative |

## 2. Acute Toxicity

Rebaudioside A (purity not specified) in 1% carboxymethylcellulose was administered *via* gastric intubation to an unspecified number of male Swiss-Webster mice, with no evidence of toxicity at doses of up to 2000 mg/kg body weight. Two weeks after dose administration, no significant differences were noted at necropsy in body or organ weights in comparison to the control group (Medon *et al.,* 1982).

## 3. Subchronic Toxicity

The subchronic oral toxicity of rebaudioside A was evaluated in a 4-week GLP-compliant study. Wistar rats ranged in age from 40 to 46 days old at the start of treatment. Body weights ranged from 125 to 167 g in males and from 98 to 140 g in females. Both food (Rat and Mouse No. 1 Maintenance diet from Special Diet Services, Witham, Essex, England) and water were provided ad libitum except during urine collection and overnight prior to blood sampling. The

rats were housed individually in polycarbonate cages with stainless steel mesh lids and allowed to acclimatize to the study conditions for 11 days before treatment commenced. Ten rats per sex received rebaudioside A in the diet at target concentrations of 0, 25,000, 50,000, 75,000, or 100,000 ppm for 4 weeks. Males consumed mean doses of 0, 3,455, 6,981, 10,180, and 14,167 mg/kg bw/day during week 1, and 0, 2,610, 5,505, 8,248, and 11,672 mg/kg bw/day in week 4. Females achieved mean doses of 0, 3,724, 7,125, 10,789, and 14,119 mg/kg bw/day during the first week of the study and 0, 2,963, 6,217, 9,189, and 13,126 mg/kg bw/day during week 4. There were no deaths or signs of adverse reactions. Reduced body weight gains, food consumption and food conversion efficiency were attributed to poor palatability, particularly early in the study, and to the decreased caloric value of the diet. No toxicologically significant changes related to rebaudioside A were observed in hematology parameters. Small but statistically significant changes in plasma creatinine were observed in all treated males and in females at the 75,000 and 100,000 ppm dose levels. These increases were accompanied by a significant increase in urine specific gravity in males at the 75,000 and 100,000 ppm dose levels, and in all treated females. Although decreases in bile acids were seen at the two highest doses in males and in all treated females compared to controls, no dose-response pattern was seen. Furthermore, after removing atypically high values from the control groups, no clear biologically significant effect of treatment was seen. Males in the two highest dose groups had significantly lower relative heart weights compared to controls; absolute testes weight were also lower at the highest dose. Lower relative adrenal weights were seen at the three highest dose groups in females, but these decreases did not follow a dose-response pattern. Upon histopathological examination, however, no corresponding changes were seen in any of the organs. Based on these data, the authors considered the no-observable-adverse-effect level (NOAEL) to be 100,000 ppm (Curry and Roberts, 2008).

In a 13-week study, 20 rats/sex/group consumed diets containing 0, 12,500, 25,000, and 50,000 ppm rebaudioside A (97% purity). Mean consumption at week 1 was reported to be 0,1,506, 3,040, and 5,828 mg/kg/day in males and 0, 1,410, 2,841, and 5,512 mg/kg/day in females, while at week 4, mean intakes were 698, 1,473, and 3,147 mg/kg bw/day and 908, 1,914, and 3704 mg/kg bw/day in males and females, respectively. There were no treatment-related deaths or changes in appearance. No dose-dependent effects on behavior or sensory reactivity were observed. No differences were seen between high-dose animals and controls in opththalmological findings. Body weight gains were significantly lower in males and females at the mid- and high-dose levels during the first 4 days of treatment. Body weight gains remained significantly lower in all male groups compared to controls until study termination. Body weight gains were slightly but significantly lower in females at the 25,000 and 50,000 ppm levels. As in the 4-week study, these reductions were attributed to poor palatability and to the decreased caloric value of the diet. No toxicologically significant changes related to rebaudioside A were observed in hematology parameters. Plasma urea and creatinine results, as well as urine specific gravity tended to be higher in both treated males and females, while urine volume was lower, especially in the higher dose groups. However, in light of other observations (*e.g.,* lack of

histopathological findings), these changes were considered to be representative of a physiological rather than toxicological effect. Lower levels of bile acids were observed in all treatment groups at various time points throughout the study, while plasma triglycerides and cholesterol levels were lower in some groups. Other statistically significant changes were seen in only one sex, did not follow a temporal or dose-dependent pattern, and/or were within the historical control values and thus were not considered to be treatment-related. Although sporadic organ weight findings were observed in high-dose males and females, they were considered to be of no toxicological significance, as no corresponding treatment-related histopathological findings were seen. The NOAEL was considered to be 50,000 ppm, or approximately 4,161 and 4,645 mg/kg bw/day in male and female rats, respectively (Curry and Roberts, 2008).

In a GLP-compliant 13-week study, Nikiforov and Eapen (2008) administered rebaudioside A (99.5% purity) in the diet to Sprague Dawley rats (20 sex/group) at concentrations intended to provide 500, 1000, or 2000 mg/kg bw/day. All animals survived the duration of the study. No treatment-related adverse effects on body weight gain, terminal body weights, clinical and functional observations, or the results of hematology, serum chemistry, or urinalysis parameters were observed. Likewise, no treatment-related effects on organ weights and no macroscopic or histopathological findings were observed. The NOAEL in this study was considered to be 2000 mg/kg bw/day, the highest dose tested.

## 4. Reproductive and Developmental Toxicity

Curry *et al.* (2008) performed a two-generation reproductive study with rebaudioside A. Male and female Han Wistar rats (30 animals/sex/group) received SDS VRF1 powdered certified rodent chow (Special Diet Services, Witham, Essex) diets supplemented with 0, 7500, 12,500 or 25,000 ppm rebaudioside from 10 weeks prior to mating until the end of lactation. Prior to mating, the rats were housed individually in 2154 polycarbonate cages. During the mating period one male and one female per cage were housed in RB3 polypropylene modified cages. For the remainder of their lifespan, males were housed individually and females were housed with their litter in polycarbonate cages until weaning. During the post-weaning phase, unselected offspring were housed in a polycarbonate cage with their littermates until necropsy.

No adverse effects on body weight, body weight gain, or food consumption were seen. Likewise, no treatment-related effects on mating performance, fertility, gestation lengths, estrous cycles, or sperm motility were seen in either the $F_0$ or $F_1$ generation. There were no significant differences in the mean numbers of implantations per litter, total litter sizes, live litter sizes on day 1 or day 25. Post-implantation survival index, live birth index, viability index, and lactation index, and sex ratio were unaffected.

Pups from the first generation ($F_1$) that were selected for continued dosing were weaned to the respective test diet and continued on that diet through mating, gestation, and lactation; $F_2$

animals were sacrificed on day 30 after birth. Survival of the $F_1$ and $F_2$ offspring and development (*e.g.*, pre-weaning body weight gains, timing of sexual maturation, overall body weight gains) were not adversely affected. No significant treatment-related gross or histopathological abnormalities were observed in select tissues. The authors concluded that the NOAEL for reproductive effects and for survival, development, and general condition of the offspring was 25,000 ppm, corresponding to intakes of 2048 to 2273 mg/kg bw/day in the $F_1$ and $F_2$ generations, respectively.

The absence of adverse effects on testes weight and spermatogenesis and the lack of testicular atrophy observed in the subchronic studies of Curry and Roberts (2008) provide supporting evidence that rebaudioside A had no effects on male reproduction.

## 5. Overview of Safety Data for Stevioside and Steviol

### a. Mutagenicity and Genotoxicity

Despite the report of mutagenic activity of steviol and some of its derivatives in *Salmonella typhimurium* strain TM677 (Matsui, 1996; Terai *et al.*, 2002), positive activity was not reproduced in a strain with normal DNA repair processes.

The findings of a single positive *in vivo* study measuring single-strand DNA breaks in the tissues of Wistar rats receiving stevioside (Nunes, 2007) were not confirmed in experiments in mice and might have measured processes other than direct DNA damage. In addition, the study was criticized because it lacked adequate controls and because stevioside failed to induce DNA strand breaks *in vitro* or in other *in vivo* studies at higher doses (Carakostas *et al.*, 2008).

JECFA (1999) noted that steviol was clearly genotoxic after metabolic activation *in vitro* and concluded at that time that the mutagenic potential of steviol had only been tested sufficiently *in vitro*. After review of new studies of DNA damage and micronucleus formation in rats, mice, and hamsters *in vivo*, JECFA (2006) concluded that steviol does not induce genotoxicity at doses of up to 8000 mg/kg bw. Similarly, based on an evaluation of the data and application of a weight-of-evidence approach, Brusick (2008) concluded that steviosides do not pose a risk of genetic damage following human consumption.

### b. Acute Toxicity

No toxicity was seen following the administration of a single 2.0 g/kg bw dose of various sweet principles isolated from *Stevia rebaudiana* (*i.e,* stevioside, steviobioside, dulcoside, and rebaudsiosides A, B, C, D, and E) in 1% carboxymethylcellulose *via* oral gavage to Swiss Webster mice. Necropsies were performed 2 weeks after administration. No significant differences in body weight or the weights of the heart, spleen, lung, pancreas, brain, kidney, or liver were seen relative to controls (Medon *et al.,* 1982). Haruo and Yukio (1975) reported an oral $LD_{50}$ of 17.07 g/kg bw in DDY-N mice.

Toskulkao *et al.* (1997) investigated the acute toxicity of steviol (90% purity) and stevioside (96% purity) in Wistar rats (50 to 60 g), Syrian golden hamsters (80 to 100 g), and Swiss albino mice (25 to 35 g). Intragastrically administered stevioside, at a dose of 15 g/kg bw, was not lethal to mice, rats, or hamsters. While similar results were obtained with steviol in the mouse and rat, the acute oral $LD_{50}$ of steviol in male and female hamsters was 5.20 and 6.10 g/kg bw, respectively. Although no lethality was seen at steviol doses up to 14 g/kg bw/day, one rat and one mouse per sex died at a dose of 15 g/kg bw/day and signs of toxicity (*e.g.*, a decline of spontaneous movement, difficulty walking, drowsiness) was seen in other animals at this dose. Additional analysis in hamsters revealed increases in serum blood urea nitrogen and creatinine, along with degeneration of the renal proximal tubular cells, suggesting that acute steviol-related renal failure may have been the cause of death in these animals. In rats and mice, histopathological lesions were absent or less severe than those in hamsters at doses of steviol of 15 g/kg bw.

### c. Subchronic Toxicity Studies

Xili *et al.* (1992) assessed the toxicity of stevioside (85% purity) administered in the diet to male and female Wistar rats (10/sex/group) at 3 to 5% for 90 days. No significant effects on body weight gain, food conversion efficiency, or signs of toxicity or abnormal behavior were observed. No changes in blood glucose levels were noted. Similarly, Haruo and Yukio (1975) reported that stevioside, administered orally at 3000 mg/kg bw/day for 3 months, produced no adverse effects in rats. Aze *et al.* (1991) reported no adverse effects following dietary administration of a 95.6% pure stevioside preparation to rats at dose levels of 0.31, 0.62, 1.25, 2.5, or 5% for 13 weeks.

### d. Chronic Toxicity and Carcinogenicity Studies

The chronic toxicity and carcinogenic potential of stevia extracts was examined by Yamada *et al.* (1985). Male and female F344 rats (70 rats/sex/group) received 0, 0.1, 0.3, or 1% stevia extracts (95.2% purity, 74.54% stevioside and 16.27% rebaudioside A) for 22 months and 24 months, respectively. No significant differences were noted between control and test animals in survival, general appearance, or behavior. Although slight growth retardation was seen at the mid-dose in both sexes, growth in the high-dose group was reduced only transiently. Several significant changes were observed in urinary, hematological, and blood chemistry evaluations and in organ weights at the 6-month interim sacrifice. However, no significant differences were seen at the 12-month sacrifice or at the end of the study. No dose-related effects on the incidence or severity of neoplastic and non-neoplastic pathological findings were seen. The no-observed effect level (NOEL) was considered to be 1% in the diet, which corresponded to an intake of 550 mg stevia extract/kg bw/day.

Xili *et al.* (1992) examined the chronic toxicity and carcinogenicity of stevioside. Male and female Wistar rats (45/sex/group) received diets containing 0, 0.2, 0.6, or 1.2 % stevioside (85% purity) for up to 2 years. Interim sacrifices of 5 rats/group were performed after 6 and 12 months of treatment. No treatment-related effects on body weight gain or food consumption or utilization

were reported. Mortality and clinical signs were similar across all groups, including controls. No dose-related, biologically significant effects on hematology or clinical biochemical parameters were observed. Likewise, no significant effects on relative organ weights were found between treated rats and controls. No lesions related to treatment with stevioside were found upon histopathological examination. The incidence of benign and malignant neoplasms was similar in treated and control groups in both sexes. Based on these data, the authors concluded the NOEL of stevioside was 1.2%, corresponding to an estimated intake of 748.8 mg/kg bw/day in males and 838,9 mg/kg/day in females.

Similarly, Toyoda *et al.* (1997) administered stevioside (95.6% purity) in the diet to male and female F344 rats (50 sex/group) for 104 weeks at concentrations of 0, 2.5, and 5% in the diet. Surviving rats were sacrificed at 108 weeks. A dose-related reduction in body weight gain was reported. High-dose males had a significantly lower survival rate compared to controls. The authors attributed this effect to the development of large granular lymphocyte (LGL) type leukemia in stevia-treated animals compared to controls. However, the final incidences of LGL-leukemia were in line with historical control data and were therefore not considered to be related to stevioside. No effects on hematological findings were seen. Absolute kidney weights were significantly reduced in high-dose males, while absolute kidney and left ovary weights and relative brain weights were increased in high-dose females compared to controls. Stevioside treatment was not associated with any significant increases in the incidence of neoplastic or non-neoplastic lesions. Decreases in the incidence of mammary tumors in stevia-treated males and the reduced severity of chronic nephropathy were attributed to reduced body weights related to the lower energy content of the stevia-supplemented diets. Based on these data, stevioside was considered to be non-carcinogenic under the conditions of this experiment. The NOAEL for stevioside in this study was considered to be 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol).

**e. Reproductive/Developmental Toxicity Studies**

A number of studies conducted with steviol, stevia leaves, or crude extracts of *Stevia rebaudiana* have reported adverse effects on fertility, reproductive structures, or development (Mazei-Planas and Kuc, 1968; Wasuntarawat *et al.*, 1998; Melis, 1999). However, the relevance of these studies to the safety assessment of purified rebaudioside is considered questionable because of the lack of characterization of the crude extracts and the use of protocols that do not meet regulatory guidelines (Curry *et al.*, 2008).

Stevioside reportedly had no effect on growth or reproduction in a 3-generation hamster study employing doses of 0, 500, 1000, and 2500 mg/kg bw/day. The duration of pregnancy, the number of fetuses, and the number of live young were not significantly affected by stevioside. Likewise, no evidence of abnormalities was seen in the reproductive tissues of any of the 3 generations upon histopathological examination (Yodyingyuad and Bunyawong, 1991). No effects were seen in developmental studies in pregnant rats with stevioside doses up to 1000

mg/kg bw/day (Takanaka *et al.*, 1991; Usami *et al.*, 1995). Administration of stevioside (95.98% purity) at doses of 0.15, 0.75, or 3% in the diet to male rats for 60 days and to female rats for 14 days prior to mating had no adverse effects on pregnancy rate or fetal development (Mori *et al*, 1981).

An aqueous extract of *Stevia rebaudiana* was negative in a dominant lethal test in rats. The extract had no toxic effect on male reproduction (sperm count, morphology, and motility) or progeny outcome (*i.e.*, number of implantation sites, number of viable fetuses, and number of dead fetuses). No increase in fetal malformations was observed compared with controls (Aritajat *et al.*, 2000).

## E. Clinical Safety of Rebaudioside A and Related Compounds

### 1. Summary of Clinical Data

Data from clinical trials examining the effects of stevia, stevioside, and rebaudioside A are presented in Table 5.

**Table 5 Clinical trials with stevia products**

| Study Design | Subjects/Dosing Regimen | Parameters Measured | Results | Reference |
|---|---|---|---|---|
| Multicenter, randomized, double-blind, placebo-controlled | 106 hypertensive patients 250 mg stevioside or placebo three times daily for 1 year | Systolic and diastolic blood pressure, blood biochemistry parameters | Significant decreases in systolic and diastolic blood pressure were seen after 3 months of treatment. Mean reductions were 12 mmHg in systolic blood pressure and 8 mmHg in diastolic blood pressure. This effect persisted for the duration of the study. Stevioside was considered to be well-tolerated and had no negative effects on subjective quality of life measures. No significant differences were noted in select hematological and biochemical parameters. | Chan *et al.* (2000) |
| Placebo-controlled, double-blind | 49 hyperlipidemic patients two 50 mg Stevioside capsules twice daily for 90 days | BMI, select biochemical parameters | No clinically relevant effects were seen on BMI, liver enzymes, cholesterol levels (total, high-, low-, and very-low density lipoprotein, triglycerides, glucose). No adverse effects were reported. | Cavalcante da Silva *et al.*, 2006 |
| Double-blind | 60 healthy volunteers, 0, 50, or 200 mg stevia extract under fed and fasting conditions. Low and high dose corresponded to 27.7 and 110.8 mg stevioside, respectively | Metabolic parameters | Dosing with stevia after overnight fast reduced plasma glucose and insulin levels, particularly at the higher dose. Post-prandial dosing increased plasma free fatty acids. Plasma potassium concentrations increased after both dosing periods. A decrease in heart rate and reduced arterial pressure was also observed post-prandially. All modifications remained within physiological limits. | Boeckh Habisch *et al.*, 1992 |
| Randomized, double-blind, placebo-controlled | Healthy subjects (n=30), subjects with type 1 diabetes (n=16) and subjects with type 2 diabetes (n=30); 250 mg stevioside three times/day for 3 months) | Blood pressure and blood glucose | Post-treatment systolic BP, diastolic BP, glucose and glycated hemoglobin (HbA1c) were not significantly different from baseline measurements, except for the placebo Type 1 diabetics group where a significant difference was observed for systolic BP and glucose. No side effects were observed in the two treatment groups. The authors concluded that oral steviol glycosides were well-tolerated and had no pharmacological effect . | Barriocanal *et al.*, 2008 |

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| Study Design | Subjects/Dosing Regimen | Parameters Measured | Results | Reference |
|---|---|---|---|---|
| Open label | 9 healthy subjects; 250 mg stevioside 3 times daily for 3 days | Blood pressure, urinary excretion of electrolytes, blood glucose/insulin, liver enzymes | No significant effects were seen in mean blood glucose and insulin levels. Urine volume and urinary excretion of liver enzymes tended to be higher after stevioside, but these differences did not reach statistical significance. No effect on liver enzyme levels was observed. | Temme *et al.*, 2004 |
| Randomized, double-blind, placebo-controlled | 122 Type 2 diabetics, 0 or 1000 mg rebaudioside A daily for 16 weeks | Glycosylated hemoglobin, blood glucose, insulin, C-peptide, blood pressure, body weight, lipids, select hematology, serum chemistry, and urinalysis parameters | Glycosylated hemoglobin levels did not differ significantly between the rebaudioside A and placebo groups. Changes from baseline for rebaudioside A and placebo, in fasting glucose, insulin, and C-peptide did not differ significantly. Assessments of changes in blood pressure, body weight, and fasting lipids indicated no differences by treatment. Although significant increases in the mean levels of alanine transaminase and gamma-glutamyl transferase were seen from baseline in the rebaudioside group, changes were small, within normal range, and judged to be of no clinical significance. Rebaudioside A was well-tolerated, and records of hypoglycemic episodes showed no excess vs. placebo. Authors concluded that results suggested that chronic use of 1000 mg rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus. | Maki *et al.*, 2008a |
| Randomized, double-blind, placebo-controlled | 100 healthy adults with normal and low-normal blood pressure; 1000 mg/day rebaudioside A or placebo for 4 weeks | Blood pressure, mean arterial pressure, heart rate | Rebaudioside A had no significant affect on resting, seated systolic or diastolic blood pressure, mean arterial pressure, heart rate, or 24-h ambulatory blood pressures responses compared with placebo, indicating that consumption of as much as 1000 mg/day of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure. | Maki *et al.*, 2008b |

000026

| Study Design | Subjects/Dosing Regimen | Parameters Measured | Results | Reference |
|---|---|---|---|---|
| Randomized, double-blinded, placebo-controlled | 55 Type 2 diabetics received 500 mg stevioside or placebo three times a day for 3 months | Glycemic control, insulin sensitivity, hormone responses, lipids, weight development, and diurnal blood pressure | Glycosylated hemoglobin and fasting blood glucose levels were significantly increased in the placebo group, but was unchanged in the stevioside group. The placebo group had a decrease in insulin level in the meal test, while no such effect was noted in the stevioside group. Insulin response was higher in the stevioside group in the glucose tolerance test compared to placebo. No difference in lipids or blood pressure was observed. | Jeppesen *et al.*, 2006 |
| Randomized, double-blinded, placebo-controlled | 174 patients with mild essential hypertension; 500 mg stevioside or placebo three times daily for 2 years | Blood pressure, left ventricular mass index, quality of life | After 2 years, the stevioside group had significant decreases in mean systolic and diastolic blood pressure compared with baseline and compared with placebo. There were no significant changes in body mass index or blood biochemistry, and the results of laboratory tests were similar in the 2 groups throughout the study. No significant difference in the incidence of adverse effects was noted between groups, and quality of life scores were significantly improved overall with stevioside compared with placebo. Neither group had a significant change in mean left ventricular mass index, however, after 2 years, 6 of 52 patients (11.5%) in the stevioside group had left ventricular hypertrophy (LVH), compared with 17 of 50 patients (34.0%) in the placebo group. Of those who did not have LVH at baseline, 3 of 46 patients (6.5%) in the stevioside group had developed LVH after 2 years, compared with 9 of 37 patients (24.3%) in the placebo group ($P < 0.001$). | Hsieh *et al.*, 2003 |

000027

| Study Design | Subjects/Dosing Regimen | Parameters Measured | Results | Reference |
|---|---|---|---|---|
| Acute, paired, cross-over | 12 Type 2 diabetic subjects; standard meal with 1 g stevioside or placebo | Post-prandial blood glucose | Compared to control, stevioside significantly reduced the incremental area under the glucose response curve. The insulinogenic index ($AUC_{(i,insulin)}/AUC_{(i,glucose)}$) was significantly increased by stevioside compared to control. Stevioside tended to decrease glucagon levels although it did not significantly alter the area under the insulin, glucagon-like peptide 1, and glucose-dependent insulinotropic polypeptide curves. The authors concluded stevioside reduces postprandial blood glucose levels in type 2 diabetic patients, indicating beneficial effects on glucose metabolism. | Gregersen *et al.*, 2004 |
| Acute, placebo-controlled | 22 healthy volunteers administered aqueous extracts of stevia leaves (20/g day as 5 g every 6 hours) or placebo for 3 days | Glucose tolerance | Plasma glucose levels were significantly lower in stevia-treated subjects than controls at all time points tested in the glucose tolerance test. | Curi *et al.*, 1986 |
| Randomized, double-blinded, placebo-controlled multiphase trial | 14 patients with mild essential hypertension; stevioside capsules or placebo were consumed twice daily before lunch and before dinner for 24 weeks. Dose levels of crude stevioside were 3.75 mg/kg/day for the first 7 weeks (Phase 1), were raised to 7.5 mg/kg/day for the next 11 weeks (Phase 2), and to 15.0 mg/kg/day for the last 6 weeks (Phase 3) | Blood pressure, body mass index, electrocardiogram, hematology, clinical chemistry, and urinalysis | Systolic and diastolic BP decreased significantly during the treatment with crude stevioside compared with baseline, but a similar effect was observed in the placebo group. As a result, the authors concluded that crude stevioside up to 15.0 mg/kg/day did not show an antihypertensive effect. No changes in electrocardiograms, blood, or urine analysis were seen. | Ferri *et al.*, 2006 |

000028

## 2. Additional Considerations

Effects on blood pressure and blood glucose concentrations have been reported following oral administration of steviol and stevioside in both experimental animals and humans. As such, a review of literature pertaining to these effects is provided herein.

### a. Effects on Glucose and Insulin

Jeppesen *et al.* (2002) studied the effects of stevioside in nonhypertensive, diabetic Goto-Kakizaki rats. Stevioside (>99.6% purity) was administered orally at a dosage of 25 mg/kg/day for 6 weeks. Blood samples were taken from 15 minutes prior to and 180 minutes after bolus injection of glucose. Prior to glucose injection, there was no significant difference in plasma glucose or insulin levels in treated vs. control groups. After glucose injection, plasma glucose was decreased in the treated group relative to controls. Plasma insulin was also increased in the treated group relative to controls. A concomitant suppression of glucagon was also seen in the treated group.

Chang *et al.* (2005) reported increased insulin sensitivity in male Wistar rats maintained on high-fructose diets treated with a single oral dose of stevioside. In addition, the authors reported that repeated oral administration of stevioside delayed the development of insulin resistance. Oral administration of stevioside at 0.2 mg/kg three times daily to streptozotocin-diabetic rats for 10 days increased the response to exogenous insulin (Chang *et al.,* 2005). In contrast, feeding of 0.025 g rebaudioside A/kg bw twice daily for 8 weeks produced no effects on glycemic control in a rat model of type 2 diabetes (Dyrskrog *et al.,* 2005). Xili *et al.* (1992) reported no change in blood glucose in rats fed a diet containing 1.2% stevioside for 24 months. Similarly, plasma glucose levels were unaffected in a rat 90-day toxicity study with rebaudioside A at dose levels up to 2000 mg/kg bw/day (Nikiforov and Eapen, 2008).

Figlewicz *et al.* (2009) compared the effects of fructose-containing beverages on feeding behavior, metabolic and serum lipid profiles, and hepatic histology and serum liver enzymes to those of non-caloric sweetened beverages. Iso-caloric solutions containing agave, fructose, high-fructose corn syrup (HFCS), HFCS and hoodia, and stevia were evaluated in the rat moderate intake model. Male albino rats (10 rats/group) received one of the sweetened beverages or water as the sole source of liquid at night (3 nights/week for 10 weeks). Although within the normal range, serum cholesterol was higher in the fructose and HFCS groups, and serum triglycerides were higher in the Agave, HFCS, and HFCS/Hoodia groups as compared to water controls. Liver histology was normal in all groups with no evidence of steatosis, inflammation, or fibrosis; however, serum alanine aminotransferase was higher in the fructose and HFCS groups vs. water-controls. Serum inflammatory marker levels were comparable among Stevia, agave, fructose, HFCS, and water-consuming groups, however, levels of IL-6 were significantly lower in association with the ingestion of Hoodia. There were no differences in terminal body weights, or glucose tolerance assessed by 120-min intravenous glucose tolerance tests performed at the end of the 10-week regimen.

The effects of stevioside (250 mg 3 times a day for 3 months) were evaluated in human subjects with Type 1 and Type 2 diabetes and in non-diabetic subjects with normal/low-normal blood pressures in a randomized, double-blind, placebo-controlled study. No significant differences in blood pressure, glucose, or glycated hemoglobin were observed after 3 months of treatment in any treatment group. No significant differences in adverse events, changes in body weight, or biochemical parameters were observed (Barriocanal *et al.,* 2008).

Anton *et al.* (2010) examined the effects of non-nutritive sweeteners, including stevia, on food intake, satiety, and postprandial glucose and insulin levels. Healthy (n=19, BMI 20.0 to 24.9) and obese (n=12, BMI 30.0 to 39.9) subjects received a preload of stevia (290 kcal), aspartame (290 kcal), or sucrose (493 kcal) before the lunch and dinner meal. The preload order was randomized, and food intake (kcal) was directly calculated. Hunger and satiety levels were reported before and after meals, and hourly thereafter throughout the afternoon. Participants provided blood samples immediately before and 20 min after the lunch preload. Despite the caloric difference in preloads (290 kcal vs. 493 kcal), participants did not compensate by eating more at their lunch and dinner meals when they consumed stevia and aspartame as compared to sucrose in preloads. Self-reported hunger and satiety levels did not differ by condition. Postprandial glucose levels were significantly reduced after the stevia preload compared to sucrose preload ($p < 0.01$). Likewise, postprandial insulin levels were significantly reduced after stevia compared to both aspartame and sucrose preloads ($p < 0.05$).

Gergersen *et al.* (2004) reported a decrease in blood glucose levels in diabetic subjects given a 1 g dose of stevioside after a meal. Fasting blood glucose levels increased in diabetic subjects given placebo, but not in those given 500 mg stevioside 3 times daily for 3 months (Jeppesen *et al.,* 2006).

Maki (2008a) administered 1000 mg/day rebaudioside A to patients with type 2 diabetes for 16 weeks. There were no significant differences between dosed and placebo groups in glucose homeostasis, body weights, fasting lipids, blood pressure, or urinalysis results.

Mixed results have been reported in non-diabetic subjects. A dose related decrease in blood glucose was seen in normal human subjects given 5 g of stevia leaves daily for 3 days (Curi, 1986). In contrast, Chan *et al.* (2000) found no effect on blood glucose following administration of 250 mg of stevioside 3 times daily for 1 year in hypertensive, non-diabetic subjects. Geuns *et al.* (2007) reported no effect in healthy subjects treated with 250 mg stevioside three times daily for 3 days.

**b. Effects on Blood Pressure**

A small but significant reduction in blood pressure (either mean arterial pressure or systolic and diastolic pressure) has been seen in normal and hypertensive experimental animals (rats and dogs) administered steviol. Jeppesen *et al.* (2003), for example, reported a significant decrease in blood pressure in nonhypertensive, diabetic Goto-Kakizaki rats receiving 25 mg stevioside/kg bw/day for 6 weeks. Liu *et al.* (2003) studied the antihypertensive effect of stevioside in

anesthetized dogs and reported significant reductions in systolic blood pressure, diastolic blood pressure, and mean arterial pressure following administration of a single nasogastric dose of 200 mg/kg of stevioside. Oral administration of stevioside to obese type 2 diabetic Zucker fatty rats at 30 mg/kg bw/day significantly reduced systolic blood pressure during eight weeks of treatment (Dyrskog *et al.,* 2005a). In contrast, rebaudioside A (25 mg/kg bw/day) produced no treatment-related effects on blood pressure in diabetic Goto-Kakizaki male rats treated for 8 weeks (Dyrskog *et al.,* 2005b).

Equivocal results have also been reported in clinical studies. Chan *et al.* (2000) and Hsieh *et al.* (2003) reported a blood pressure lowering effect in hypertensive humans dosed with 250 mg of stevioside three times daily for 1 year or 500 mg 3 times daily for 2 years. In contrast, no effect on blood pressure was seen in Type 2 diabetics given stevioside at a level of 500 mg three times a day for 3 months (Jeppesen *et al*, 2006). Similarly, Geuns (2007) reported no effect in non-hypertensive subjects treated with 250 mg of stevioside, 3 times a day for 3 days. Ferri *et al.* (2006) reported no effect on the blood pressure of mildly hypertensive subjects given up to 15 mg stevioside/kg bw/day for 6 weeks. Maki *et al.* (2008b) observed no statistically significant effects on resting, seated systolic or diastolic blood pressure, mean arterial pressure, heart rate, or 24-hour ambulatory blood pressures following the administration of 1000 mg/day rebaudioside A for 4 weeks to patients with low-normal to normal blood pressures compared with placebo.

Food Standards Australia New Zealand recently reviewed the available data on blood pressure and blood glucose effects and concluded that mild effects on these parameters may only be expected to occur when parameters are initially elevated and are not expected to pose a risk at dietary concentrations of stevioside (FSANZ, 2008).

## F. Summary and Basis for GRAS Conclusion

Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd.'s GRAS determination that rebaudioside A derived from *Stevia rebaudiana* intended for use as a general purpose non-nutritive sweetener in a variety of food products at levels determined by current good manufacturing practices (cGMPs) such that intakes are consistent with the acceptable daily intake (ADI) of 0 to 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), would not be harmful under the intended conditions is based on scientific procedures and includes the evaluation of data from safety studies conducted on products compositionally similar to Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd.'s product, as well as other related steviol glycosides.

Using published dietary exposure data for other high intensity sweeteners (*e.g.*, aspartame) with adjustment for their relative sweetness intensities, Renwick (2008) estimated rebaudioside A exposure in various population subgroups. The predicted rebaudioside A exposure is 1.3 mg/kg

body weight/day for average consumers and 3.4 mg/kg body weight/day for high consumers. Children and individuals with diabetes would be expected to have the highest exposures. These intakes are below the ADI established by JECFA for steviol glycosides (JECFA, 2008).

Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. will notify each food manufacturer purchasing rebaudioside A that the use of this ingredient in foods is generally recognized as safe (GRAS), provided consumption of such foods does not result in daily intakes exceeding 4 mg steviol equivalents per kg body weight per person, as established by JECFA (2008).

The safety of rebaudioside A derived from *Stevia rebaudiana* has been demonstrated in the absence of mutagenic or genotoxic activity *in vitro* and *in vivo*. The subchronic toxicity potential of rebaudioside A has also been evaluated in rats. No adverse effects were seen at dietary concentrations of up to 100,000 ppm (9,938 and 11,728 mg/kg bw/day for males and females, respectively) for 4 weeks or 50,000 ppm (4,161 and 4,645 mg/kg bw/day for males and females, respectively) for 13 weeks (Curry and Roberts, 2008). A multi-generational reproductive and developmental study conducted with rebaudioside A did not show any reproductive or developmental toxicity in Han Wistar rats and hamsters (Curry *et al.*, 2008). These data are supported by evidence of the safety of related compounds, stevioside and steviol, in experimental animals and by evidence of safety of these compounds in humans.

Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. have concluded that rebaudioside A derived from *Stevia rebaudiana* is GRAS under the intended conditions of use on the basis of scientific procedures. This determination is further supported by an Expert Panel evaluation of the safety of rebaudioside A derived from *Stevia rebaudiana* under the intended conditions of use.

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# APPENDICES

# REBAUDIOSIDE A DERIVED FROM STEVIA REBAUDIANA GRAS NOTIFICATION

Submitted: November, 2010

Notifier: Shanghai Freemen, LLC

# Report for GRAS Expert Evaluation of Rebaudioside A derived from *Stevia rebaudiana* for the Use as a General Purpose Non-Nutritive Sweetener

The undersigned, an independent Panel of recognized Experts (hereinafter referred to as the Expert Panel), qualified by scientific training and relevant international experience to evaluate the safety of food and food ingredients, was commissioned by Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freeman Americas, LLC to evaluate the Generally Recognized as Safe (GRAS) status of rebaudioside A derived from *Stevia rebaudiana* for use as a general purpose non-nutritive sweetener at levels determined by current good manufacturing practices (cGMPs), such that intakes are consistent with the acceptable daily intake (ADI) of 0 to 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).

The Expert Panel independently and collectively critically evaluated information contained in a comprehensive package of scientific information and data compiled from the literature and other sources through May, 2010 by Cantox U.S. Inc. The Expert Panel convened by teleconference on 08 July 2010 and unanimously concluded that the intended uses of rebaudioside A derived from *Stevia rebaudiana,* meeting the food grade specifications presented in the dossier, are Generally Recognized As Safe (GRAS) based on scientific procedures for the intended use.

The stevia plant (*Stevia rebaudiana* Bertoni) is a member of the Compositae (Asteraceae) family. It is native to South America, but has also been distributed to Southeast Asia. Extracts of the plant, consisting of various combinations of its glycosides, have been consumed for hundreds of years by humans and are used as sweeteners in South America, Japan, and China. Rebaudioside A is intended for use at levels determined by current good manufacturing practices (cGMPs), such that intakes are consistent with the acceptable daily intake (ADI) of 0 to 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). Certificates of analysis for 3 non-consecutive batches of rebaudioside A show that Zhucheng Haotian Pharm Co. and Shanghai Freeman Americas' manufacturing process results in a product that reproducibly meets manufacturing and quality specifications.

Preclinical safety data available in the published scientific literature demonstrate rebaudioside A, as well as related steviol glycosides, exhibit low toxicity at levels well-above the estimated intakes from the intended uses. Acute gavage administration of rebaudioside A at a dose of 2 g/kg bw to male mice produced no toxic effects. No adverse effects were observed following the administration of rebaudioside A (97% purity) in the diet to male and female rats at doses of approximately 4,161 and 4,645 mg/kg bw/day in male and female rats, respectively. Multi-generational reproductive and developmental studies conducted with rebaudioside A purified stevioside have not shown any reproductive or developmental toxicity.

The available long-term toxicity/carcinogenicity data on steviol glycosides and Stevia extract show no evidence of carcinogenic potential or any indication of toxicity associated with prolonged high-dose dietary exposure. A NOAEL of 970 mg/kg body weightl/day was established for stevioside based on the results of the 2-year dietary rat study. Rebaudioside A has consistently demonstrated absence of mutagenic or genotoxic activity *in vitro* and *in vivo*. The genotoxic potential of stevioside and steviol also has been assessed extensively and are not anticipated to pose a risk of genetic damage following human consumption.

Pharmacokinetic data reviewed by the panel demonstrate that rebaudioside A and stevioside have similar metabolism and pharmacokinetic profile in the rat. Therefore, the results of toxicology studies on stevioside provide further support of rebaudioside A's safety.

Collectively, the results of multiple human clinical trials conducted with rebaudioside A and/or stevioside demonstrate that steviol glycosides are safe and well-tolerated in groups of normotensive or hypotensive individuals and type-2 diabetics following long-term consumption at doses of up to 1.5 g/day, or about 25 mg/kg body weight/day, with no effects on glucose homeostasis or blood pressure at doses of up to 1,000 mg/day (about 16 mg/kg body weightlday), a dose more than 10-fold greater than the predicted intake of rebaudioside A in children with diabetes (approximately 1.5 mg/kg body weightlday).

In addition, the panel noted that other recent GRAS notifications (*e.g.* GRAS Notice Nos. 252, 253, 278, 282, 287, 303, 304, and 318), all pertaining to rebaudioside A have been reviewed by Expert Panels. The FDA has not objected to these Panels' conclusions.

## Conclusion

We, the Expert Panel, have independently and collectively critically evaluated the information summarized above and unanimously conclude that there is reasonable certainty that no harm will result from the use of Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freeman Americas, LLC's rebaudioside A derived from *Stevia rebaudiana* as a general purpose non-nutritive sweetener at levels determined by current good manufacturing practices (cGMPs) such that intakes are consistent with the acceptable daily intake (ADI) of 0 to 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). We further conclude that this intended use of Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freeman Americas, LLC's rebaudioside A derived from *Stevia rebaudiana*, manufactured consistent with cGMP and meeting the food grade specifications presented in the dossier, is Generally Recognized As Safe (GRAS) based on scientific procedures.

(b) (6) [redacted]

07/09/10

Robert J. Nicolosi, Ph.D. — Date
Professor Department of Clinical Laboratory & Nutritional Sciences
Director - Center for Health & Disease Research & Division of NanoMedicine
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(b) (6) [redacted]

7/12/10

John A. Thomas, Ph.D. — Date
Adjunct Professor
Indiana University School of Medicine Indianapolis, IN

(b) (6) [redacted]

7/19/10

David Bechtel, Ph.D., DABT — Date
Vice President & Senior Scientific Consultant
CANTOX U.S. Inc., Bridgewater, NJ

(b) (6)

# Certificate of Analysis

| | | | |
|---|---|---|---|
| Name of Product | Rebaudioside A | Batch No. | 20100503 |
| Manufacturing Date | May 18,2010 | specification | RA≥97.0% |
| Expiry Date | 2 years from Manufacturing Date | Batch Quantity | 250kgs |
| Standard based on | U S P – FCC 6 | Storage Conditions | Keep dry, and store in tight containers at ambient temperature. |

| Parameter | Methods | Specification | Result |
|---|---|---|---|
| Appearance | Visual | White Powder | Approved |
| Identification | IR | The spectrum of the sample exhibits maxima at the samewavelengths as those in the spectrum of the Reference standard. | Approved |
| Rebaudioside A,%(wt/wt) on anhydrous basis | HPLC | ≥97.0 | 100.69 |
| Related steviol glycosides,% | HPLC | ≤5 | 0.10 |
| Arsenic,ppm | AAS | ≤1 | Not detected |
| Lead,ppm | AAS | ≤1 | Not detected |
| Residual Ethanol,% | GC | ≤0.50 | 0.003 |
| Residual Methanol,% | GC | ≤0.020 | 0.004 |
| PH | Ch.P. | 4.5-7.0 | 6.76 |
| Residue on Ignition,% | Ch.P. | ≤1 | 0.05 |
| Water,% | Ch.P. | ≤6 | 0.5 |
| Total plate count,CFU/g | Ch.P. | ≤1000 | <10 |
| Mould and yeast ,CFU/g | Ch.P. | ≤100 | <10 |
| Escherichia coli,CFU/g | Ch.P. | Absent | Not Detected |
| Conclusion | This product are complied with all the specifications | | |

Analyzed by: (b) (6) (b) (6) Checked by: (b) (6) Approved by: (b) (6)



BEST ORIGINAL COPY

(b) (6)

# Certificate of Analysis

| | | | |
|---|---|---|---|
| Name of Product | Rebaudioside A | Batch No. | 20100501 |
| Manufacturing Date | May 15,2010 | specification | RA≥97.0% |
| Expiry Date | 2 years from Manufacturing Date | Batch Quantity | 250kgs |
| Standard based on | U S P – FCC 6 | Storage Conditions | Keep dry, and store in tight containers at ambient temperature. |

| Parameter | Methods | Specification | Result |
|---|---|---|---|
| Appearance | Visual | White Powder | Approved |
| Identification | IR | The spectrum of the sample exhibits maxima at the samewavelengths as those in the spectrum of the Reference standard. | Approved |
| Rebaudioside A,%(wt/wt) on anhydrous basis | HPLC | ≥97.0 | 99.81 |
| Related steviol glycosides,% | HPLC | ≤5 | 0.18 |
| Arsenic,ppm | AAS | ≤1 | Not detected |
| Lead,ppm | AAS | ≤1 | 0.005 |
| Residual Ethanol,% | GC | ≤0.50 | Not detected |
| Residual Methanol,% | GC | ≤0.020 | 0.005 |
| PH | Ch.P. | 4.6–7.0 | 5.71 |
| Residue on Ignition,% | Ch.P. | ≤1 | 0.05 |
| Water,% | Ch.P. | ≤6 | 0.5 |
| Total plate count,CFU/g | Ch.P. | ≤1000 | <10 |
| Mould and yeast ,CFU/g | Ch.P. | ≤100 | <10 |
| Escherichia coli,CFU/g | Ch.P. | Absent | Not Detected |
| Conclusion | This product are complied with all the specifications | | |

Analyzed by: (b) (6) Checked by: (b) (6) Approved by: (b) (6)

(b) (6)

# Certificate of Analysis

| | | | |
|---|---|---|---|
| Name of Product | Rebaudioside A | Batch No. | 20100505 |
| Manufacturing Date | May 23,2010 | specification | RA≥97.0% |
| Expiry Date | 2 years from Manufacturing Date | Batch Quantity | 250kgs |
| Standard based on | U S P – FCC 6 | Storage Conditions | Keep dry, and store in tight containers at ambient temperature. |

| Parameter | Methods | Specification | Result |
|---|---|---|---|
| Appearance | Visual | White Powder | Approved |
| Identification | IR | The spectrum of the sample exhibits maxima at the samewavelengths as those in the spectrum of the Reference standard. | Approved |
| Rebaudioside A,%(wt/wt) on anhydrous basis | HPLC | ≥97.0 | 100.17 |
| Related steviol glycosides,% | HPLC | ≤5 | 0.05 |
| Arsenic,ppm | AAS | ≤1 | Not detected |
| Lead,ppm | AAS | ≤1 | 0.003 |
| Residual Ethanol,% | GC | ≤0.50 | 0.009 |
| Residual Methanol,% | GC | ≤0.020 | 0.004 |
| PH | Ch.P. | 4.5–7.0 | 6.11 |
| Residue on ignition,% | Ch.P. | ≤1 | 0.07 |
| Water,% | Ch.P. | ≤6 | 0.4 |
| Total plate count,CFU/g | Ch.P. | ≤1000 | <10 |
| Mould and yeast ,CFU/g | Ch.P. | ≤100 | <10 |
| Eschierichia coli,CFU/g | Ch.P. | Absent | Not Detected |
| Conclusion | This product are complied with all the specifications | | |

Analyzed by: (b) (6) Checked by: (b) (6) Approved by: (b) (6)

BEST ORIGINAL COPY

SUBMISSION END

**Belay, Negash**

**From:** Tom Tchang [ttchang@shanghaifreemen.com]
**Sent:** Friday, March 18, 2011 2:03 PM
**To:** Belay, Negash
**Cc:** 'David Zhang'; Haisong Li
**Subject:** GRAS Notice for rebaudioside A
**Attachments:** Shanghai Freemen - Reb A, corrected copy page 8, Section B.pdf

**Dr. Negash Belay**
**Division of Biotechnology and GRAS Notice Review**
**Office of Food Additive Safety**
**U.S. Food and Drug Administration**

**Dr. Belay,**

**Per our telephone discussion, I am respectfully submitting the following modifications for the Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. GRAS Exemption claim for rebaudioside A derived from Stevia rebaudiana for use as a general purpose non-nutritive sweetener submitted on November 22, 2010.**

1. **Paragraph 1 in the cover letter, we would like to further clarify the third sentence starting with "This determination is based on ....." and add "Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. wish to clarify that the intended use of their rebaudioside A derived from Stevia rebaudiana for use as a general purpose non-nutritive sweetener does not include intended use in meats, poultry or other USDA-regulated products."**

2. **Section B, page 8, I have attached a corrected copy of page 8 with the corrected heading for Section B, "Estimated Intake for Rebaudioside A."**

3. **Zhucheng Haotian and Shanghai Freemen Lifescience enlisted the services of CANTOX U.S. Inc, 1011 US Highway 22W., Suite 200, Bridgewater, NJ 08807-2950 as a consultant to prepare the GRAS exemption submission.**

4. **Please note the suite number is wrong for Shanghai Freemen Americas, LLC. The correct address should be 377 Hoes Lane, Suite 240, Piscataway, NJ 08854.**

**If you need more information, please feel free to contact me.**

**Best regards,**
**Tom Tchang**
**President**

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## B. Estimated Intake of Rebaudioside A

Zhucheng Haotian Pharm Co., Ltd. and Shanghai Freemen Lifescience Co., Ltd. are interested in establishing that rebaudioside A derived from *Stevia rebaudiana* is generally recognized as safe (GRAS) when used as a general purpose non-nutritive sweetener in a variety of food products in the United States. Rebaudioside A is intended for use at levels determined by current good manufacturing practices (GMPs) such that intakes are consistent with the ADI of 0 to 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).

Using published dietary exposure data for other high intensity sweeteners (*e.g.*, aspartame) with adjustment for their relative sweetness intensities, Renwick (2008) estimated rebaudioside A exposure in various population subgroups. Rebaudioside A was assumed to have relative sweetness 200 times that of sucrose. Table 2 shows the projected intakes by average and high consumers in different population groups.

**Table 2 Estimated Intake of Rebaudioside A by Population Group (mg/kg body weight/day)**

| Population group | Intake of intense sweeteners[1] | | Projected intakes of rebaudioside A | | Steviol equivalents[2] | |
|---|---|---|---|---|---|---|
| | Average Consumer | High Consumer | Average Consumer | High Consumer | Average Consumer | High Consumer |
| Non-diabetic adults | 255 | 675 | 1.3 | 3.4 | 0.4 | 1.1 |
| Diabetic adults | 280 | 897 | 1.4 | 4.5 | 0.5 | 1.5 |
| Non-diabetic children | 425 | 990 | 2.1 | 5.0 | 0.7 | 1.7 |
| Diabetic children | 672 | 908 | 3.4 | 4.5 | 1.1 | 1.5 |

[1] Expressed as sucrose equivalents.
[2] Values are estimated as one-third of rebaudioside A values since the molecular weight of rebaudioside A is about three times that of steviol (*i.e.*, 967 *vs.* 318).

As shown in the table above, the predicted rebaudioside A exposure is 1.3 mg/kg body weight/day for average consumers and 3.4 mg/kg body weight/day for high consumers. These intakes are equivalent to 0.4 and 1.1 mg/kg as steviol. Children and individuals with diabetes would be expected to have the highest exposures. These intakes are below the ADI established by JECFA for steviol glycosides expressed as steviol equivalents (JECFA, 2008).

## Belay, Negash

**From:** Tom Tchang [ttchang@shanghaifreemen.com]
**Sent:** Tuesday, May 10, 2011 8:18 PM
**To:** Belay, Negash
**Cc:** li.haisong@freemen.sh.cn
**Subject:** Stevia GRAS Application FDA No. 369

**Dr. Belay,**

**We spoke earlier with regards to the GRAS application No. 369 for Shanghai Freemen Americas, LLC. I'm not sure if you are the right person to address my question.**

**The stevia is a joint project between Shanghai Freemen and Zucheng Haotian Pharm Co. Is it possible to include Zucheng Haotian Pharm Co.'s name on the application along with Shanghai Freemen Americas, LLC.**

**Thank you for your attention to this request.**

**Best regards,**
**Tom Tchang**
**President**
**Shanghai Freemen Americas, LLC**

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## Belay, Negash

**From:** Tom Tchang [ttchang@shanghaifreemen.com]
**Sent:** Thursday, May 12, 2011 3:21 PM
**To:** Belay, Negash; Erives, Gladys*
**Cc:** david.zhang@freemen.sh.cn; li.haisong@freemen.sh.cn; 'Erika Tchang'; 'Felix Sun'
**Subject:** GRN No. 369 - Stevia - Shanghai Freemen

**Dr. Belay,**

**I have sent your request for additional information to both our partner, Zucheng Haotian and our consultant, Cantox. I hope to have all your information shortly and will email it to both you and Dr. Gladys Erives.**

**Also, on the GRAS Notice Inventory, GRN No. 369, Shanghai Freemen Americas, LLC is listed as the company. We would like to include the name of our partner, Zucheng Haotian Pharm Co., Ltd.**

**Can you change the name to:**

**Zucheng Haotian Pharm Co., Ltd./Shanghai Freemen Americas, LLC**

**Thank you for your attention to this request.**

**Best regards,**
**Tom Tchang**
**President**
**Shanghai Freemen Americas, LLC**

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**Belay, Negash**

**From:** Tom Tchang [ttchang@shanghaifreemen.com]
**Sent:** Saturday, May 14, 2011 9:02 AM
**To:** Belay, Negash
**Cc:** li.haisong@freemen.sh.cn; david.zhang@freemen.sh.cn; jzannoni@optonline.net
**Subject:** Shanghai Freemen

**Dr. Belay,**

**I hate to trouble you again especially because of my error but one of my colleagues informed me that I spelled our partner's name wrong.**

**The correct spelling is Zhucheng Haotian Pharm Co., Ltd.**

**Please adjust our GRAS Notice No. 369 accordingly.**

**Thanks again for your assistance,**
**Tom**

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**Erives, Gladys***

**From:** Tom Tchang [ttchang@shanghaifreemen.com]
**Sent:** Friday, June 17, 2011 2:23 PM
**To:** Erives, Gladys*
**Cc:** 'David Zhang'; jzannoni@shanghaifreemen.com; 'Hanks Li'; Erika Tchang; sun@zcht.cc; 'David Bechtel Intertek'
**Subject:** Zhucheng Haotian - Shanghai Freemen Stevia
**Attachments:** Zhucheng Haotian - Shanghai Freemen Stevia Spec - Reb A 97%.doc; Zucheng Haotian - Shanghai Freemen Stevia Process Description.doc

**Dr. Erives,**

**My apologies again for the delay in getting you the additional information for the Zhucheng Haotian – Shanghai Freemen stevia Reb A 97%..**

**I have attached a more detailed process description and a product specification with the test methods which are FCC VII, USP and China Pharmacopeia methods.**

**If you need more information, please feel free to contact me.**

**Also, I had discussed earlier with Dr. Belay that the FDA GRAS Notice List had only the Shanghai Freemen name and we would like to add our partner Zhucheng Haotian Pharm Co. to the list.**

**Lastly, can you indicate when we can expect a decision as a result of the delay In obtaining the additional information.**

**Thanks,**
**Tom Tchang**

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# Product Specification

## Rebaudioside A 97%

Source: Dried Leaves of *Stevia rebaudiana* (Bertoni)

Country of Origin: China Shelf life: 2 Years from date of manufacture

Packaging: 20 kg fiber drum (2 x 10 kg plastic bags)

Storage: Keep dry, and store in tight containers at ambient temperature

| Item | Method | Specification |
|---|---|---|
| Appearance | Visual | White fine powder |
| Assay, Rebaudioside A, % | FCC VII | ≥ 97.0 |
| Other Related Steviol Glycosides, % | FCC VII | ≤ 3.0 |
| pH | FCC VII | 4.5 to 7.0 |
| Water, % | FCC VII | ≤ 5.0 |
| Residue on Ignition, % | FCC VII | ≤ 0.2 |
| Specific Rotation, ° | USP <781> | -29.0 to -33.0 |
| Heavy Metals, ppm | USP <231> | ≤ 10 |
| Arsenic, ppm | AAS (ChP 1, APP. IX B) | ≤ 0.1 |
| Lead, ppm | AAS (ChP 1, APP. IX B) | ≤ 0.1 |
| Cadmium, ppm | AAS (ChP 1, APP. IX B) | ≤ 0.1 |
| Mercury, ppm | AAS (ChP 1, APP. IX B) | ≤ 0.1 |
| Residual Solvents, ppm | FCC VII | Methanol ≤ 200<br>Ethanol ≤ 3,000 |
| Total Plate Count, cfu/g | ChP Part 2 App. XI J | ≤ 1,000 |
| Molds & Yeasts, cfu/g | ChP Part 2 App. XI J | ≤ 100 |
| Total Coliform, cfu/g | ChP Part 2 App. XI J | Negative |
| Escherichia coli, MPN/g | ChP Part 2 App. XI J | Negative |
| Salmonella sp. | ChP Part 2 App. XI J | Negative / 25 g |
| Staphylococcus aureus | ChP Part 2 App. XI J | Negative |
| Pesticide Residues | GC/MS/MS or HC/MS/MS | Negative |
| Issue Date: June 6, 2011 | Revision #2 | |

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## Production Process

Zhucheng Haotian Pharm Co., Ltd and Shanghai Freemen Americas, LLC's high purity rebaudioside A is obtained by extracting leaves of *Stevia rebaudiana* Bertoni. The production process of rebaudioside A is demonstrated by flow diagram in Figure 2-2. All solvents, processing aids used in the process, the adsorption resin and the ion-exchange resins are food grade. The related residues in the finished product are absent or meet the related food safety regulations. The filters and membranes used meet FDA requirements for food contact.

Preparation: The starting materials in the production of Rebaudioside A are leaves of *Stevia rebaudiana* Bertoni. The leaves are dried and needed to be picked out the foreign material (gravel, string, dead branches & leaves, etc.) before using.

Extraction: The dried Stevia leaves are extracted with deionized and filtered water for 1 hour.

Flocculation & Precipitation & Filtration A: Food grade Ferrous Sulphate and $Ca(OH)_2$ are added, followed by filtration through a food contact approved cloth frame filter to remove solids.

Adsorption & Desorption: The filtrate A passes through food contact approved adsorption absorption resin.

Desorption with Ethanol & Water: Food grade ethanol is added.

Concentration with Nanofiltrtion Membrane: The ethanol-water eluant is concentrated with a food contact approved organic nanofitration membrane. The ethanol content is concentrated to between 30% and 35%.

Refining with Ion-Exchange Resins: The concentrate is desalinated and decolorized by passing through a food contact approved ion-exchange resin.

Evaporation & Concentration & Filtration B: Concentrating the eluant from ion-exchange column using a single and double effect evaporator. Solids content is increased to between 45% and 55% (W/V), followed by filtration through a food contact approved cloth frame filter to remove solids.

Spray Drying: Filtrate is then diluted with deionized and filtered water followed by spray drying.

Recrystallization & Centrifugation: Spray dried extracts is dissolved in food grade ethanol-water mix and recrystallized.

Centrifugation. Precipitated crystals are separated in a centrifuge and washed with a food grade ethanol-water mix.

Drying: The crystals from the centrifuge are dried by microwave and forced hot-air.

Milling & Sieving: The dried crystals are milled and sieved to the desired specification using food

contact approved processing equipment.
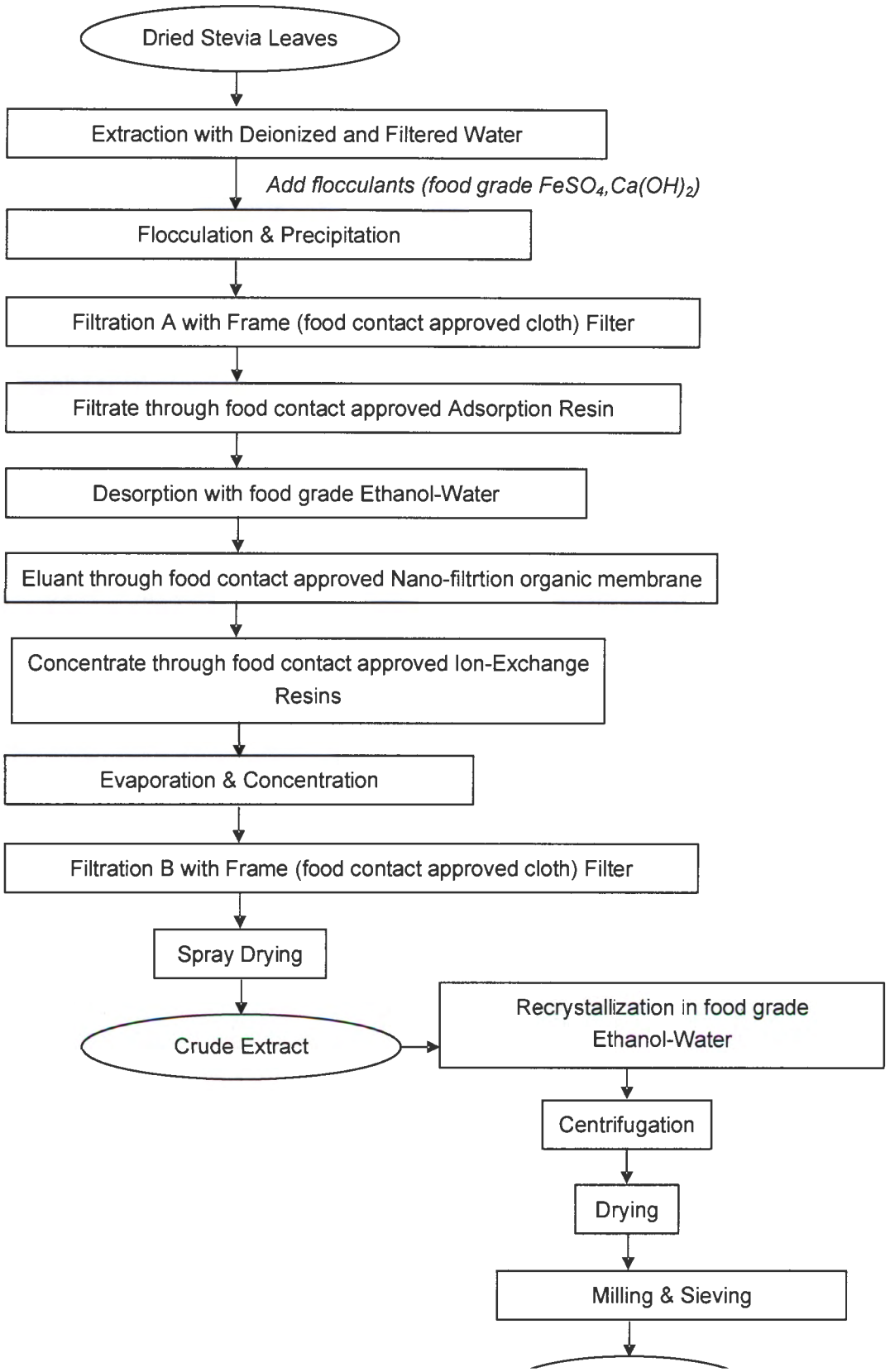
**Figure 2-2 Production Process of rebaudioside A**