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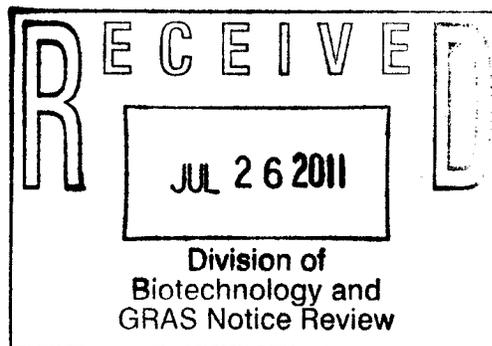
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20482 Jacklight Lane  
Bend, OR 97702-3074  
541-678-5522  
mcquate@gras-associates.com

July 19, 2011

Food and Drug Administration  
Center for Food Safety & Applied Nutrition  
Office of Food Additive Safety (HFS-255)  
5100 Paint Branch Parkway  
College Park, MD 20740-3835



Attention: Dr. Mary D. Ditto

Re: GRAS Notification – High Purity Rebaudioside A ( $\geq 97\%$ )

Dear Dr. Ditto:

On behalf of Daeyung Co., Ltd. of South Korea, we are submitting for FDA review a GRAS notification for high purity Rebaudioside A ( $\geq 97\%$ ). The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

(b) (6)

Robert S. McQuate, Ph.D.  
CEO & Co-Founder  
GRAS Associates, LLC  
20482 Jacklight Lane  
Bend, OR 97702-3074  
541-678-5522  
[mcquate@gras-associates.com](mailto:mcquate@gras-associates.com)  
[www.gras-associates.com](http://www.gras-associates.com)

Enclosure: GRAS Notification for Daeyung Co., Ltd. – High Purity Rebaudioside A ( $\geq 97\%$ ) (in triplicate)



**GRAS ASSESSMENT**

**HIGH PURITY REBAUDIOSIDE A ( $\geq 97\%$ )**

**Food Usage Conditions for General Recognition of Safety**

**for**

**DAEPYUNG Co., Ltd.**

**Bundang-Gu, Seongnam-Si, Gyeonggi-Do  
Republic of South Korea (463-864)**

Evaluation by

Richard C. Kraska, Ph.D., DABT  
Robert S. McQuate, Ph.D.  
Robert W. Kapp, Jr., Ph.D., Fellow ATS

July 18, 2011



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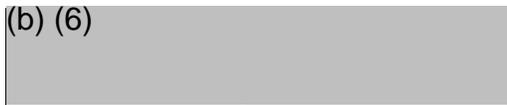
## I. GRAS EXEMPTION CLAIM

### A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)<sup>1</sup>

Daepyeong Co., Ltd. has determined that its high purity rebaudioside A (≥ 97%) product, which is referred to as Rebaten 97%, and which meets the specifications as described below, is Generally Recognized As Safe (GRAS) in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the following sections. The evaluation accurately reflects the conditions of the stevia-derived sweetener's intended uses in foods.

Signed:

(b) (6)



Robert S. McQuate, Ph.D.  
GRAS Associates, LLC  
20482 Jacklight Lane  
Bend, OR 97702-3074

July 19, 2011  
Date

### B. Name & Address of Notifier

Daepyeong Co., Ltd.  
Leaders B/D 604, #274-4 Seohyeon-Dong  
Bundang-Gu, Seongnam-Si, Gyeonggi-Do  
Republic of South Korea (463-824)

As the notifier, Daepyeong Co., Ltd. ("Daepyeong") accepts responsibility for the GRAS determination that has been made for its purified rebaudioside A product<sup>2</sup> as described in the subject notification; consequently, these rebaudioside A preparations, i.e., having purities of no less than 97% rebaudioside A, meeting the conditions described herein are exempt from pre-market approval requirements for food ingredients.

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<sup>1</sup> See 62 FR 18938 (17 April 1997). <http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm>.

<sup>2</sup> DAEPYUNG Co., Ltd. refers to its high purity rebaudioside A product from leaves of *Stevia rebaudiana* Bertoni with the tradename of Rebaten 97%.

### **C. Common Name & Identity of Notified Substance**

High purity rebaudioside A, commonly abbreviated as reb A or Reb A, is the common name for the notified substance; also see Section III.A.

### **D. Conditions of Intended Uses in Food**

The high purity rebaudioside A preparations are intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into foods in general, other than infant formulas and meat and poultry products, at per serving levels that reflect good manufacturing practices principles in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

### **E. Basis for the GRAS Determination**

Pursuant to 21 CFR 170.30, Daepyeong's standardized rebaudioside A preparation from the leaves of *Stevia rebaudiana* Bertoni has been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

### **F. Availability of Information**

The data and information that serve as the basis for this GRAS notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074.

## II. INTRODUCTION

### A. Objective

At the request of Daepyeong, GRAS Associates, LLC (“GA”) has undertaken an independent safety evaluation of Daepyeong’s Rebaten 97% preparation. The preparation is composed of high purity rebaudioside A, which is extracted from the leaves of *Stevia rebaudiana* Bertoni and purified to yield rebaudioside A with a purity of ≥ 97%. The purpose of the evaluation is to ascertain whether or not the intended food uses of rebaudioside A as a non-nutritive general purpose sweetener as described in Section IV.A are generally recognized as safe, i.e., GRAS.

### B. Foreword

Daepyeong provided GA with background information needed to enable the GRAS assessment to be undertaken. In particular, the information that was provided addressed the safety/toxicity of steviol glycosides; the history of use of stevia in food; and compositional details, specifications, and method of preparation of its purified rebaudioside A. Daepyeong was asked to provide adverse reports, as well as those that supported conclusions of safety. Safety/toxicity studies performed with animals were noted to have value, along with available human testing. Daepyeong was also asked to supply past and present human food use information. Knowing how much steviol glycosides has been safely consumed, i.e., the use levels, is critical in extrapolating to safe exposures for rebaudioside A when consumed as a food ingredient. The composite safety/toxicity studies, in concert with exposure information, ultimately provide the specific scientific foundation for the GRAS determination.

In addition to the product specifications, chemical properties, manufacturing, and safety related information, Daepyeong also provided some consumption/exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through July 15, 2011. A GRAS assessment based on the composite safety information, i.e., based on scientific procedures, was undertaken. Those references that were deemed pertinent to the objective at hand are listed in Section VIII.

### C. Summary of Regulatory History of Stevia

Sweeteners derived from stevia are permitted as food additives in South America and in several countries in Asia, including China, Japan, and Korea. In recent years, the subject sweeteners have received food usage approvals in Mexico, Australia, New Zealand, Switzerland, France and Hong Kong. Steviol glycosides have been used as a dietary supplement in the US since 1995 (Geuns, 2003). Based on the available information, no New Dietary Ingredient Notification for dietary supplement use of purified rebaudioside A has been made to the US FDA. Since 1989 and prior to 2008, at least two GRAS petitions seeking authorization for the addition of stevioside or steviol glycosides to foods had been submitted to FDA. However, no authorizations had been

issued by FDA in response to these filings, and these petitions were withdrawn. It appears that the previously available safety data—including purity considerations—for stevia, stevioside, or steviol glycosides were inadequate.

Based on information from FDA's GRAS Notice Inventory<sup>3</sup> website as of July 15, 2011, the agency has received 20 notices on rebaudioside A or steviol glycosides. Thirteen of these notices have received "no questions" letters from the FDA, while 7 notices are under the agency review. In May 2008, Merisant and Cargill independently submitted GRAS notifications for rebaudioside A, highly purified forms of the steviol glycosides, to FDA. On December 17, 2008, FDA issued "no questions" letters for each of these GRAS notices. Since December 2008, a series of GRAS notifications was submitted to FDA for stevia-derived sweetener products by the following companies: McNeil Nutritionals, LLC; Blue California; Sweet Green Fields, LLC; Wisdom Natural Brands; Sunwin and Wild Flavors (two notifications); Pyure Brands, LLC; PureCircle USA, Inc.; GLG Life Tech, Ltd.; NOW Foods; and Sinochem Qingdao Co., Ltd. Each of these firms received a "no questions" letter from FDA.<sup>4</sup> Additionally, 7 notifications submitted to FDA by different manufacturers are pending with the agency.

The Food Standards Australia New Zealand (FSANZ) completed its evaluation of an application for use of steviol glycosides in foods in 2008. FSANZ recommended that the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) amend the Australia New Zealand Food Standards Code to allow the use of steviol glycosides in food (FSANZ, 2008).

The Joint Expert Committee on Food Additives (JECFA) has reviewed steviol glycosides at its 51<sup>st</sup>, 63<sup>rd</sup>, 68<sup>th</sup> and 73<sup>rd</sup> meetings. In 2000, JECFA published the original review on steviol glycosides (WHO, 2000). JECFA established a temporary ADI (acceptable daily intake) of 0-2 mg/kg (on a steviol basis) at its 63<sup>rd</sup> meeting (WHO, 2006). Additionally, JECFA finalized food grade specifications (FAO, 2007a), although they were subsequently updated in 2008 (FAO, 2008) and 2010 (FAO, 2010) (see below). At the 69<sup>th</sup> meeting, the temporary status of the ADI was removed, and the ADI was raised to 0-4 mg/kg bw/day (on a steviol basis) as a result of the JECFA review of recently completed clinical studies with steviol glycosides (WHO, 2008). In 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009).

In early 2009, a number of parties, including the government of Australia and the Calorie Control Council, submitted a request to the Codex Committee on Food Additives in which it was proposed that the JECFA specifications for steviol glycosides should be modified to allow inclusion of Rebaudioside D and Rebaudioside F as specifically named acceptable glycosides that would be considered as part of the minimum 95% steviol glycosides composition (CCFA, 2009). This

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<sup>3</sup> Accessible at: <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true>

<sup>4</sup> 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, GRAS notification 337 was submitted by NOW Foods, and GRAS notification 367 was submitted to Sinochem Qingdao Co., Ltd.; information pertaining to these notifications are listed on FDA's website at [http://www.accessdata.fda.gov/scripts/fcn/gras\\_notices/GRN000329.pdf](http://www.accessdata.fda.gov/scripts/fcn/gras_notices/GRN000329.pdf), along with their respective "no questions" letters.

proposed modification was endorsed by the Codex Alimentarius Committee in July 2009; it was on the agenda for discussion at the JECFA meeting in June, 2010 (WHO, 2009), and JECFA recently took final action in approving the modified steviol glycosides specifications to include Rebaudioside D and Rebaudioside F (FAO, 2010; Appendix A).

In 2008, Switzerland's Federal Office for Public Health (2008) approved the use of stevia as a sweetener citing the favorable actions of JECFA. Subsequently, France published its approval for the food uses of rebaudioside A with a purity of 97% (AFSSA, 2009).

As of May 2010, the government of Hong Kong amended its food regulations to allow the use of steviol glycosides as a permitted sweetener in foods (Hong Kong Centre for Food Safety, 2010). This action followed in the aftermath of the detailed safety evaluation and favorable findings as reported by JECFA.

On September 18, 2009, based on a review of the international regulation of *Stevia rebaudiana* and the clinical evidence for safety and efficacy, the Natural Health Products Directorate, Health Canada (2009) has adopted the following guidelines for the use of stevia and steviol glycosides in Natural Health Products (NHPs). The revised recommendation for the maximum limit for steviol glycosides in NHPs is in accordance with the full ADI (acceptable daily intake) of 4 mg steviol/kg bw established by WHO (2008).

In light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission for European Food Safety Authority (EFSA) to deliver a scientific opinion on the safety of steviol glycosides as a sweetener for use in the food categories specified in the dossiers from the three petitioners, EFSA reexamined the safety of steviol glycosides (EFSA, 2010). After considering all the data on stability, degradation products, metabolism and toxicology, the EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day, which is similar to JECFA's determination.<sup>5</sup> In addition on May 25, 2011 EFSA published a determination that the daily dietary intake for use of rebaudioside A as a flavoring substance in a variety of foods would be less than the ADI for steviol glycosides (EFSA, 2011b).

The international community continues to exhibit much interest in the food uses of steviol glycosides, with additional advances reported in early July, 2011. The Codex Alimentarius Commission has adopted proposed maximum use levels for steviol glycosides in all major food and beverage categories, and this action is expected to favorably influence authorizations of stevia uses in India, Indonesia, Thailand, and the Philippines (FoodNavigator, 2011). Furthermore, the International Alliance of Dietary/Food Supplement Associations (IADSA) reported that the Codex Alimentarius Commission agreed to adopt the use of steviol glycosides

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<sup>5</sup> From a historical perspective, it is noted that the UK's Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food on September 24, 1998 rejected an application for use of steviol glycosides as a sweetener in herbal teas because "the applicant had not provided all of the information necessary to enable an assessment to be made." <http://archive.food.gov.uk/maff/archive/food/novel/980924.htm>. In 1999, the Scientific Committee on Food for the European Commission concluded that "there are no satisfactory data to support the safe use of these stevia plants and leaves" (European Commission, 1999a). In another opinion also dated June 17, 1999, the Committee also reiterated "its earlier opinion that stevioside is not acceptable as a sweetener on the presently available data" (European Commission, 1999b).

for addition to chewable food supplements as had been requested by IADSA (NewHope360, 2011). It is anticipated that more details of these actions will be released in the near future.

#### **D. FDA Regulatory Framework**

Since 1995, steviol glycosides (or stevioside) have been used in dietary supplements in the US (Geuns, 2003). These supplements are widely available to consumers in the US through retail outlets and Internet purchases (Al-Achi and Greenwood, 2000). According to FDA regulation of foods, dietary supplements cannot legally be added to conventional foods. In order for their uses in conventional foods, dietary supplements must undergo premarket approval by FDA as food additives or, alternatively, the ingredients must be determined to be generally recognized as safe (GRAS). The authority to make GRAS determinations is not restricted to FDA. In fact, GRAS determinations may be provided by experts who are qualified by scientific training and experience to evaluate the safety of food and food ingredients under the intended conditions of use.<sup>6</sup>

In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process. At that time, the petitioning process was replaced with a notification procedure.<sup>7</sup> While outlining the necessary content to be considered in making a GRAS determination, FDA encouraged that such determinations be provided to FDA in the form of a notification. However, notifying FDA of such determinations is strictly voluntary.

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<sup>6</sup> See 21 CFR 170.3(i)(3).

<sup>7</sup> See 62 FR 18938 (17 April 1997). <http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm>

### III. CHEMISTRY & MANUFACTURE OF DAEPYUNG'S REBATEN 97%

#### A. Common or Usual Name

The common or usual name for the products that are the subject of this notification is high purity rebaudioside A, which is derived from the leaves of *Stevia rebaudiana* Bertoni. Rebaudioside A is one of the common steviol glycosides found in nature. The rebaudioside A content of the broadly available commercial products is equal to or higher than 95%. Rebaten 97% is the commercial name used by Daepyoung 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 reb A---like stevioside---is the common name for another one of the specific glycosides that is extracted from stevia leaves.

#### B. Description

In 2010, Food Chemicals Codex (FCC) prepared a monograph with a description and specifications for rebaudioside A. In this monograph, rebaudioside A is described as a white to off-white, hygroscopic fine crystal, granule, or powder having a sweet taste (FCC, 2010). 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* Bertoni plant in a multistep separation and purification process. The principal steps of manufacturing include extraction of steviol glycosides from the leaves using an aqueous or aqueous alcoholic (ethanol or methanol) solvent, and purification of rebaudioside 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 rebaudioside A, a glycoside of the *ent*-kaurenoid diterpenoid aglycone known as steviol (FCC, 2010).

#### C. Chemistry of Rebaudioside A

At its 51<sup>st</sup> meeting, JECFA reviewed the safety related information on steviol glycosides, including the identity and chemistry of these compounds. The following description is taken from the original JECFA monograph (WHO, 2000).

Steviol glycosides are natural constituents of the plant *Stevia rebaudiana* Bertoni, belonging to the Compositae family. The leaves of *S. rebaudiana* Bertoni contain eight different steviol glycosides, the major constituent being stevioside (triglucosylated steviol), constituting about 5-10% in dry leaves. Other main constituents are rebaudioside A (tetraglucosylated steviol), rebaudioside C, and dulcoside A. *S. rebaudiana* is native to South America and has been used to sweeten beverages and food for several centuries. The plant has also been distributed to Southeast Asia. Stevioside has a sweetening potency 250-300 times that of sucrose and is stable to heat. In a 62-year-old sample from a herbarium, the intense sweetness of *S. rebaudiana* was conserved, indicating the stability of stevioside to drying, preservation, and storage (Soejarto et al., 1982; Hanson and De Oliveira, 1993).

Of the nine different steviol glycosides, the two principal sweeteners of stevia extracts have been identified as rebaudioside A and stevioside. The chemical identities and key chemical identifiers for the two major components are presented in Table 1.

The chemical structure of rebaudioside A is presented in Figure 1. JECFA (FAO, 2007b) identified the sweetener components of stevia and updated the list of common glycosides and their chemical structures (Figure 2) that are slightly different than compounds shown in other older publications (Nanayakkara et al., 1987; Suttajit et al., 1993). The structures of the components of stevia glycosides were also described in reviews by Kinghorn and Soejarto (1985), Kennelly (2002), and Geuns (2003). Other substances that lack sweetness include the labdane diterpenes, triterpenes, sterols and flavonoid glycosides.

#### **D. Accepted Identity Specifications for Food Grade Steviol Glycosides**

In addition to the manufacturing process, the composition of *Stevia rebaudiana* Bertoni extract depends upon the composition of the harvested leaves, which, in turn, is influenced by soil, climate, etc. (FAO, 2007b). As discussed in Section III.E.1., JECFA recommended that food grade specifications for steviol glycosides consist of a minimum of 95% on a dried weight basis of seven specific steviol glycosides (FAO, 2007a), and this has more recently been expanded to include the original seven specific steviol glycosides plus Reb D and Reb F (FAO, 2010; see Appendix A). The component glycosides of particular interest for their sweetening property are rebaudioside A and stevioside. In addition to the newly added Reb D and Reb F, the other five glycosides that are found at substantially lower levels in the preparations of steviol glycosides and recognized by JECFA consist of Reb C, dulcoside A, rubusoside, steviolbioside, and Reb B.

#### **E. Manufacturing Processes**

Based on available scientific and patent literature, several manufacturing processes for steviol glycosides have been reported. These processes are summarized below along with that utilized by Daepyeong for their Rebaten 97%; their manufacturing process is also specifically discussed in Section III.E.2.

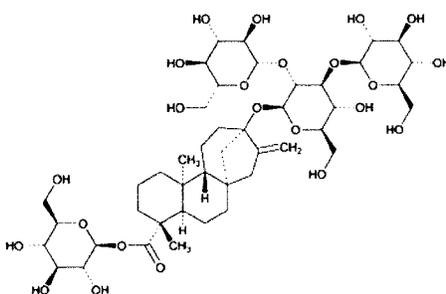
##### **1. Scientific & Patent Literature**

In general, steviol glycosides are obtained by extracting leaves of *Stevia rebaudiana* Bertoni with hot water or alcohols (ethanol or methanol). The extract is a dark particulate solution containing all the active principles along with leaf pigments, soluble polysaccharides, and other impurities. Some processes remove the “grease” from the leaves with solvents such as chloroform or hexane before extraction occurs (Kinghorn and Soejarto, 1985). There are several extraction patents for the isolation of steviol glycosides. Kinghorn and Soejarto (1985) have categorized the extraction patents into those based on solvent, solvent plus a decolorizing agent, adsorption and column chromatography, ion exchange resin, and selective precipitation of individual glycosides.

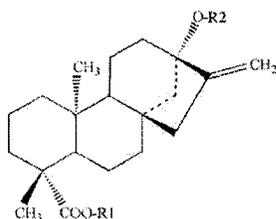
**Table 1. Chemical Identity of Rebaudioside A & Stevioside**

<b>REBAUDIOSIDE A</b>	
<b>Common name</b>	Rebaudioside A
<b>Chemical name</b>	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
<b>Chemical formula</b>	C <sub>44</sub> H <sub>70</sub> O <sub>23</sub>
<b>Formula weight</b>	967.03
<b>CAS Number</b>	58543-16-1
<b>STEVIOSIDE</b>	
<b>Common Name</b>	Stevioside
<b>Chemical name</b>	13-[2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
<b>Chemical formula</b>	C <sub>38</sub> H <sub>60</sub> O <sub>18</sub>
<b>Formula weight</b>	804.88
<b>CAS Number</b>	57817-89-7

**Figure 1. Chemical Structure of Rebaudioside A**



**Figure 2. Chemical Structures of Various Steviol Glycosides<sup>a, b</sup>**



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

<sup>a</sup> From FAO, 2007b.

<sup>b</sup> The indicated C.A.S. No. for Rubusoside as reported in the cited reference is incorrect and should be 64849-39-4.

In recent patents, methods using ultrafiltration, metallic ions, supercritical fluid extraction with CO<sub>2</sub> and extract clarification with zeolite are employed.

At the 68<sup>th</sup> JECFA meeting, steviol glycosides were defined as the products obtained from the leaves of *Stevia rebaudiana* Bertoni. As described by JECFA, the typical manufacture starts with extracting leaves 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 methanol to release the glycosides and the product is recrystallized with methanol. Ion-exchange resins may be used in the purification process. The final product is commonly spray-dried.

## 2. Daepyeong's Manufacturing Process for Rebaten 97%

For the manufacture of Rebaten 97%, Daepyeong employs a fairly typical process that is used in the industry for the production of stevia-derived sweeteners. The source of Daepyeong's Rebaten 97% preparations is the leaves of the *Stevia rebaudiana* Bertoni plant. In order to extract rebaudioside A from the leaves of stevia, Daepyeong has developed a state-of-the-art process. As

summarized by flow charts in Appendix B-1 and B-2, the production of Rebaten 97% is carried out in two stages. In the first stage, stevia extract powder containing 30% - 60% rebaudioside A is prepared. The powder obtained from the first step is further purified to obtain Rebaten 97% in the second stage.

In the first stage, dried/crushed leaves from selected varieties of *S. rebaudiana* Bertoni are extracted in water to obtain stevia extract powder containing 30% - 60% rebaudioside A. In order to facilitate the precipitation of the glycosides, ferric chloride and calcium hydroxide are used (see certificates of analyses in Appendices B-3 and B-4). The extraction solution is filtered by centrifugation, and the filtrate is concentrated. This is followed by adsorption onto polar resin, and the glycosides are subsequently eluted with methanol. The eluate is concentrated using a film evaporator and is subjected to decolorization using charcoal. The concentrate is spray dried or vacuum dried to obtain the stevia extract powder.

The stevia extract powder obtained in the first stage is further processed as depicted in the flow chart found in Appendix B-2 to obtain the high purity rebaudioside A. The stevia extract powder is dissolved in aqueous methanol or ethanol and kept at room temperature for 1 to 2 hours for the first recrystallization. Subsequently, the solution is centrifuged (filtered) using high-speed fixed angle rotors. The mother liquor undergoes a second crystallization step at room temperature for 24 hours. This is followed by centrifugation, and the precipitate is collected. The recrystallized product is concentrated using a batch-type concentrator. The concentrate thus obtained is sterilized and spray-dried to obtain the final product.

The ethanol and methanol used in the purification process comply with Food Chemicals Codex 7<sup>th</sup> Edition (FCC, 2010) specifications for these solvents. The Rebaten 97% is prepared in accordance with current Good Manufacturing Practices (cGMP). A Publicly Available Specification (PAS) has been prepared by the Korea Food & Drug Administration (KFDA) to specify requirements for prerequisite programs and operational prerequisite programs to assist in controlling food safety hazards under Korean Food Sanitation Law. This PAS is under review by experts familiar with US FDA GMP requirements (21 CFR 110) and is in the process of being upgraded to support management systems designed to meet the requirements. In addition, since January 23, 2007, the Daepyeong manufacturing facility is a DHHS/FDA registered food facility (#16411116026 - See Appendix B-5).

## **F. Product Specifications & Supporting Methods**

### **1. JECFA Specifications for Steviol Glycosides**

The composition of extracts of *Stevia rebaudiana* Bertoni depends upon the composition of the harvested leaves, which are, in turn, influenced by soil, climate, and the manufacturing process itself (FAO, 2007b).

In 2007, JECFA recommended that the method of assay should include a minimum requirement of 95% of the total of 7 specific steviol glycosides on a dried weight basis, and JECFA finalized food grade specifications at the 68<sup>th</sup> JECFA meeting with publication in the FAO JECFA

Monograph 4 (FAO, 2007a). Stevioside and rebaudioside A are the major component glycosides of interest because of their sweetening property. The 5 other associated glycosides found in preparations of steviol glycosides accepted by the JECFA specifications with the 95% requirement are rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B. These, however, are typically found at much lower levels than stevioside or rebaudioside A. JECFA updated the specifications for steviol glycosides in 2008 (FAO, 2008), and then again in 2010 when the specifications were expanded to include the original seven specific steviol glycosides plus Reb D and Reb F (FAO, 2010); also see Appendix A.

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 between 4.5 - 7.0. The product should not have more than 1% ash with no more than a 6% loss on drying at 105°C for 2 hours. Any residual methanol levels should not exceed 200 ppm, and ethanol residues should not exceed 5000 ppm. Arsenic levels should not exceed 1 ppm as determined by the atomic absorption hydride technique. Lead levels should not exceed 1 ppm.

## **2. Specifications for Daepyeong's Rebaten 97% & Supporting Methods**

Daepyeong has adopted product specifications for its purified Rebaten 97% that meets or exceeds JECFA recommendations while also complying with Food Chemicals Codex (FCC, 2010) specifications for rebaudioside A. A comparison of the specifications provided by Daepyeong and those from JECFA and FCC is presented in Table 2. Results of analyses performed by Daepyeong quality control laboratories at the manufacturing site demonstrating that five production batches of Rebaten 97% meet the required specifications are provided in Food Master File 849, pages 5-10. A detailed analytical report pertaining to the quantitation of the rebaudioside A in these the same five lots of Rebaten 97%, along with details of the methodology, are included in Food Additive Master File 849, pages 16-75. A test report for analyses of pesticide residues for the intermediate stevia extract in one production lot is included in Appendix C-1. Appendix C-2 lists the limits of detection for the relevant pesticides noted in the C-1 pesticide analyses. The collection of these reports demonstrates that the substance is well characterized and meets the purity criteria.

## **G. Stability Data**

### **1. Stability Data on Steviol Glycosides**

Stevioside has been reported to be stable over the pH range 3 - 9 and can be heated at 100°C for 1 hour, but at pH levels greater than 9 under these conditions it rapidly decomposes (Kinghorn and Soejarto, 1985). These investigators also speculated that at pH 10 steviolbioside would be the major decomposition product produced from stevioside by alkaline hydrolysis. In another study, Chang and Cook (1983) investigated the stability of pure stevioside and rebaudioside A in carbonated phosphoric and citric acidified beverages. Some degradation of each sweetening component after 2 months of storage at 37°C was noted. However, no significant change at room

temperature or below following 5 months of storage of stevioside and 3 months of storage of rebaudioside A was noted. Exposure to 1 week of sunlight did not affect stevioside, but resulted in approximately 20% loss of rebaudioside A. Heating at 60°C for 6 days resulted in 0-6% loss of rebaudioside A.

Merisant (2008) conducted stability testing on rebaudioside A (1) as a powder, (2) as a pure sweetener in solution, and (3) on both cola-type and citrus carbonated beverages. In these investigations no degradation was detected when the powder was stored at 105°C for 96 hours. It was concluded that the powder was stable when stored for 26 weeks at 40±2°C with relative humidity of 75±5%. Both published and unpublished testing results from Merisant revealed that rebaudioside 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 rebaudioside 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).

Cargill (2008) also conducted extensive stability testing on rebaudioside A as a powder under various storage conditions and under a range of pH and temperatures. Additionally, Cargill also investigated rebaudioside A stability in several representative food matrices at room temperature and elevated temperatures. Stability profiles were created for table top sweetener applications, mock beverages including cola, root beer and lemon-lime, thermally processed beverages, yogurt, and white cake. The results of stability testing revealed some degradation products that had not been detected in bulk rebaudioside A. These degradation products were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana* Bertoni. All the degradation products were found to share the same steviol aglycone backbone structure as found in stevioside and rebaudioside A, but they differ by virtue of the glucose moieties present.

The results of stability testing revealed that rebaudioside A is stable in various food matrices following several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, rebaudioside A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

In photostability studies of the dry powder and mock beverages to ascertain rebaudioside A behavior under defined conditions of fluorescent and near UV light exposure, rebaudioside A was found to be photostable under the defined conditions of analysis (Clos et al., 2008).

In addition to the above described stability reports for purified rebaudioside A, in a GRAS notification by Sunwin and WILD Flavors (2010) on purified steviol glycosides with rebaudioside A and stevioside as the principal components, stability was investigated using a 0.04% solution of Reb A 80% in acidic solutions between pH 2.81 and 4.18. In this study, the solutions were stored at 32°C for 4 weeks, and the Reb A content was determined at 1, 2 and 4 weeks. Reb A 80% was found to be very stable at pH 3.17 and above. At pH 2.81, after 4 weeks of storage under accelerated conditions only a 7% loss of Reb A was noted. Sunwin and WILD Flavors also studied the stability of Reb A 80% in simulated beverages using 0.1 % citric acid (pH 3.2). The solutions were pasteurized and stored for 8 weeks at 4° and 32°C, and little difference in sweetness perception was found under these conditions.

**Table 2. Specifications for Daepyeong’s Rebaten 97% Product**

PARAMETER	JECFA <sup>a</sup> SPECIFICATIONS STEVIOL GLYCOSIDES	FCC <sup>b</sup> SPECIFICATIONS REBAUDIOSIDE A	DAEPYUNG SPECIFICATIONS REBATEN 97%	METHODS
Appearance	White to light yellow powder	White to off-white, hygroscopic fine crystal, granule, or powder	White powder to off-white	Visual
Sweetness	200-300 times sweeter than sucrose	NA	275-325	Gustatory
Rebaudioside A	NA	NLT 95%	≥ 97%	JECFA HPLC
Total Steviol Glycosides	NLT 95%	NA	≥ 97%	JECFA HPLC
Other Related Steviol Glycosides (such as Stev, Reb A, B, C, Dulc A, Rub & SB) on dry weight basis	NLT 95%	NMT 5% <sup>c</sup>	NS	JECFA, 2007
Residue on Ignition	NS	NS	NS	USP
Moisture (loss on drying)	NMT 6%	NMT 6%	≤ 6%	USP
Ash	NMT 1%	NMT 1%	< 0.2%	JECFA Vol. 4
Optical rotation	NS	NS	NS	USP
Solubility	Freely soluble in water and ethanol	Freely soluble in water:ethanol (50:50)	Freely soluble in water and ethanol	USP
pH (1% solution)	4.5 - 7.0	4.5 - 7.0	4.5-7.0	USP
<b>RESIDUAL SOLVENT LEVELS</b>				
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	< 200 ppm	USP
Residual Ethanol	NMT 5000 mg/kg	NMT 0.5%	< 5000 ppm	USP
<b>HEAVY METALS</b>				
Lead	NMT 1 mg/kg	NMT 1 mg/kg	< 1 ppm	ICP-MS AOAC
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	< 1 ppm	ICP-MS AOAC
<b>MICROBIOLOGICAL</b>				
Total Plate Count	NA	NA	≤ 1000 cfu/g	AOAC 990.12
Yeast and Mold	NA	NA	≤ 100 cfu/g	AOAC 990.02
Total coliform	NA	NA	NS	
<i>Salmonella</i>	NA	NA	Negative	AOAC 990.02
<i>Escherichia coli</i>	NA	NA	Negative	AOAC 990.02
<i>Staphylococcus aureus</i>	NA	NA	Negative	

<sup>a</sup> Prepared at 69<sup>th</sup> JECFA (WHO, 2008).

<sup>b</sup> FCC, 2010. Rebaudioside A monograph. Food Chemicals Codex (7th Ed.)

<sup>c</sup> Excludes Reb A but includes additional two glycosides Reb D and Reb F; Abbreviations: St = Stevioside; Reb A = Rebaudioside A; Reb B = Rebaudioside B; Reb C = Rebaudioside C; Dulc A = Dulcoside A; Rub = Rubusoside; SB = Steviolbioside; NS = not specified; NA = not applicable; NLT = not less than; NMT = not more than.

The stability data in the scientific literature for stevioside, the JECFA report, and the extensive stability testing presented by Merisant, Cargill and Sunwin and WILD Flavors, along with Daepyung's stability testing results support the position that Daepyung's Rebaten 97% preparations are well-suited for the intended food uses as reported by Daepyung.

## **2. Stability Data on Rebaten 97%**

Daepyung's Rebaten 97% was tested for shelf-life stability from February 10, 2009 to February 10, 2011. The report results indicate no significant changes in purity or moisture content over a two-year period. These data are found in Appendix D.

## IV. INTENDED DIETARY USES

### A. Intended Uses

The subject Daepyeong Rebaten 97% preparations with rebaudioside A (≥ 97%) as the principal component are intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener in various foods other than infant formulas and meat and poultry products. The intended use will be as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19).<sup>8</sup> The intended use levels will vary by actual food category, but the actual levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts of Rebaten 97% 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.<sup>9</sup>

### B. Food Uses As Addressed by JECFA, Merisant & Cargill

As part of its safety deliberations, JECFA reviewed various estimates of possible daily intake of steviol glycosides (WHO, 2006). These estimates are presented in Table 3. Merisant also listed intended use levels of rebaudioside A for various food applications in their GRAS Notification (Table 4). Merisant utilized food consumption survey data from 2003-2004 NHANES to determine the estimated daily intake from the proposed uses of rebaudioside A. On a per user basis, the mean and 90<sup>th</sup> percentile daily consumption of rebaudioside A was estimated as 2.0 and 4.7 mg/kg bw/day, respectively. In its notification, Cargill (2008) utilized a different approach in estimating dietary intake figures for rebaudioside A when incorporated as a general sweetener in a broad cross-section of processed foods. Cargill considered that with a few minor exceptions rebaudioside A uses and use levels would be comparable to those of aspartame uses in the US. Using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008), Cargill performed a side-by-side consumption analysis for rebaudioside A versus aspartame. Findings from the above-described different sources along with FSANZ estimates are further discussed in Section IV.C, and the intake estimates are presented in Table 5.

### C. Estimated Daily Intake

The very conservative consumer intake estimates provided by JECFA as shown in Table 3 were utilized to gauge the potential human exposures of steviol glycosides and rebaudioside A in foods as reported in the US and in other countries. As rebaudioside A is about twice as sweet as the mixed glycosides, these levels can be adjusted accordingly. Daepyeong intends to use Rebaten 97% in a number of food categories at levels that comply with GMP uses. The application of Rebaten 97% to the same foods and at the same levels as those described in earlier FDA notices by Merisant and Cargill is unlikely to affect the dietary intake of rebaudioside A from introduction

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<sup>8</sup> Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.

<sup>9</sup> See 21 CFR 182.1(b)(1).

into the market by another supplier who will have to compete in essentially the same markets and foods. This also negates the need for cumulative intake analysis.

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

FOOD TYPE	MAXIMUM USE LEVEL REPORTED <sup>a</sup> (MG STEVIOL GLYCOSIDES /KG OF FOOD)	MAXIMUM USE LEVEL CALCULATED FOR REBAUDIOSIDE A <sup>b</sup> MG REBAUDIOSIDE A /KG OF FOOD	MAXIMUM USE LEVEL CALCULATED FOR REBAUDIOSIDE A <sup>b</sup> MG STEVIOL EQUIVALENTS /KG OF FOOD
Desserts	500	250	83
Cold confectionery	500	250	83
Pickles	1000	500	167
Sweet corn	200	100	33
Biscuits	300	150	50
Beverages	500	250	83
Yogurt	500	250	83
Sauces	1000	500	167
Delicacies	1000	500	167
Bread	160	80	27

<sup>a</sup> Reproduced from WHO, 2006.

<sup>b</sup> Calculated by Expert Panel assuming twice the sweetness intensity for rebaudioside A and three-fold difference in molecular weight between rebaudioside A and steviol.

**Table 4. Proposed Uses & Levels of Rebaudioside A by Merisant<sup>a</sup>**

FOOD GROUP	REBAUDIOSIDE A (PPM)
Tabletop sweeteners	30,000 <sup>b</sup>
Sweetened ready-to-drink teas	90-450
Fruit juice drinks	150-500
Diet soft drinks	150-500
Energy drinks	150
Flavored water	150
Cereals (oatmeal, cold cereal, cereal bars)	150

<sup>a</sup> Merisant, 2008.

<sup>b</sup> Reb A content of sachet prior to dilution and not representative of "as consumed."

**Table 5. Summary of Estimated Daily Intake Assessments for Rebaudioside A & Calculation of Rebaudioside A Values from JECFA & FSANZ Estimates of the EDI**

SCENARIOS	EDI		
	AS STEVIOL <sup>a</sup> (MG/KG BW/DAY)	AS REBAUDIOSIDE A <sup>b</sup> (MG/KG BW/DAY)	TOTAL DAILY INTAKE <sup>c</sup> (MG/DAY)
<b>JECFA</b>			
100% Reb A replacement of sugars	5.0	7.5	450
20-30% Reb A replacement of sugars	1.0 - 1.5	1.5 - 2.3	90 - 140
<b>FSANZ</b>			
100% Reb A replacement of sugars	0.3 - 1.0	0.5 - 1.5	30 - 90
<b>MERISANT</b>			
		2.0 - 4.7 <sup>d</sup>	120 - 282
<b>CARGILL</b>			
		1.3 - 3.4 <sup>d</sup>	78 - 204

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

Further consideration was given to anticipated human exposures as projected independently and with different approaches by JECFA (WHO, 2006), Merisant (2008), and Cargill (2008). As described below, the multiple approaches tended to converge to yield estimated daily intakes (EDIs) in the range of 1.3 – 4.7 mg/kg bw/day that, when compared to the acceptable daily intake (ADI), constitutes an integral component in the subject GRAS evaluation.

JECFA evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that were prepared for the European Commission by the Scientific Committee on Food. JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars, at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1. The intakes ranged from 1.3 mg/kg bw/day with the African diet to 3.5 mg/kg bw/day with the European diet. Additionally, JECFA also estimated the per capita exposure derived

from disappearance (poundage) data supplied by Japan and China. The Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US. The exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee are summarized in Table 6.

**Table 6. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)**

ESTIMATE	EXPOSURE (mg/kg BW/DAY)
GEMS/Food (International) <sup>a</sup>	1.3–3.5 (for a 60 kg person)
Japan, Per Capita	0.04
Japan, Replacement Estimate <sup>b</sup>	3
US, Replacement Estimate <sup>b</sup>	5

<sup>a</sup> WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme.

<sup>b</sup> These estimates were prepared in parallel to those for the international estimates; it was assumed that all dietary sugars in diets in Japan and the US would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200:1.

In its assessment, JECFA concluded that the replacement estimates were highly conservative as the calculated dietary exposure overestimates likely consumption and that true dietary intakes of steviol glycosides (as steviol) would probably be 20 – 30% of these values or 1.0 - 1.5 mg/kg bw/day on a steviol basis, or 3.0 – 4.5 mg/kg bw/day for rebaudioside A based on the molecular weight adjustment. Furthermore, by adjusting for the 400-fold increased sweetness of rebaudioside A relative to sucrose compared to the mixed steviol glycosides sweetness factor of 200-fold relative to sucrose assumed by JECFA, the estimated dietary intake of rebaudioside A would likely be about 1.5 to ~ 2.3 mg/kg bw/day.

Similar to JECFA, FSANZ (2008) also estimated steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario which resulted in estimated exposures of 0.3 - 1.0 mg/kg bw/day on a steviol basis, or 0.5 – 1.5 mg/kg bw/day for rebaudioside A when making both the molecular weight and sweetness equivalency calculations. Merisant also calculated a dietary estimate for rebaudioside A of 2.0 mg/kg bw/day for the average consumer of the foods listed in Table 4 and 4.7 mg/kg bw/day for a 90<sup>th</sup> percentile consumer. In another review conducted on behalf of Cargill and included in their GRAS notification, the intake of rebaudioside A when used as a complete sugar replacement was estimated at 1.3 – 3.4 mg/kg bw/day when calculated as rebaudioside A (Renwick, 2008). The estimated daily intake assessments have been compiled in Table 5. These different assessments suggest that total daily consumption of rebaudioside A for specified food categories and as a general purpose sweetener is unlikely to exceed 5 mg/kg bw/day, for a total daily dietary exposure of up to 300 mg rebaudioside A for an adult weighing 60 kg.

In October 2009, Cargill applied to FSANZ to increase the maximum usage levels of high purity steviol glycosides in the high volume food categories of ice cream and various beverages. Cargill supported its application with increased usage levels by presenting market share analyses which overestimate actual intake while remaining well below the generally accepted ADI. In December

2010, FSANZ recommended accepting the increased usage levels as requested since no public health and safety issues were identified (FSANZ, 2010). Subsequently, FSANZ approved the Cargill application to increase the allowed maximum permitted level (MPL) of steviol glycosides (expressed as steviol equivalents) in ice cream, water based beverages, brewed soft drinks, formulated beverages and flavored soy beverages up to 200 mg/kg and in plain soy beverages up to 100 mg/kg (FSANZ, 2011).

On January 13, 2011, EFSA revised its dietary exposure assessment of steviol glycosides. For high consumers, revised exposure estimates to steviol glycosides remain above the established acceptable daily intake (ADI) of 4 mg/kg bw (steviol equivalent). For European children (aged 1-14), revised intake estimates ranged from 1.7 to 16.3 mg/kg bw/day; and for adults, the range was from 5.6 to 6.8 mg/kg bw/day (EFSA, 2011a).

There have been many scholarly estimates of potential dietary intake of replacement sweeteners-- including steviol glycosides---that have been published (FSANZ, 2008; Renwick, 2008; WHO, 2003) or submitted to FDA (Merisant, 2008). In GRAS notification 301, a simplified estimate was proposed to and accepted by FDA, based on the estimates of exposure in "sucrose equivalents" (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90<sup>th</sup> percentile consumer of a sweetener which is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg/kg bw/day for any population subgroup. As noted in Table 2, the minimum sweetness intensity for Daepyoung's Rebaten 97% preparation is 275-fold that of sucrose. Therefore, the 90<sup>th</sup> percentile consumer of this reb A preparation would consume approximately 3.6 mg/kg bw/day. Based on an estimate that reb A preparations consist of 40% steviol equivalents,<sup>10</sup> the consumption would be less than 1.5 mg/kg/ bw/day on a steviol equivalents basis.

The extent that stevia-based sweeteners will penetrate the US food supply and the extent the market will select mixed steviol glycoside products versus reb A products remains uncertain. Furthermore, many competing non-caloric sweeteners are currently available to consumers, which have been successful in the marketplace, most notably aspartame and sucralose.

Based on the totality of dietary intake considerations presented above, the intake estimates are viewed as being conservative. When comparing these EDI assessments for steviol glycosides, we see that total daily consumption of the steviol glycosides and reb A for defined food uses and as a general purpose sweetener is expected to be substantially less than the acceptable daily intake values discussed at length in Section VI.C.

#### **D. Other Information on Human Exposure to Stevia: Use as Food Ingredient & Other Uses**

For about 20 years, consumers in Japan and Brazil, where stevia has long been approved as a food additive, have been using stevia extracts as non-caloric sweeteners.<sup>11</sup> It was previously reported that 40% of the artificial sweetener market in Japan is stevia based and that stevia is

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<sup>10</sup> Calculated by Expert Panel by multiplying by the ratio of molecular weight of steviol to molecular weight of rebaudioside A.

<sup>11</sup> See Raintree Nutrition Tropical Plant Database. <http://www.rain-tree.com/stevia.htm>.

commonly used in processed foods in Japan (Lester, 1999). Although there are no reported uses of rebaudioside A as a dietary supplement, use of steviol glycosides as a dietary supplement is presently permitted in the US, Australia, and New Zealand and as a natural health product in Canada. It has wide use in China and Japan in food and in dietary supplements. In 2005, it was estimated that sales of stevia in the US reached \$45 million (The Food Institute Report, 2006). More recent reports of consumption figures for stevia reveal pronounced increases in global consumption. Worldwide, Zenith International estimates stevia sales of 3500 metric tons in 2010 which represents a 27% increase over 2009 figures. The market value is estimated to have increased to \$285 million (Zenith, 2011).

Hawke (2003) reported that stevia is commonly used as a treatment for Type 2 diabetes in South America. However, for its therapeutic effects elevated doses in the range of 1 g/person/day or more were reported to be necessary (Gregersen et al., 2004).

## V. SAFETY DATA FOR REBAUDIOSIDE A

### A. Safety Data on Steviol Glycosides: Recent Reports & Reviews by Expert Bodies & Other Scientists

Daepyeong's Rebaten 97% contains rebaudioside A as its major component. Given the structural similarity among rebaudioside A, stevioside and other steviol glycosides and metabolic considerations, the scientific data on stevia and its other components are relevant to the present safety assessment.

Stevia and steviol glycosides have been extensively investigated for their biological, toxicological, and clinical effects (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002). Additionally, the national and international regulatory agencies have thoroughly reviewed the safety of stevia and its glycosides. Most notably, over the years JECFA has evaluated stevia and steviol glycoside multiple times (WHO, 2000, 2006, 2007, 2008). Recently FSANZ (2008) also evaluated steviol glycosides for use in food. The JECFA reviews, as well as the other reviews completed before 2008, primarily focused on mixtures of steviol glycosides typically and were not specific for purified rebaudioside A.

From the safety perspective, some of the earliest studies on steviol glycosides were of limited value as the actual compositions of materials investigated and their questionable purities undermined drawing firm toxicological conclusions. These early studies reported a decrease in fertility with crude stevia preparations and increased mutagenic activity of the principle metabolite, steviol. Based on these and other questions raised about safety by studies with materials of lesser purity and by studies with unusual protocols in *in vivo* and *in vitro* systems usually employing high doses or high concentrations of test materials, FDA was reluctant to authorize the use of stevia. These concerns included renal toxicity, effects on glucose metabolism, and inhibition of mitochondrial enzymes. Over the last decade and a half, the safety of steviol glycosides and rebaudioside A in particular have been extensively investigated employing comprehensive and modern toxicology protocols using scientifically accepted dosing regimens of purified and standardized test substances. The findings from these investigations are discussed below.<sup>12</sup>

Reviews on safety of steviol glycosides by expert bodies such as JECFA, FSANZ and EFSA are presented in Section V.A.1., V.A.2., and V.A.3, respectively. The key toxicology and clinical data on rebaudioside A are described in Section V.B. JECFA encouraged the further elucidation of clinical effects on blood pressure and glucose metabolism on hypertensive and diabetic individuals, respectively, in parallel with normal human subjects. By 2006, sufficient data were generated for JECFA to satisfactorily establish a temporary ADI, which was finalized in 2008. Additional details on the JECFA reviews are discussed below.

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<sup>12</sup> Recently, an additional subchronic study was published that investigated the effects of 97% pure stevioside in drinking water on body weight, organ relative weight, hematological and biochemical parameters, and enzyme activities in Sprague Dawley rats. This study is summarized in Appendix E and is discussed by the Expert Panel in Section VI.B.

## 1. Summary of JECFA Reviews

Earlier at its 51<sup>st</sup> meeting, JECFA (WHO, 2000) expressed the following reservations about the safety data available at that time for steviol glycosides:

The Committee noted several shortcomings in the information available on stevioside. In some studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was available on other constituents or contaminants. Furthermore, no studies of human metabolism of stevioside and steviol were available. In addition, data on long-term toxicity and carcinogenicity were available for stevioside in only one species. The mutagenic potential of steviol has been tested sufficiently only *in vitro*.

Subsequently, additional data were generated on the metabolism of steviol glycosides and submitted to JECFA. This information suggested that the common steviol glycosides are converted to steviol by intestinal bacteria and then rapidly converted to glucuronides that are excreted. The committee now had a molecular basis to become comfortable with studies on test materials which consisted of variable composition but were relatively high purity mixtures of the common steviol glycosides. The new information also revealed that in *in vitro* studies steviol is mutagenic, while *in vivo* condition it is not mutagenic. The committee became convinced that purified steviol glycosides did not impair reproductive performance as did crude preparations of stevia and that there were sufficient chronic studies in rats with adequate no observed effect levels (NOEL) that could support a reasonable acceptable daily intake (ADI) in the range of doses that would be encountered by the use of steviol glycosides as a sugar substitute. However, JECFA wanted more clinical data to rule out pharmacological effects at the expected doses. The following excerpt was taken from the report of the 63<sup>rd</sup> meeting (WHO, 2006):

The Committee noted that most of the data requested at its fifty-first meeting, e.g., data on the metabolism of stevioside in humans, and on the activity of steviol in suitable studies of genotoxicity *in vivo*, had been made available. The Committee concluded that stevioside and rebaudioside A are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*.

The NOEL for stevioside was 970 mg/kg bw/day in a long-term study (Toyoda et al., 1997) evaluated by the Committee at its fifty-first meeting. The Committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to about 12.5–25 mg/kg bw/day (equivalent to 5–10 mg/kg bw/day expressed as steviol). The evidence available at present was inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g., those with hypotension or diabetes).

The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg/kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg/kg bw/day (or 383 mg/kg bw/day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. The Committee noted that this temporary ADI only applies to products complying with the specifications.

The Committee required additional information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics.

In 2007, at its 68<sup>th</sup> meeting, JECFA (WHO, 2007) concluded that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008. Subsequently, sufficient data had been received by JECFA to revise and finalize food additive specifications for steviol glycosides (FAO, 2007a). The Chemical and Technical Assessment report written after the 2007 meeting, explained the Committee's thinking which resulted in flexibility in the identity specifications (FAO, 2007b).

In response to the call for data on "stevioside" for the 63rd meeting of the Committee, submissions from several countries showed that the main components of the commercially available extracts of stevia are stevioside and rebaudioside A, in various amounts ranging from about 10-70% stevioside and 20-70% rebaudioside A. The information indicated that most commercial products contained more than 90% steviol glycosides with the two main steviol glycosides comprising about 80% of the material. The 63rd JECFA required that the summed content of stevioside and rebaudioside A was not less than 70% and established a minimum purity of 95% total steviol glycosides. Analytical data showed that most of the remaining 5% could be accounted for by saccharides other than those associated with the individual steviol glycosides.

Noting that the additive could be produced with high purity (at least 95%) and that all the steviol glycosides hydrolyze upon ingestion to steviol, on which the temporary ADI is based, the 68th JECFA decided it was unnecessary to maintain a limit for the sum of stevioside and rebaudioside content. The Committee recognized that the newly revised specifications would cover a range of compositions that could include, on the dried basis, product that was at least 95% stevioside or at least 95% rebaudioside A.

In 2008, based on additional clinical studies, at its 69<sup>th</sup> meeting, JECFA finalized the evaluation of steviol glycosides (WHO, 2008) and raised the ADI to 0 – 4 mg/kg bw/day and removed the "temporary" designation. The summary of the Committee's key conclusions in the final toxicology monograph addendum (WHO, 2009) were stated 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 per 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 per day (383 mg/kg bw per 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 per day (5–10 mg/kg bw per 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 per 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.

## 2. Summary of FSANZ Review of Steviol Glycosides

In 2008, FSANZ completed a review of the safety of steviol glycosides for use as a sweetener in foods. FSANZ concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day. The FSANZ review discussed the adequacy of the existing database and several new studies, including the clinical studies reviewed by JECFA in the summer of 2007, most notably the work of Barriocanal et al. (2008), which was later published in 2008.

In their draft document, FSANZ also indicated that the new data in humans provides a basis for revising the uncertainty factors that were used by 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, FSANZ established an ADI of 4 mg/kg bw/day for steviol glycosides 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 (FSANZ, 2008).

## 3. Summary of EFSA Review of Steviol Glycosides

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 rebaudioside A) as a food additive. Earlier—in 1984, 1989 and 1999—the Scientific Committee for Food (SCF) evaluated stevioside as a sweetener. At the time, the SCF concluded that the use of stevioside was “toxicologically not acceptable” due to insufficient available data to assess its safety. However, in light of JECFA’s 2008 findings and in response to a June 2008 request by the European Commission, EFSA reevaluated the safety of steviol glycosides as a sweetener. As both rebaudioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for both glycosides, the EFSA Panel agreed that the results of toxicology studies on either stevioside or rebaudioside A are applicable for the safety assessment of steviol glycosides. Considering the available safety data (*in vitro* and *in vivo* animal studies and some human tolerance studies), the EFSA Panel concluded that steviol glycosides, complying with JECFA specifications, are not carcinogenic, genotoxic, or associated with any reproductive/developmental toxicity. The EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on the application of a 100-fold uncertainty factor to the NOAEL in the 2-year carcinogenicity study in the rat when administering 2.5% stevioside in the diet. This is equal to 967 mg stevioside/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day). Conservative estimates of steviol glycosides exposures both in adults and in children suggest that the ADI could possibly be exceeded by European consumers of certain ages and geographies at the maximum proposed use levels.

Recently, EFSA (2011a) revised its exposure assessment of steviol glycosides from its uses as a food additive for children and adults and published the reduced usage levels in 16 foods by a factor of 1.5 to 3, with no changes for 12 food groups. Additionally, 15 other foods were removed,

mainly within the category of desserts and other products, while 3 new food uses were added. The mean estimated exposure to steviol glycosides (equivalents) in European children (aged 1-14 years) ranged from 0.4 to 6.4 mg/kg bw/day and from 1.7 to 16.3 mg/kg bw/day at the 95<sup>th</sup> percentile. A correction was considered to be necessary for the consumption of non-alcoholic flavored drinks (soft drinks) by children, and the corrected exposure estimate at the 95<sup>th</sup> percentile for children ranged from 1.0 to 12.7 mg/kg bw/day. For adults, the mean and 97.5<sup>th</sup> percentile intakes were estimated to range from 1.9 to 2.3 and 5.6 to 6.8 mg/kg bw/day, respectively. Non-alcoholic flavored drinks (soft drinks) are the main contributors to the total anticipated exposure to steviol glycosides for both consumer categories. For high consumers, EFSA noted that revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg/kg bw (steviol equivalent).

In addition, EFSA (2011b) recently accepted rebaudioside A as a flavoring agent in a variety of foods. EFSA reviewed the available safety data on rebaudioside A and agreed that the ADI of 4mg/kg bw/day established for steviol glycosides applied also to rebaudioside A in a purified form. The dietary intake for use as a flavoring agent was calculated by two different methods, and EFSA determined that the worst case exposure would be 10,888 microgram/person/day, which is equivalent to 181 microgram rebaudioside A/kg bw/day, for a person weighing 60 kg. This corresponds to a daily intake of 60 microgram steviol/kg bw/day, using a conversion factor of 0.33 for converting the amount of rebaudioside A into steviol equivalents.

## **B. Safety Data on Rebaudioside A<sup>13</sup>**

Since 2008, several well-designed toxicology studies that followed the current regulatory and other guidelines for such studies have been reported on purified rebaudioside A although it is uncertain whether or not these studies were considered by JECFA during its 2008 deliberations. These investigations included additional subchronic studies in rats and one in dogs, mutagenicity studies, reproduction and developmental studies in rats, and comparative pharmacokinetic studies with stevioside in rats and humans, as well as additional clinical studies.

### **1. Absorption, Distribution, Metabolism & Excretion (ADME) Studies**

In three recently completed studies, absorption and fate of rebaudioside A was systematically investigated in rats and humans.

For comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, rebaudioside A, toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol were examined in rats (Roberts and Renwick, 2008). Orally administered single doses of the radiolabelled compounds were

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<sup>13</sup> Questions about the safety of rebaudioside A were previously raised by Huxtable (2002) and Kobylewski and Eckhart (2008). Their respective concerns, as well as opposing views supporting the safety of designated food uses of rebaudioside A expressed by Expert Panels have been outlined in other GRAS notifications that were submitted to FDA. A more detailed account can be found in GRAS notifications 278, 287, 303, and 304.

extensively and rapidly absorbed with plasma concentration-time profiles following similar patterns for stevioside and rebaudioside A. Within 72 hours of administration, elimination of radioactivity from plasma was essentially complete. 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 less than 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 authors concluded that the overall data on toxicokinetics and metabolism indicate that rebaudioside A and stevioside are handled in an almost identical manner in the rat after oral dosing.

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 following single oral doses of rebaudioside A and stevioside. Following administration of rebaudioside A or stevioside, steviol glucuronide appeared in the plasma of all subjects, with median  $T_{max}$  values of 12.00 and 8.00 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 rebaudioside 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 rebaudioside 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 rebaudioside A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that both rebaudioside 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 (rebaudioside A: 0.04%; stevioside: 0.02%). The investigators concluded that rebaudioside 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 (Wheeler et al., 2008).

Another pharmacokinetic investigation was done as a toxicokinetic (TK) phase of a dietary study to determine the potential of rebaudioside A toxicity in rats at levels up to 2000 mg/kg bw/day (Sloter, 2008a). Rebaudioside A and total steviol were detected in peripheral blood of rats during daily administration of 2000 mg/kg bw/day of rebaudioside A at extremely low levels, with mean plasma concentrations of approximately 0.6 and 12 ug/mL, respectively. Estimates of absorbed dose for rebaudioside 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 rebaudioside A and measured hydrolysis products

expressed as Total Rebaudioside A Equivalents compared to daily administered dose results in an estimate of percent of dose recovered ≈ 84%.

## 2. Subchronic Toxicity Studies

Recently, Curry and Roberts (2008) reported the results of two repeat dose studies of rebaudioside A in Wistar rats. The results of these investigations suggest that administration of rebaudioside A to Han Wistar rats at dietary concentrations of up to 100,000 ppm (9938 and 11,728 mg/kg bw/day for males and females, respectively) for 4 weeks or 50,000 ppm (4161 and 4645 mg/kg bw/day for males and females, respectively) for 13 weeks did not present any evidence of systemic toxicity. In the 4-week study, rebaudioside A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000 and 100,000 ppm to male and female rats. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In the 13-week study, Wistar rats were fed diets containing rebaudioside A at dietary concentrations of 0, 12,500, 25,000 and 50,000 ppm. In high-dose male and females groups, reductions in body weight gain attributable to initial taste aversion and lower caloric density of the feed were observed. Inconsistent reductions in serum bile acids and cholesterol were attributed to physiological changes in bile acid metabolism due to excretion of high levels of rebaudioside A *via* the liver. All other hepatic function test results and liver histopathology were within normal limits. No significant changes in other clinical pathology results, organ weights and functional observational battery test results were noted. Macroscopic and microscopic examinations of all organs 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 (Curry and Roberts, 2008).

In another 90-day dietary admix toxicity study, effects of rebaudioside A (99.5% purity) at target exposure levels of 500, 1000 and 2000 mg/kg bw/day were tested in Crl:CD(SD) rats (Nikiforov and Eapen, 2008; Eapen, 2007). Each group consisted of 20/animals/sex. No treatment related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters were noted. 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. At the end of the dosing period, the body weight in males was 9.1% lower than the control group. Due to the small magnitude of difference from the control group value, the investigators did not consider this result to be adverse. The decrease was most likely due to the large proportion of the diet represented by the test material. The NOAEL was determined as ≥2000 mg/kg bw/day.

A 6-month dietary toxicity study in Beagle dogs (4/sex/group) was conducted to investigate the potential adverse effects of rebaudioside A (97.5% purity) at dosage levels of 0, 500, 1000 or 2000 mg/kg bw/day (Eapen, 2008). There were no unscheduled deaths during the course of the study. No treatment-related clinical observations were noted. Administration of rebaudioside A did not affect home cage, open field observations and functional observations and measurements. No differences in hematology findings, serum chemistry findings, or urinalysis findings between the groups were noted. Additionally, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. The investigators concluded that no systemic toxicity of rebaudioside A was

observed at dosage levels up to 2000 mg/kg bw/day and the assigned NOAEL was ≥ 2000 mg/kg bw/day.

### 3. Mutagenicity Studies

In a set of *in vitro* and *in vivo* genotoxicity assays covering mutation, chromosome damage and DNA strand breakage, rebaudioside A consistently and uniformly revealed negative results (Pezzuto et al, 1985; Nakajima, 2000a; Nakajima, 2000b; Sekihashi et al., 2002. These studies are critically reviewed by Brusick (2008). JECFA also reviewed an unpublished chromosome aberration assay of rebaudioside A in cultured mammalian cells (Nakajima, 2000a) and did not find increases in chromosome aberrations.

Additionally, FDA also reviewed three unpublished studies on rebaudioside 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) submitted by Merisant as part of the GRAS Notification. All three studies demonstrated lack of mutagenic or genotoxic activity. Additionally, Williams and Burdock (2009) also reported lack of genotoxicity in another set of published studies that included *in vitro* mutagenicity assays with *Salmonella*, *E. coli*, and mouse lymphoma cells. These investigators also reported lack of *in vitro* clastogenic effects in Chinese hamster V79 cells and the absence of *in vivo* effects in a mouse micronucleus assay and a rat study for unscheduled DNA synthesis. The key mutagenicity testing results for rebaudioside A are summarized in Table 7.

### 4. Reproduction & Developmental Studies

In a two-generation reproductive toxicity study, rebaudioside A (97% purity) at 0, 7,500, 12,500, and 25,000 ppm was administered in diet to male and female Han Wistar rats (Curry et al., 2008). Administration of rebaudioside A was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. Similarly, administration of rebaudioside A did not affect reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology in either the F<sub>0</sub> or F<sub>1</sub> generations. The survival and general condition of the F<sub>1</sub> and F<sub>2</sub> offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by rebaudioside A treatment. The NOAEL for reproductive effects was 25,000 ppm and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm or 2,048 to 2,273 mg/kg body weight/day (the highest dose tested).

The results from two unpublished studies with rebaudioside A (Sloter 2008a, b) further support the above described findings from published studies. In a two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were fed either basal diet or the diet containing rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter 2008a). For the F<sub>0</sub> and F<sub>1</sub> generations rebaudioside A doses were 0, 500, 1000 and 2000 mg/kg/day. At initiation of study, F<sub>0</sub> animals were approximately 7 weeks of age. The test diet was offered to the offspring selected to become the F<sub>1</sub> generation following weaning [beginning on postnatal day (PND) 21]. The F<sub>0</sub> and F<sub>1</sub> males continued to receive rebaudioside A

**Table 7. Mutagenicity & Genotoxicity Studies on Rebaudioside A**

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCENTRATION / DOSE	RESULT	REFERENCE
Bacterial Mutagenicity	5 Salmonella strains with and without exogenous metabolic activation system	Reb A	99.5	1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate	No mutagenic response	Wagner and Van Dyke (2006)
Bacterial Mutagenicity	4 Salmonella strains and 1 <i>E. coli</i> strain with and without exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg per plate	No mutagenic response	Williams and Burdock (2009)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence and presence of exogenous metabolic activation system	Reb A	99.5	Cloning conc. of 500, 1000, 2000, 3000, 4000 and 5000 µg/mL	No mutagenic or clastogenic response	Clarke (2006)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence and presence of exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Chromosome Aberration	Human lymphocytes in absence and presence of exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Mouse Micronucleus	Micronucleus study in groups of 5 male and 5 female ICR mice	Reb A	99.5	500, 1000 and 2000 mg/kg bw	No increase in micronuclei formation	Krsmanovic and Huston (2006)
Mouse Micronucleus	Micronucleus study in groups of 5 male and 5 female NMRI mice	Reb A	95.6	Up to 750 mg/kg bw	No increase in micronuclei formation	Williams and Burdock (2009)
Unscheduled DNA Synthesis	Unscheduled DNA synthesis in one group of 4 Wistar rats	Reb A	95.6	Up to 2000 mg/kg bw	No increase in unscheduled DNA synthesis	Williams and Burdock (2009)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevio-side, 52%; Reb A, 22%	250 - 2000 mg/kg bw	Negative <sup>a</sup>	Sekihashi et al. (2002)
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Reb A	NS	1.2 - 55 mg/mL	Negative <sup>b</sup>	Nakajima (2000a)
Micronucleus formation	BDF1 mouse bone marrow	Reb A	NS	500-2000 mg/kg bw per day for 2 days	Negative <sup>c</sup>	Nakajima (2000b)
Forward mutation	<i>S. typhimurium</i> TM677	Reb A	NS	10 mg/plate	Negative <sup>b</sup>	Pezzuto et al. (1985)

NS = Not specified.

<sup>a</sup> Sacrificed at 3 hours and 24 hours.

<sup>b</sup> With or without metabolic activation (source not specified in original monograph).

<sup>c</sup> Sacrificed at 30 hours after 2nd administration.

throughout mating, continuing through the day of euthanasia. The F<sub>0</sub> and F<sub>1</sub> females continued to receive rebaudioside A throughout mating, gestation and lactation until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females as evaluated by

estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints. Both for parental systemic and reproductive toxicity a dose level ≥ 2000 mg/kg bw/day (highest dose administered) was assigned to be the NOAEL.

In an embryo/fetal developmental toxicity study in rats (Sloter, 2008b), effects of rebaudioside A administered via gavage was tested. Rebaudioside A administration did not affect intrauterine growth and survival, and there were no test article-related fetal malformations or developmental variations at any dosage level. In the absence of maternal or developmental toxicity a dose level ≥2000 mg/kg bw/day (highest dose administered) was considered to be the NOAEL for maternal and embryo/fetal developmental toxicity.

## 5. Clinical Studies on Rebaudioside A

In a four week randomized, double-blind, placebo controlled trial, hemodynamic effects of rebaudioside A at a dose of 1000 mg/day rebaudioside A (97% purity) or placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) were investigated (Maki et al., 2008a). Subjects were predominantly female (76%, rebaudioside 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 rebaudioside A and placebo groups, respectively. Compared with placebo, administration of rebaudioside A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-hour ambulatory blood pressure responses. The investigators concluded that consumption of 1000 mg/day of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

In another trial, effects of 16 weeks of consumption of 1000 mg rebaudioside A (97% purity, n = 60) were compared to placebo (n = 62) in men and women (33-75 years of age) with type 2 diabetes mellitus (Maki et al., 2008b). Changes in glycosylated hemoglobin levels did not differ significantly between the rebaudioside A ( $0.11 \pm 0.06\%$ , mean  $\pm$  standard error) and placebo ( $0.09 \pm 0.05\%$ ;  $p = 0.355$ ) groups. Similarly, no significant ( $p > 0.05$  for all) changes from baseline for rebaudioside A and placebo, respectively, in fasting glucose ( $7.5 \pm 3.7$  mg/dL and  $11.2 \pm 4.5$  mg/dL), insulin ( $1.0 \pm 0.64$   $\mu$ U/mL and  $3.3 \pm 1.5$   $\mu$ U/mL), and Cpeptide ( $0.13 \pm 0.09$  ng/mL and  $0.42 \pm 0.14$  ng/mL) were noted. No treatment related changes in blood pressure, body weight, and fasting lipids were noted. Rebaudioside A was well-tolerated, and records of hypoglycemic episodes showed no excess versus placebo. Based on these results, the investigators suggested that chronic use of 1000 mg rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

## VI. GRAS CRITERIA & REVIEWED INFORMATION

### A. GRAS Criteria

FDA defines “safe” or “safety” as it applies to food ingredients as:

“...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance.”<sup>14</sup>

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance and appropriate safety factors. It is FDA’s operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that:

“...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.”

“General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information.”<sup>15</sup>

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called “common knowledge element,” in terms of the two following component elements:<sup>16</sup>

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

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<sup>14</sup> See 21 CFR 170.3(i).

<sup>15</sup> See 21 CFR 170.30(a).

<sup>16</sup> See 62 FR 18938 (17 April 1997).

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

The apparent imprecision of the terms “appreciable”, “at the time” and “reasonable certainty” demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu 1988; Renwick 1990).

As noted below, the safety assessment to ascertain GRAS status for rebaudioside A with the defined food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

## B. Expert Safety Reviews of Steviol Glycosides

Because of their sweetness characteristics, steviol glycosides are unique in that they have viable uses as a non-nutritive sweetener in foods.<sup>17</sup> Periodic reviews by JECFA over the years indicate the progress of knowledge on the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple investigations with *in vivo* and *in vitro* models which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably, the initially reported concerns related to the effects of stevia leaves or crude extracts on fertility were refuted by the well-designed reproductive studies with purified steviol glycosides. All other concerns failed to manifest themselves at the doses employed in the long-term rat studies.

As discussed in Section V, at its fifty-first meeting, JECFA determined that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), to establish a temporary ADI of 0 - 2 mg/kg bw/day with an adequate margin of safety. The committee also critically reviewed the lack of carcinogenic response in well-conducted studies. These studies justified the Committee conclusion that the *in vitro* mutagenic activity of steviol did not present a risk of carcinogenic effects *in vivo* and, therefore, all common steviol glycosides which share the same basic metabolic and excretory pathway and that the use of high purity preparations of various steviol glycosides are safe to use as a sugar substitute. Subsequently, the additional clinical data reviewed by JECFA allowed the Committee to establish a permanent ADI of 0 - 4 mg/kg bw/day

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<sup>17</sup> It has also been reported that steviol glycosides may have pharmacological properties, which can be used to treat certain disease conditions such as hypertension and Type 2 diabetes. Chatsudhipong and Muanprasat (2009) published a comprehensive review where they note that such therapeutic applications have not been firmly established as being due to steviol glycosides. The reviewers point out that the effects occur at higher doses than would be used for sweetening purposes. Furthermore, many effects noted in older studies may have been due to impurities in preparations that do not meet the contemporary purity specifications established by JECFA for use as a sweetener. If oral doses of steviol glycosides impart pharmacological effects, such effects would undoubtedly occur due to actions of the principle metabolite, steviol, but the pharmacological effects of steviol have not been comprehensively investigated.

(based on steviol equivalents) or 0 - 12 mg/kg bw for rebaudioside A. The GRAS Expert Panel critically reviewed the JECFA assessment and agrees with this reasoning.

The Panel has reviewed the findings from human clinical studies. The Panel noted that as regards to the clinical effects noted in humans, in order to corroborate the observations in these studies that these effects of steviol glycosides only occur in patients with either elevated blood glucose or blood pressure (or both), JECFA called for studies in individuals that are neither hypertensive nor diabetic (WHO, 2006). The new data presented to JECFA and also published by Barriocanal et al. (2008) demonstrate the lack of pharmacological effects of steviol glycosides at 11 mg/kg bw/day in normal individuals or approximately slightly more than 4 mg/kg bw on the basis of steviol equivalents. It is possible that JECFA may also have reviewed the preliminary results associated with the recently published clinical studies on rebaudioside A (Maki et al., 2008a, b). The Panel concludes that there will be no effects on blood pressure and glucose metabolism in humans at the doses of rebaudioside A expected from its use in food as a non-nutritive sweetener.

JECFA's review also included anticipated dietary patterns and the use concentrations expected in various foods in order to calculate an estimated daily intake (EDI) (WHO, 2003, 2006). Based on the assumption of 100% substitution of steviol glycosides for sugar, an EDI of 5 mg/kg bw/day of steviol was calculated for US consumption. JECFA noted that the replacement estimates were highly conservative and that this calculated intake of steviol glycosides (as steviol) would more likely be 20% – 30% of these values. Except for the scenario developed by JECFA with 100% replacement of sugars by steviol glycosides, and as discussed in Section IV.C and summarized in Table 5, the highest dietary estimate for use in foods for rebaudioside A is 4.7 mg/kg bw/day. The Panel agrees with the JECFA ADI of 4 mg/kg bw/day based on steviol equivalents which corresponds to 12 mg/kg bw/day for rebaudioside A and notes that the estimates as contained in Table 5 of anticipated dietary intake are below the ADI.

### **C. Safety of Rebaudioside A**

Since July 2008, over ten papers describing the results of a comprehensive research program by different groups on rebaudioside A have been published. These and some other unpublished studies formed the basis of the two initial GRAS notifications to FDA each by Cargill (GRN 253) and Merisant (GRN 252). Prior to this, a limited number of toxicology studies specifically on rebaudioside A were conducted. Even before these new studies were completed and as noted in the previous section, JECFA concluded that 7 (which was later expanded to 9) common steviol glycosides are safe for use as sweetener preparations when present in any combination as long as the combined purity of 95% or more was established.

Since a majority of the previous pharmacokinetic research was conducted with steviol glycosides, the presumed strategy adopted for the more recent research on rebaudioside A was to conduct a limited number of well-designed and executed toxicology studies on rebaudioside A itself and to demonstrate in rats and in humans that it is handled pharmacokinetically similarly to stevioside. This approach appears to have been undertaken to justify the JECFA-generated ADI without having to conduct a chronic study in rats with rebaudioside A. Additionally, the Merisant group

conducted three mutagenicity assays on rebaudioside A that FDA generally considers to be most predictive for carcinogenicity potential. The Cargill group conducted two clinical studies to assure that rebaudioside A does not have potentially problematic pharmacological effects on blood glucose and blood pressure.

In a review article, Carakostas et al. (2008) summarized the most recent research on rebaudioside A. This review summarized the findings of the Cargill research program as follows:

- Steviol glycosides, rebaudioside A, and stevioside are not genotoxic *in vitro*.
- In well-conducted *in vivo* assays, steviol glycosides, rebaudioside A, and stevioside have not been found to be genotoxic.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes, et al., 2007) and was improperly interpreted as a positive response.
- Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.
- The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- The pharmacokinetic similarity between rebaudioside A and stevioside justifies the use of the ADI established by JECFA that was determined on studies employing stevioside as the main component as the ADI for rebaudioside A.
- The dietary levels expected from consumption of rebaudioside A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.

The Panel concurs that the consumption estimates described by both JECFA and Renwick (2008) very conservatively represent a potential high user of rebaudioside A if this non-nutritive sweetener becomes widely available in food. As part of the present GRAS evaluation, the Panel adopts the JECFA EDI for application to DAEPYUNG's Rebaten 97%.

Regarding the available aggregate safety information, the Panel has concluded that JECFA has critically and extensively evaluated the use of steviol glycosides in foods and agrees that, at the present time, the ADI for steviol glycosides of adequate purity as defined by JECFA specifications has been properly determined to be 4 mg/kg bw/person as steviol equivalents, which corresponds to 12 mg/kg bw/day for rebaudioside A on a dry weight basis. The Panel agrees that unwanted pharmacological effects are not likely to occur at this level and that high consumers of rebaudioside A are not likely to exceed this level. Therefore, the Panel adopts the JECFA-derived ADI as a safe exposure for rebaudioside A and that food uses meeting the specifications within the limits determined by this esteemed international body of food safety experts can be considered to be generally recognized as safe (GRAS).

The Panel recognizes that JECFA is composed of dozens of scientists that are internationally known experts on food ingredient safety that have established ADIs for food ingredients over the last 40 years. Both Merisant and Cargill took rather rigorous scientific approaches to demonstrate the safety of rebaudioside A. The studies were equally well conducted. The safety

profiles compiled by Merisant and Cargill differ somewhat, yet the results are complementary and are mutually reinforcing of rebaudioside A safety.

The studies conducted by Cargill provided significant insight into the pharmacokinetics of rebaudioside A while demonstrating clinical safety of rebaudioside A regarding lack of effects on blood pressure and glucose metabolism that could result from doses expected from use in food. The Merisant notification augmented genotoxicity data in three systems recognized by FDA as good predictors of carcinogenic potential. Two of these assays were conducted in mouse systems. Additional mutagenicity and genotoxicity studies have been published on rebaudioside A (Williams and Burdock, 2009). Merisant added a subchronic study in dogs and a teratology study in rats. Both Cargill and Merisant relied on the JECFA ADI for steviol glycosides as determined largely by published chronic studies in rats. Both groups justified the use of the ADI on pharmacokinetic arguments showing the similarity of stevioside and rebaudioside A metabolism and excretion.

The Panel discussed findings from a recently published exploratory subchronic toxicity study in rats by Awney et al. (2010), where a number of toxicological effects of stevioside treatment were reported. Critical review of the publication revealed the poor study design that included insufficient numbers of animals, group-housing with the potential for stress-related changes, unreliable access to steviol *via* drinking water resulting in suspect dosing calculations in group-housed cages, no indication of fasting prior to blood collection which affects many chemistry and hematological values, no urine collection and no histopathological evaluations for confirmation of findings beyond the controls. Additionally, the report did not adequately describe mean or individual organ weight data and lacked comparison of study findings against laboratory historical control data. In contrast to the data presented by Awney et al. (2010), several well-designed and well-conducted subchronic toxicity studies did not reveal any adverse effects from rebaudioside A consumption.

The Panel also noted from a recent study that DNA damage was seen in a variety of organs as assessed by comet assay in rats given drinking water containing 4 mg/mL steviol glycosides for up to 45 days (Nunes et al., 2007). The methodology used in this study was questioned by several experts in the field (Geuns, 2007; Williams, 2007; Brusick, 2008). The Panel has reviewed the cited publications and agrees and discounts the importance of the Nunes et al. (2007) study.

In summary, the Panel agrees with the conclusion of JECFA and the Cargill and Merisant Expert Panels that there are a sufficient number of good quality health and safety studies to support the determination that the intended use of purified preparations of steviol glycosides, including rebaudioside A, when added to food at levels up to full replacement of sugar on a sweetness equivalency basis, meets FDA's definition of safe. In addition, the Panel has compared the specifications of Daepyeong's Rebaten 97% to the composition of the test materials used in all the published studies. The Panel agrees that the Daepyeong product is sufficiently similar to those used in all key studies reviewed by JECFA and those on rebaudioside A subsequently reviewed by FDA, and there is no need for further studies to be conducted on the Daepyeong product. The Panel also has reviewed the expected levels of dietary intake and agrees that there is sufficient information to conclude that the Daepyeong product can be safely used as a table top sweetener

and as a general purpose non-nutritive sweetener in various foods other than infant formulas and meat and poultry products

#### **D. Common Knowledge Elements of GRAS Determination**

The first common knowledge element for a GRAS determination is that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing published, peer-reviewed scientific journals. The majority of studies reviewed as part of this safety assessment have been published in the scientific literature as reported in Section V. Most of the literature relied upon by JECFA has also been published, most importantly the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website. Thus, these studies become generally available to the scientific community. JECFA only reviewed a limited number of studies conducted specifically on rebaudioside A. The collection of supporting data on rebaudioside A has recently been enhanced by a series of studies published during 2008 and cited earlier. The newest clinical studies that address JECFA's concern on unwanted pharmacological effects with steviol glycosides (Barriocanal et al., 2008) and with rebaudioside A (Maki et al., 2008 a, b) are also published in the peer-reviewed scientific literature.

The Panel recognizes that the safety of steviol glycoside in human foods has been the subject of interest for many years. In addition to the reported substantial history of consumption of stevia, especially in South America and Asia, many scientific studies have been conducted and published. Some of the earlier studies have raised concerns about the safety, and the Panel has given careful attention to such concerns. The overriding evidence has diminished the Panel's concerns based on better study designs, better execution, or simply updated investigations that better reflect state-of-the art toxicological principles and findings.

The remaining common knowledge element for a GRAS determination is that there must be a basis to conclude that there is consensus among qualified scientists about the safety of the substance with its intended use. The JECFA opinion largely meets the common knowledge test on its own. The Panel is cognizant of the scientific rigor and broad base of scientific expertise that resides with the prestigious JECFA. JECFA is composed of expert scientists from various regulatory agencies around the world, as well as other scientists chosen because of their specific expertise on various classes of food ingredients. In addition, FDA participated in the JECFA deliberations.

The JECFA conclusion has been reviewed and validated by other respected regulatory agencies including FSANZ, the Switzerland Office of Public Health, and France's Agence Francais De Securite Sanitaire Des Alimenta (FSANZ, 2008; Switzerland Office of Public Health, 2008; AFSSA, 2009). Furthermore, the favorable scientific opinion on the safety of steviol glycosides use as a sweetener in foods as issued by EFSA in 2010 reinforces the safety determinations of many other qualified organizations (EFSA, 2010). In addition, a number of individual well-respected scientists have indicated that steviol glycosides are safe for human consumption at

doses in the range of the JECFA ADI (Xili et al., 1992; Toyoda et al., 1997; Geuns, 2003; Williams, 2007).

The common knowledge element has been embellished by the many respected scientists that participated in the Cargill-sponsored new research conducted on rebaudioside A, most notably Brusick and Renwick. An assertion of “general recognition of safety” was made by Carakostas et al. (2008). In summary, there are many diverse groups of scientists from all corners of the globe that together provide strong fulfillment of the consensus requirement. Of particular significance from the perspective of establishing consensus for the safety of high purity steviol glycosides are the mid-December 2008 “no questions” determinations by FDA for the GRAS notifications for rebaudioside A as submitted by Merisant and Cargill and the more recent comparable findings by FDA with the additional GRAS notifications cited elsewhere.

While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Panel believes that a wide consensus does exist in the scientific community to support the GRAS conclusion on rebaudioside A as outlined in this evaluation. The broader scientific community has concluded that past concerns expressed by others over the years (Huxtable, 2002) and earlier safety issues noted by FDA have been resolved by newer data on more purified test materials and the rigid specifications for purity published by JECFA for steviol glycosides, including rebaudioside A. Indeed, scientists from FDA are members of JECFA and have not objected to the safety decision on steviol glycosides. There is also a wider consensus that the body of new research on rebaudioside A is sufficient as opposed to the small group of scientists that argue that more studies need to be done before the sweetener is made available in the US.

## VII. CONCLUSIONS<sup>18</sup>

**Daepyeong's high purity rebaudioside A (≥ 97%), referred to as Rebaten 97%, as expressed on a dry weight basis, is Generally Recognized As Safe when consumed as a general purpose non-nutritive sweetener in foods other than infant formulas and meat and poultry products when: (1) it is produced in accordance with FDA Good Manufacturing Practices requirements; (2) it meets or exceeds the JECFA purity specifications for steviol glycosides as identified in Table 2; and (3) it is consumed within the designated JECFA ADI of 12 mg/kg bw/day on a rebaudioside A basis. In order to remain within the designated ADI, it is important to observe good manufacturing practices principles in that the quantity of a substance added to food shall not exceed the amount reasonably required to accomplish its intended technical effect.**

This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

(b) (6)

Richard C. Kraska, Ph.D., DABT  
Chair

(b) (6)

Robert S. McQuate, Ph.D.

(b) (6)

Robert W. Kapp, Jr., Ph.D., Fellow ATS

July 18, 2011

<sup>18</sup> The detailed educational and professional credentials for the individuals serving on the Expert Panel can be found on the GRAS Associates website at [www.gras-associates.com](http://www.gras-associates.com). Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Kapp's curriculum vitae can be accessed at: <http://www.biotox.net>. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety. Each individual has previously served on multiple GRAS Expert Panels. Dr. Kraska served as Chair of the Panel.

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## **APPENDIX A**

### **JECFA Steviol Glycosides Specifications & Analytical Method**

#### **2010 – JECFA Specifications for Steviol Glycosides**

## STEVIOLE GLYCOSIDES

Prepared at the 73<sup>rd</sup> JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 69<sup>th</sup> 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 69<sup>th</sup> 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

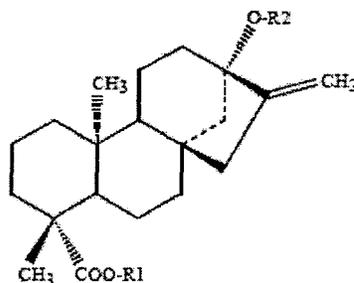
Stevioside: 57817-89-7  
Rebaudioside A: 58543-16-1

### Chemical formula

Stevioside: C<sub>38</sub>H<sub>60</sub>O<sub>18</sub>  
Rebaudioside A: C<sub>44</sub>H<sub>70</sub>O<sub>23</sub>

Structural Formula

The nine named steviol glycosides:



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

**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**

Solubility (Vol. 4) Freely soluble in water

Stevioside and rebaudioside A The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.

pH (Vol. 4) Between 4.5 and 7.0 (1 in 100 solution)

**PURITY**

Total ash (Vol. 4) Not more than 1%

Loss on drying (Vol. 4) Not more than 6% (105°, 2h)

Residual solvents (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)

Arsenic (Vol. 4) Not more than 1 mg/kg  
Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)

Lead (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.

Reagents

Acetonitrile: more than 95% transmittance at 210 nm.

Standards

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

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 C<sub>18</sub> 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<sub>S</sub> is the amount (mg) calculated on the dried basis of stevioside in the standard solution;

W<sub>R</sub> 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<sub>S</sub> is the peak area for stevioside from the standard solution;

A<sub>R</sub> is the peak area for rebaudioside from the standard solution;

$A_x$  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).

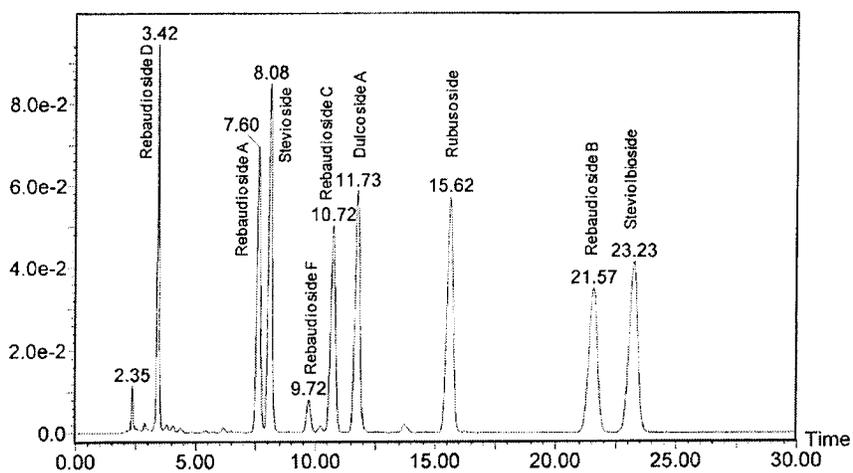


Figure. Chromatogram of mixture of nine steviol glycosides standard solution

Column: Capcell pak C<sub>18</sub> MG II

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

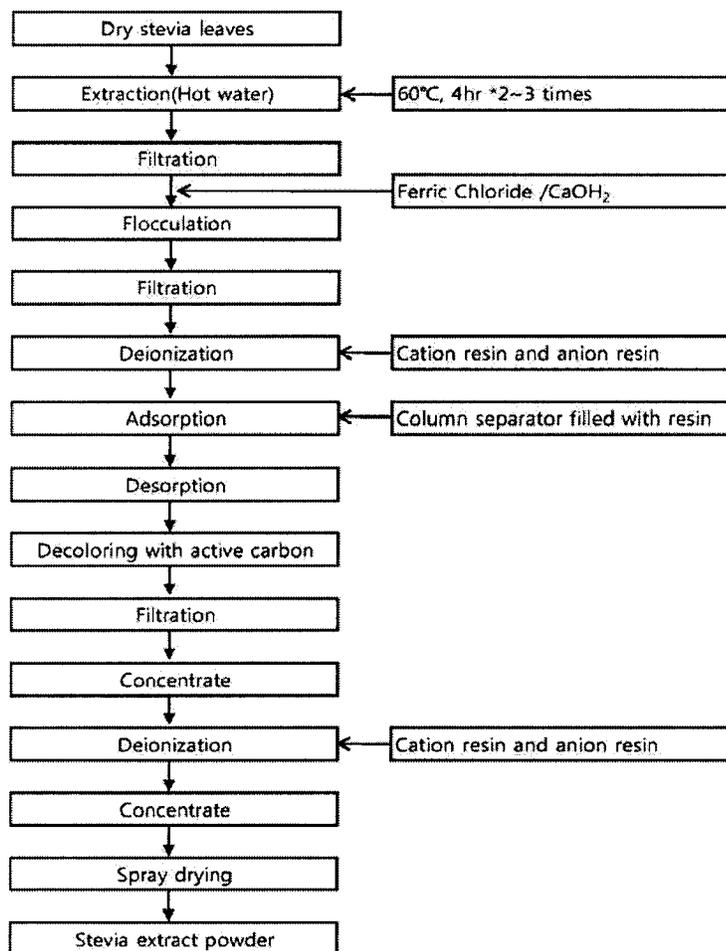
## **APPENDIX B**

### **Manufacturing Information for Production of Rebaten 97%**

- B-1 Process Flow Diagram for Primary Stevia  
Extract Powder containing 30%-60%  
Rebaudioside A**
- B-2 Manufacturing Flow Chart of Rebaten 97%**
- B-3 Certificate of Analysis: Ferric Chloride:  
Qufu Haigen Stevia Products Co., Ltd.**
- B-4 Certificate of Analysis: Calcium Hydroxide:  
Longyou Haifa Calcium Co., Ltd.**
- B-5 DHHS/FDA -- Food Facility Registration Form**

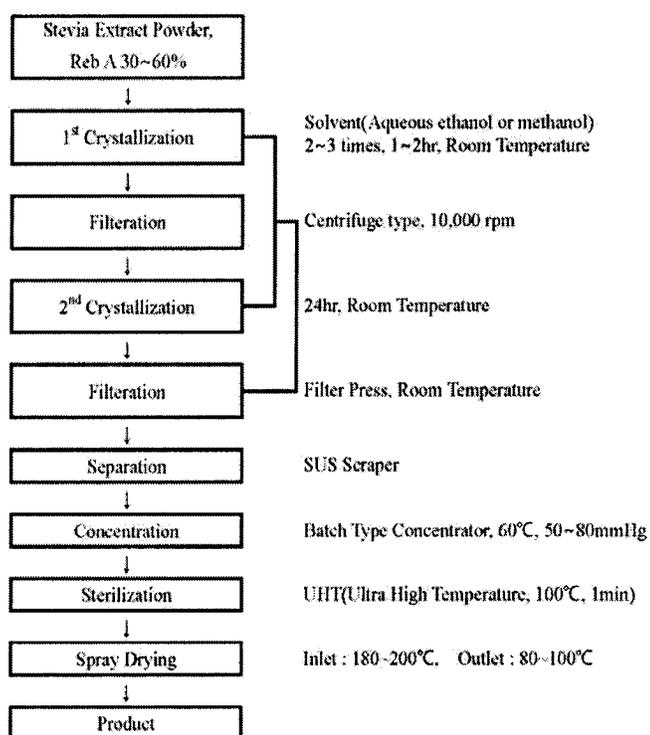
### APPENDIX B-1 Process Flow Diagram for Primary Stevia Extract Powder Containing 30%-60% Rebaudioside A

Processing overview for primary stevia extract



## APPENDIX B-2 Manufacturing Flow Chart of Rebaten 97%

### MANUFACTURING FLOW CHART OF Steviol Glycoside 95%



**APPENDIX B-3**  
**Certificate of Analysis: Ferric Chloride: Qufu Haigen Stevia Products Co., Ltd.**

青岛产品质量监督检验所检验报告附页

No. QJY012000847

共四页 第 2 页

检验项目 (TEST ITEM)	技术要求 (Specification)	检验判定 (Result)	
Description	Yellowish brown crystals or lumps with hygroscopic properties	Pass	
Ferric Chloride Content	98.5-102.0	99.3	
Identification	Responds to the tests by Chloride fume test and ferric salt	Pass	
Purity	1) Clarity and color of solution	Show slightly low level of turbid or better	Pass
	2) Free acid	No sign of acid	Pass
	3) Nitrate	Remain blue more than 2 minutes	Pass
	4) Sulfate	Not to more than the amount that correspond to 0.01N sulfuric acid	Pass
	5) Heavy metal	Less than 20ppm	Pass
	6) Zinc	Less than 30ppm	Pass
	7) Arsenic	less than 4ppm	Pass
	8) Free chloride	Not turn blue	Pass

**BEST ORIGINAL COPY**

**APPENDIX B-4**  
**Certificate of Analysis: Calcium Hydroxide: Longyou Haifa Calcium Co., Ltd.**

龙游海发钙业有限公司

检验报告单

产品名称	食品级氢氧化钙	取样地点	成品名称
检验容器	Q/LYHF 001-2009	袋装	海龙
数量		批号	101229
检验项目 (TEST ITEM)	技术要求 (Specification)	单项判定 (Result)	
Description	White powder	Pass	
CA (OH)2 Content	95.0 ~ 100.0	97.5	
Water	1.0	0.05	
Arsenic	NMT 3mg/kg	Pass	
Carbonate	No more than a slight effervescence is observed	Pass	
Chloride	≤ 0.005%	Pass	
Loss	≤ 0.05	Pass	
CL %	≤ 0.05	≤ 0.05	
SO4 %	≤ 0.5	≤ 0.5	
Magnesium and Alkali Salts	NMT 4.8%	Pass	
Acid Insoluble Substances	NMT 0.5%	Pass	

**BEST ORIGINAL COPY**

APPENDIX B-5  
DHHS/FDA – Food Facility Registration Form



팩 시 밀 리 전 송 표 지

발 송 : 썬 켈론 FDA Korea 일 자 : 2007 년 01 월 24 일  
대표이사 안 승 현 FAX.NO. (02) 543-4746  
수 신 : 썬 대평  
장 이 석 과장님 FAX.NO. (031) 709-7756  
제 목 : FDA 식품시설등록(Food Facility Registration) 번호 통보  
\* 이 표지를 포함하여 8 장을 전송합니다.

안녕하십니까?

- 1. FDA 식품시설등록(Food Facility Registration) 번호를 알려드립니다.
  - ※ Facility Registration Number: 16411116026
  - ※ PIN Number: 9JxE40ed
  - (숫자 9, 알파벳 대문자 J, 소문자 X, 대문자 E, 숫자 4, 숫자 0, 소문자 E, 소문자 D)

문의사항 있으면 언제든지 전화주시요.  
안녕히 계십시오.

첨부: FDA 식품시설등록(Food Facility Registration) 자료 7매

\* 이 팩시밀리 수신결과 상태가 분명치 않은 곳이 있으면, 전화 (02) 568-7744로 문의 바랍니다.

DHHS/FDA Food Facility Registration

페이지 1 / 3

## DHHS/FDA - FOOD FACILITY REGISTRATION FORM

**Please review the registration.**

DATE: January 23, 2007 (MM/DD/YYYY)

**Section 1 - TYPE OF REGISTRATION**

1a. Foreign Registration

1b. FACILITY REGISTRATION NUMBER:

16411116026

PIN: 9JxE40ed

1c. PREVIOUS OWNER'S NAME:

PREVIOUS OWNER'S REGISTRATION NUMBER:

**Section 2 - FACILITY NAME / ADDRESS INFORMATION**

NAME: DAEPYUNG CO., LTD.

FACILITY STREET ADDRESS, Line 1: #417 ODONG-RI, HAMCHANG

FACILITY STREET ADDRESS, Line 2:

CITY: SANGJU

STATE / PROVINCE: Chungcheongbugdo [Ch ungch  
ongbuk-do]

ZIP CODE (POSTAL CODE): 742-804

COUNTRY: KOREA, REPUBLIC OF

PHONE NUMBER (Include Area/Country Code): 82 54 5419015

FAX NUMBER (OPTIONAL; Include Area/Country Code): 82 54 5419016

E-MAIL ADDRESS (OPTIONAL): simonshin@daepyeong.co.kr

**Section 3 - PREFERRED ADDRESS MAILING INFORMATION (Optional)**

NAME:

ADDRESS, Line 1:

ADDRESS, Line 2:

CITY:

STATE / PROVINCE:

ZIP CODE (POSTAL CODE):

COUNTRY:

PHONE NUMBER (Include Area/Country Code):

FAX NUMBER (OPTIONAL; Include Area/Country Code):

E-MAIL ADDRESS (OPTIONAL):

**Section 4 - PARENT COMPANY NAME / ADDRESS INFORMATION**

NAME OF PARENT COMPANY:

STREET ADDRESS, Line 1:

STREET ADDRESS, Line 2:

CITY:

STATE / PROVINCE:

ZIP CODE (POSTAL CODE):

COUNTRY:

PHONE NUMBER (Include Area/Country Code):

FAX NUMBER (OPTIONAL; Include Area/Country Code):

E-MAIL ADDRESS (OPTIONAL):

**Section 5 - FACILITY EMERGENCY CONTACT INFORMATION**

INDIVIDUAL'S NAME (Optional): SIMON SHIN

TITLE (Optional): SENIOR DIRECTOR

EMERGENCY CONTACT PHONE (Include Area/Country Code): 82 10 92708993

E-MAIL ADDRESS (Optional): simonshin@daepyeong.co.kr

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DHHS/FDA Food Facility Registration

페이지 3 / 3

<b>The owner, operator, or agent in charge of the facility, or an individual authorized by the owner, operator, or agent in charge of the facility, must submit this form. By submitting this form to FDA, or by authorizing an individual to submit this form to FDA, the owner, operator, or agent in charge of the facility certifies that the above information is true and accurate. An individual (other than the owner, operator or agent in charge of the facility) who submits the form to the FDA also certifies that the above information submitted is true and accurate and that he/she is authorized to submit the registration on the facility's behalf. An individual authorized by the owner, operator, or agent in charge must below identify by name the individual who authorized submission of the registration. Under 18 U.S.C 1001, anyone who makes a materially false, fictitious, or fraudulent statement to the U.S. Government is subject to criminal penalties.</b>	
<b>NAME OF THE SUBMITTER:</b> SIMON SHIN	
<b>CHECK ONE BOX:</b>	
<input checked="" type="radio"/> <b>A. OWNER, OPERATOR OR AGENT IN CHARGE</b>	
<input type="radio"/> <b>B. INDIVIDUAL AUTHORIZED TO SUBMIT THE REGISTRATION</b>	
<b>IF YOU CHECKED BOX B ABOVE, INDICATE WHO AUTHORIZED YOU TO SUBMIT THE REGISTRATION:</b>	
<input type="radio"/> <b>OWNER, OPERATOR OR AGENT IN CHARGE</b>	
<input checked="" type="radio"/> <b>(INDIVIDUAL AUTHORIZED TO SUBMIT THE REGISTRATION)</b>	
<b>FACILITY STREET ADDRESS, Line 1:</b>	
<b>FACILITY STREET ADDRESS, Line 2:</b>	
<b>CITY:</b>	
<b>STATE / PROVINCE:</b>	<b>ZIP CODE (POSTAL CODE):</b>
<b>COUNTRY:</b>	
<b>PHONE NUMBER (Include Area/Country Code):</b>	
<b>FAX NUMBER (OPTIONAL; Include Area/Country Code):</b>	
<b>E-MAIL ADDRESS (OPTIONAL):</b>	

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DHHS/FDA Food Facility Registration

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<b>Section 6 - TRADE NAMES</b>	
ALTERNATE TRADE NAME #1:	
ALTERNATE TRADE NAME #2:	
ALTERNATE TRADE NAME #3:	
ALTERNATE TRADE NAME #4:	

<b>Section 7 - UNITED STATES AGENT</b>	
NAME OF U.S. AGENT: STAR PACIFIC TRADING CO., LTD.	
TITLE (Optional): MR. DEREK J. CHING	
STREET ADDRESS, Line 1: 3075 ALA POHA PL	
STREET ADDRESS, Line 2:	
CITY: Honolulu	
STATE: HAWAII	ZIP CODE: 96818
U.S. AGENT PHONE NUMBER (Include Area Code): 808 3712558	
EMERGENCY CONTACT PHONE (Include Area Code): 808 3712558	
FAX NUMBER (OPTIONAL; Include Area Code): 808 5960940	
E-MAIL ADDRESS (Optional):	

<b>Section 8 - SEASONAL FACILITY DATES OF OPERATION (Optional)</b>
DATES OF OPERATION:

<b>Section 9 - TYPE OF ACTIVITY CONDUCTED AT THE FACILITY (Optional)</b>
Warehouse/Holding Facility (e.g. storage facilities, including storage tanks, grain elevators)
Manufacturer/Processor
Repacker/Packer
Salvage Operator (Reconditioner)

<b>Section 10 - TYPE OF STORAGE</b>
Ambient Storage (Including heated Storage)

<b>Section 11a - GENERAL PRODUCT CATEGORIES - FOOD FOR HUMAN CONSUMPTION</b>
Food Additives, Generally Recognized as Safe (GRAS) Ingredients, or Other Ingredients Used for Processing

<b>Section 11b - GENERAL PRODUCT CATEGORY - FOOD FOR ANIMAL CONSUMPTION</b>
---

<b>Section 12 - OWNER, OPERATOR, OR AGENT IN CHARGE INFORMATION</b>	
NAME OF ENTITY OR INDIVIDUAL WHO IS THE OWNER, OPERATOR, OR AGENT IN CHARGE: SIMON SHIN	
STREET ADDRESS, Line 1: #417 ODONG-RI, HAMCHANG	
STREET ADDRESS, Line 2:	
CITY: SANGJU	
STATE / PROVINCE: Chungcheongbugdo [Ch ungch ongbuk-do]	ZIP CODE (POSTAL CODE): 742-804
COUNTRY: KOREA, REPUBLIC OF	
PHONE NUMBER (Include Area/Country Code): 82 54 5419015	
FAX NUMBER (OPTIONAL; Include Area/Country Code): 82 54 5419016	
E-MAIL ADDRESS (OPTIONAL): simonshin@daepyeong.co.kr	

<b>Section 13 - CERTIFICATION STATEMENT</b>
---

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**Web Entry Summary Confirmation**

Print this Web Entry Summary Confirmation and present it to U.S. Customs and Border Protection (CBP) or the Food and Drug Administration (FDA) at the Port of Arrival. The Prior Notice Confirmation Number must accompany food carried by or otherwise accompanying an individual (1.279(f)).

**WEB ENTRY**

Envelope Number: F07X02828395      Entry Type: Transportation and Exportation, Express Courier  
 Entry Identifier: F07X02828395      Anticipated Arrival: 01/25/2007 09:00  
 Port of Arrival: Charleston, SC (1801)      Mode of Transportation: Express Courier - Air  
 Number of Intended Prior Notices: 1

**Submitter**

SIMON SHIN  
 DAEPYUNG CO., LTD.  
 #417 ODONG-RI HAMCHANG  
 SANGJU, Gyeongsangbuk-do [Kyöngsangbuk-do] 742-804  
 KOREA, REPUBLIC OF (SOUTH)

**Carrier**

FEDERAL EXPRESS      Airway Bill or Tracking Number: 855071275542  
 KOREA, REPUBLIC OF (SOUTH)  
 Carrier Code (IATA): FX

**PRIOR NOTICES**

Article	Product	Country	HTS	Submitted	Confirmation
0001	STEVTEN FRESH	KR		01/23/2007 03:20:49	070056969135

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FACILITY REGISTRATION PRINT PAGE

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Please review the registration.

Date: 01/23/2007 3:03:37  
 Last Updated: 01/23/2007 03:03:01 Last Modified by: Seung-Hyun Ahn  
 Registration Status: VALID Last Modified by Company: Chemron FDA Korea Co., L

**SECTION 1 TYPE OF REGISTRATION**  
 1a.  DOMESTIC REGISTRATION  FOREIGN REGISTRATION  
 1b. FACILITY REGISTRATION NUMBER: 1641116026 PIN: 6JxE40ed  
 1c. Previous owner's name: Previous owner's registration number:

**SECTION 2 FACILITY NAME ADDRESS INFORMATION**  
 FACILITY NAME: DAEPYUNG CO., LTD.  
 FACILITY STREET ADDRESS, Line 1: #417 ODONG-RI, HAMCHANG  
 FACILITY STREET ADDRESS, Line 2:  
 CITY: SANGJU  
 STATE / PROVINCE / TERRITORY: Gyeongsangbugdo [Kyongsangbuk-do] ZIP CODE (POSTAL CODE): 742-804  
 COUNTRY: KOREA, REPUBLIC OF  
 PHONE NUMBER (Include Area/Country Code): 082 54 5419015  
 FAX NUMBER (OPTIONAL; Include Area/Country Code): 082 54 5419016  
 E-MAIL ADDRESS (OPTIONAL): slmonshin@daepyeong.co.kr

**SECTION 3 PREFERRED MAILING ADDRESS INFORMATION (Optional)**  
 Complete this section only if different from Section 2, Facility Name/Address Information. (OPTIONAL)  
 NAME:  
 ADDRESS, Line 1:  
 ADDRESS, Line 2:  
 CITY:  
 STATE / PROVINCE / TERRITORY: ZIP CODE (POSTAL CODE):  
 COUNTRY:  
 PHONE NUMBER (Include Area/Country Code):  
 FAX NUMBER (Include Area/Country Code):  
 E-MAIL ADDRESS:

**SECTION 4 PARENT COMPANY NAME ADDRESS INFORMATION**  
 Complete this section only if applicable and if different from sections 2 and 3. If information is same as another section, check which section:  
 Facility Address Information  Preferred Mailing Information  
 NAME OF PARENT COMPANY:  
 STREET ADDRESS OF PARENT COMPANY, Line 1:  
 STREET ADDRESS OF PARENT COMPANY, Line 2:  
 CITY:  
 STATE / PROVINCE / TERRITORY: ZIP CODE (POSTAL CODE):

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- 17. Molasses
- 18. Non-protein Nitrogen Products
- 19. Peanut Products
- 20. Recycled Animal Waste Products
- 21. Screenings
- 22. Vitamins
- 23. Yeast Products
- 24. Mixed Feed (Poultry, Livestock, and Equine)
- 25. Pet Food
- 26. Most/All Animal Food Product Categories

**SECTION 12: OWNER, OPERATOR OR AGENT IN CHARGE INFORMATION**

Provide the following information, if different from all other sections of the form. If information is same as another section, check which section:

- Facility Address Information
- Preferred Mailing Information
- Parent Company Address Information
- United States Agent

NAME OF ENTITY OR INDIVIDUAL WHO IS THE OWNER, OPERATOR, OR AGENT IN CHARGE: SIMON SHIN

STREET ADDRESS, Line 1: #417 ODONG-RI, HAMCHANG

STREET ADDRESS, Line 2:

CITY: SANGJU

STATE / PROVINCE / TERRITORY: Gyeongsangbukdo  
[Kyongsangbuk-do]

ZIP CODE (POSTAL CODE): 742-804

COUNTRY: KOREA, REPUBLIC OF

PHONE NUMBER (Include Area/Country Code): 082 54 5419015

FAX NUMBER (OPTIONAL; Include Area/Country Code): 082 54 5419016

E-MAIL ADDRESS (OPTIONAL): simonshin@daepyeong.co.kr

**SECTION 13: CERTIFICATION STATEMENT**

The owner, operator, or agent in charge of the facility, or an individual authorized by the owner, operator, or agent in charge of the facility, must submit this form. By submitting this form to FDA, or by authorizing an individual to submit this form to FDA, the owner, operator, or agent in charge of the facility certifies that the information is true and accurate. An individual (other than the owner, operator or agent in charge of the facility) who submits the form to the FDA also certifies that the above information submitted is true and accurate and he/she is authorized to submit the registration on the facility's behalf. An individual authorized by the owner, operator, or agent in charge must below identify by name the individual who authorized submission of the registration. Under 18 U.S.C 1001, anyone who makes a materially false, fictitious, or fraudulent statement to U.S. Government is subject to criminal penalties.

NAME OF THE SUBMITTER: SIMON SHIN

CHECK ONE BOX:

- A. Owner, Operator or Agent in Charge
- B. Individual authorized to submit the registration

IF YOU CHECKED BOX B ABOVE, INDICATE WHO AUTHORIZED YOU TO SUBMIT THE REGISTRATION:

OWNER, OPERATOR OR AGENT IN CHARGE

\_\_\_\_\_ (NAME OF INDIVIDUAL WHO AUTHORIZED REGISTRATION ON BEHALF OF OWNER, OPERATOR, OR AGENT IN CHARGE)

ADDRESS INFORMATION FOR THE AUTHORIZING INDIVIDUAL:

AUTHORIZING INDIVIDUAL ADDRESS, Line 1:

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COUNTRY:  
PHONE NUMBER (Include Area/Country Code):  
FAX NUMBER (OPTIONAL; Include Area/Country Code):  
E-MAIL ADDRESS (OPTIONAL):

**SECTION 5 FACILITY EMERGENCY CONTACT INFORMATION**  
(OPTIONAL for foreign facilities; FDA will use your U.S. Agent as your emergency contact unless you choose designate a different contact here)  
INDIVIDUAL'S NAME (OPTIONAL): SIMON SHIN  
TITLE (OPTIONAL): SENIOR DIRECTOR  
EMERGENCY CONTACT PHONE (Include Area/Country code): 082 10 92708993  
E-MAIL ADDRESS (OPTIONAL): simonshin@daepyung.co.kr

**SECTION 6 TRADE NAMES**  
(If this facility uses trade names other than the Facility Name, list them below. e.g. "Also doing business as," "Facility also known as"):  
ALTERNATE TRADE NAME #1:  
ALTERNATE TRADE NAME #2:  
ALTERNATE TRADE NAME #3:  
ALTERNATE TRADE NAME #4:

**SECTION 7 UNITED STATES AGENT**  
(To be completed by facilities located outside any state or territory of the United States, the District of Columbia, or the Commonwealth of Puerto Rico.)  
NAME OF U.S. AGENT: STAR PACIFIC TRADING CO., LTD.  
TITLE (OPTIONAL): MR. DEREK J. CHING  
ADDRESS, Line 1: 3075 ALA POHA PL  
ADDRESS, Line 2:  
CITY: Honolulu  
STATE: HAWAII ZIP CODE: 96818  
COUNTRY: United States  
US AGENT PHONE NUMBER (Include Area Code): 808 3712558  
EMERGENCY CONTACT PHONE NUMBER (Include Area Code): 808 3712558  
FAX NUMBER (OPTIONAL; Include Area Code): 808 5960940  
E-MAIL ADDRESS (OPTIONAL):

**SECTION 8 SEASONAL FACILITY DATES OF OPERATION (Optional)**  
(Give the Approximate dates during that your facility is open for business, if its operations are on a seasonal basis)  
(OPTIONAL)  
DATES OF OPERATION:

**SECTION 9 TYPE OF ACTIVITY CONDUCTED AT FACILITY (Optional)**

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(Check ALL types of operations that are performed at this facility regarding the Manufacturing, Processing, Packaging or Holding of Food)

- Warehouse/Holding Facility (e.g. storage facilities, including storage tanks, grain elevators)
- Acidified/Low Acid Food Processor
- Interstate Conveyance Caterer/Catering Point
- Molluscan Shellfish Establishment
- Commissary
- Contract Sterilizer
- Labeler/Relabeler
- Manufacturer/Processor
- Repacker/Packer
- Salvage Operator (Reconditioner)
- Animal food manufacturer/processor/holder

**SECTION 10 - TYPE OF STORAGE (FOR FACILITIES THAT ARE PRIMARY HOLDERS/CO-OWNERS)**

- Ambient (neither frozen nor refrigerated) Storage
- Refrigerated Storage
- Frozen Storage

**SECTION 11 - GENERAL PRODUCT CATEGORY - FOOD FOR HUMAN CONSUMPTION**

To be completed by all human food facilities. IF NONE OF THE MANDATORY CATEGORIES BELOW APPL SELECT BOX 37

- 1. Alcoholic Beverages  
[21 CFR 170.3 (n) (2)]
- 2. Baby (Infant and Junior) Food Products Including Infant Formula  
(Optional Selection)
- 3. Bakery Products, Dough, Mixes, or Icings  
[21 CFR 170.3 (n) (1), (9)]
- 4. Beverage Bases  
[21 CFR 170.3 (n) (3), (16), (35)]
- 5. Candy without Chocolate, Candy Specialties and Chewing Gum  
[21 CFR 170.3 (n) (6), (9), (25), (38)]
- 6. Cereal Preparations, Breakfast Foods, Quick Cooking/Instant Cereals  
[21 CFR 170.3 (n) (4)]
- 7. Cheese and Cheese Products  
[21 CFR 170.3 (n) (5)]
- 8. Chocolate and Cocoa Products  
[21 CFR 170.3 (n) (3), (9), (36), (43)]
- 9. Coffee and Tea  
[21 CFR 170.3 (n) (3), (7)]
- 10. Color Additives for Foods  
[21 CFR 170.3 (o) (4)]

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- 11. Dietary Conventional Foods or Meal Replacements (includes Medical Foods)  
[21 CFR 170.3 (n) (31)]
- 12. Dietary Supplements
  - Proteins, Amino Acids, Fats and Lipid Substances  
[21 CFR 170.3 (o) (20)]
  - Vitamins and Minerals  
[21 CFR 170.3 (o) (20)]
  - Animal By-Products and Extracts  
(Optional Selection)
  - Herbs and Botanicals  
(Optional Selection)
- 13. Dressing and Condiments  
[21 CFR 170.3 (n) (8), (12)]
- 14. Fishery/ Seafood Products  
[21 CFR 170.3 (n) (13), (15), (39), (40)]
- 15. Food Additives, Generally Recognized as Safe (GRAS) Ingredients, or Other Ingredients Use Processing  
[21 CFR 170.3 (n) (42); 21 CFR 170.3 (o) (1), (2), (3), (5), (6), (7), (8), (9), (10), (11), (12), (13), (14), (16), (17), (18), (19), (22), (23), (24), (28), (26), (27), (28), (29), (30), (31), (32)]
- 16. Food Sweeteners (Nutritive)  
[21 CFR 170.3 (n) (9), (41); 21 CFR 170.3 (o) (21)]
- 17. Fruits and Fruit Products  
[21 CFR 170.3 (n) (16), (27), (28), (35), (43)]
- 18. Gelatin, Rennet, Pudding Mixes, or Pie Fillings  
[21 CFR 170.3 (n) (22)]
- 19. Ice Cream and Related Products  
[21 CFR 170.3 (n) (20), (21)]
- 20. Imitation Milk Products  
[21 CFR 170.3 (n) (10)]
- 21. Macaroni or Noodle Products  
[21 CFR 170.3 (n) (23)]
- 22. Meat, Meat Products and Poultry (FDA Regulated)  
[21 CFR 170.3 (n) (17), (18), (29), (34), (40)]
- 23. Milk, Butter, or Dried Milk Products  
[21 CFR 170.3 (n) (12), (30), (31)]
- 24. Multiple Food Dinners, Gravies, Sauces, and Specialties  
[21 CFR 170.3 (n) (11), (14), (17), (18), (23), (24), (29), (34), (40)]
- 25. Nut and Edible Seed Products  
[21 CFR 170.3 (n) (26), (32)]
- 26. Prepared Salad Products  
[21 CFR 170.3 (n) (11), (17), (18), (22), (29), (34), (35)]

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- 27. Shell Egg and Egg Products  
[21 CFR 170.3 (n) (11), (14)]
- 28. Snack Food Items (Flour, Meal, or Vegetable Base)  
[21 CFR 170.3 (n) (37)]
- 29. Spices, Flavors, and Salts  
[21 CFR 170.3 (n) (26)]
- 30. Soups  
[21 CFR 170.3 (n) (39),(40)]
- 31. Soft Drinks and Waters  
[21 CFR 170.3 (n) (3), (35)]
- 32. Vegetables and Vegetable Products  
[21 CFR 170.3 (n) (19), (36)]
- 33. Vegetable Oils (includes Olive Oil)  
[21 CFR 170.3 (n) (12)]
- 34. Vegetable Protein Products (Simulated Meats)  
[21 CFR 170.3 (n) (33)]
- 35. Whole Grains, Milled Grain Products (Flours) or Starch  
[21 CFR 170.3 (n) (1), (23)]
- 36. Most/All Human Food Product Categories  
(Optional Selection)
- 37. None of the Above Mandatory Categories

SECTION 11B GENERAL PRODUCT CATEGORY - FOOD FOR ANIMAL CONSUMPTION

- 1. Grain Products (e.g. barley, grain sorghums, maize, oat, rice, rye and wheat)
- 2. Oilseed Products (e.g. cottonseed, soybeans, other oil seeds)
- 3. Alfalfa and Lespedeza Products
- 4. Amino Acids
- 5. Animal-derived Products
- 6. Brewer Products
- 7. Chemical Preservatives
- 8. Citrus Products
- 9. Distillery Products
- 10. Enzymes
- 11. Fats and Oils
- 12. Fermentation Products
- 13. Marine Products
- 14. Milk Products
- 15. Minerals
- 16. Miscellaneous and Special Purpose Products

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## **APPENDIX C**

### **PESTICIDE ANALYSES**

**C-1 Stevia Extract Pesticide Residue Analysis**

**C-2 Pesticide Limits of Detection**



<b>Gyeongsangbuk-DO, Provincial Government Institute of Health &amp; Environment</b>				
59-17 Wanje-ni, Geumho-eup, Yeongcheon city, Gyeongsangbuk-do, Korea http://inhen.gb.go.kr TEL : 82-54-339-8151 FAX : 82-54-339-8159				
<b>Test Report</b>				
Report No.	R20110609-0001	Receipt No.	201105001004-0001	
Client Name	Kim, kyung-Jae	Date of Receipt	19/05/2011	
Client Tel.(FAX)	Blank	Date of Issue	25/06/2011	
Client Address	417, Odong-ri, Hanchang-eup, Sangju-si, Gyeongsangbuk-do, Rep of Korea	Use of Report	Blank	
Name of Product	STEVIA EXTRACT	Lot No.		
Date of Manufacture or Shelf life	Blank	Manufacturer	DAEPYUNG CO., LTD.	
Test Item(s)	Unit	Specification	Test Result	Test method used
Pesticides(178 Species)	mg/kg		Not detected	Korea Food Code
Blank				
* Additional information				
<ul style="list-style-type: none"> <li>* The above merchandise was submitted and identified by the client.</li> <li>* The results shown in this test report refer only to the sample tested and it does not cover the quality of all products</li> <li>* No one can use this report for the purpose of public information, advertisement, and litigation without GHEE's consent.</li> <li>* This document cannot be reproduced except in full, without prior written approval of the client.</li> </ul>				
Tested by (b) (6)		Approved by (b) (6)		
Analyst Drug & chemistry analysis team		Director General or Laboratory Manager		
<b>Gyeongsangbuk-DO, Provincial Government Institute of Health &amp; Environment</b>				

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Test Item	Test Item
Acetochlor	Dicloran
Acrinathrin	Dicofol
Alachlor	Diethofencarb
Aldrin & Dieldrin	Dimethenamid
Anilazin	Dimethipin
Anilofos	Dimethoate
Azinphos-methyl	Dimethylvinphos
Azoxystrobin	Dimiconazole
BHC $\alpha$ , $\beta$ , $\gamma$ , $\delta$ -BHC	Diphenamid
Bifenox	Diphenylamine
Bifenthrin	Disulfoton
Bromacil	Dithiopyr
Bromopropylate	Diuron
Buprofezin	Edifenphos
Butachlor	Endosulfan $\alpha, \beta$
Cadusafos	Endrin
Captafol	EPN
Captan	Esprocarb
Carbophenothion	Ethalfuralin
Chinomethionat(Oxythioquinox)	Ethion
Chlorfenapyr	Ethoprophos
Chlorfenwinphos	Etoxazole
Chlorobenzilate	Etrinfos
Chlorothalonil	Fenamidone
Chlorpyrifos	Fenamiphos
Chlorpyrifos-methyl	Fenarimol
Chlorsulfuron	Fenhexamid
Cyfluthrin	FenitrothionMEP
Cyhalothrin	Fenobucarb
Cypermethrin	Fenoxanil
Cyproconazole	Fenpropathrin
Cyprodinil	Fensulfothion
DDT	FenthionMPP
Deltamethrin	Fenvalerate
Diazinon	Fipronil
Dichlobenil	Fluazinam
Dichlofluanid	Flucythrinate
Dichlorvos:DDVP	Fludioxonil
Diclofop-methyl	Flufenacet

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Test Item	Test Item
Flufenoxuron	Nitrapyrin
Fluquinconazole	Norflurazon
Fusilazole	Nuarimol
Flutolanil	Oxadiazon
Fluvalinate	Oxadixyi
Folpet	Oxyfluorfen
Formethion	Paclbutrazol
Fthalide	Parathion-Methyl
Furathiocarb	Penconazole
Heptachlor	Pendimethalin
Hexaconazole	Pentachloroaniline
Hexaflumuron	Permethrin
Hexazinone	PhenthoatePAP
Imazalil	Phosalone
Imibenconazole	Phosmet(PMP)imidan
Indanofan	Phosphamidone
Indoxacarb	Pirimicarb
Iprobenfos	Pirimiphos-ethyl
Iprodione	Pirimiphos-methyl
Isazofos	Pretilachlor
Isofenphos	Probenazole
Isoprothiolane	Prochloraz
Kresoxim-methyl	Procymidone
Lufenuron	Profenofos
Malathion	Prometryn
Mecarban	Propanil
Mefenacet	Propiconazole
Mepanipyrim	Propisochlor
Mepronil	Prothiofos
Metaxyl	Pyrazophos
Metconazole	Pyridaben
Methidathion	Pyridaphenthion
Methoxychlor	Pyrimethanil
Metobromuron	Pyrimidifen
Metolachlor	Quintozene
Metribuzin	Simazine
Mevinphos	Spirodiclofen
Molinate	Tebuconazole
Myclobutanil	Tebufenpyrad

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**APPENDIX C-2**  
**Pesticide Limits of Detection**



Pesticide Limit of Detection

Substance	Limit(mg/kg)
Alachor	0.05
Aldrin and dieldrin(sum of)	0.01
Azinphos-methyl	0.1
Bromopropylate	0.5
Chlordane (sum of cis- and trans- isomers and oxychlordane)	0.02
Chlorfenwinphos	0.05
Chlorpyrifos	0.01
Chlorpyrifos-methyl	0.05
Cypermethrin(and isomers)	0.05
DDT(sum of p,p'-DDT, o,p'-DDT, p,p'-DDE, and p,p'-TDE))	0.05
Deltamethrin	0.01
Diazinon	0.05
Dichlorvos	0.05
Dithiocarbamates(as CS <sub>2</sub> )	0.05
Endosulfan (sum of endosulfan isomers and endosulfan sulfate)	0.05
Endrin	0.01
Ethion	0.01
Fenitrothion	0.02
Fenvalerate	0.05
Fonofos	-
Heptachlor(Sum of heptachlor and heptachlor epoxide)	0.01
Hexachlorobenzene	-
Hexachlorocyclohexane isomers(other than γ)	-
Lindane(γ-hexachlorocyclohexane)	0.01
Malathion	0.5
Methidathion	0.02
Parathion	0.05
Parathion-methyl	0.01
Permethrin	0.05
Phosalone	0.1
Piperonyl butoxide	0.05
Pirimiphos-methyl	0.05
Pyrethrins(sum of)	1.0
Quintozene(sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulfide)	0.01

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## **APPENDIX D**

### **Report of Shelf-Life of Reb A 97%**

## **REPORT of Shelf-Life of Reb-A 97%**

File No. : DP-20110210

Date : 10. FEB. 2011

Prepared by : R.K. Kim /R&D

Prepared by : S.J. Kim /QA

Approved by : K.J. Kim /President



**DAEPYUNG CO., LTD.**

## **Table of contents**

1. General
2. Bibliography
3. Personnel
4. Testing conditions
5. Target product
6. Sampling
7. Testing procedures
8. Analysis conditions
9. Results
10. Testing lab

## 1. General

This document is prepared in order to establish and confirm the shelf-life of Reb-A 97%, Daepyeong Co., Ltd. Manufactures, and its packaging. It focuses on the lifetime of the functions of Reb-A 97%.

The study of the Korea Food & Drug Administration Food Sanitation Law, food additives and Health supplements was conducted in accordance with standards set expiration date.

The results of this study will determine the ability to mark the packages with an expiration date when stored under normal temperature and humidity conditions for 2 years.

## 2. Bibliography

This protocol is prepared and the following documents are referenced.

Korea Food & Drug Administration Food Sanitation act, food, food additives and health food by the expiration date set.

## 3. Personnel

3.1 Prepared by : Rae-Kyoung Kim

3.2 Reviewed by : Su-Jeong Kim

3.3 Approved by: Kyung-Jae Kim

## 4. Testing conditions

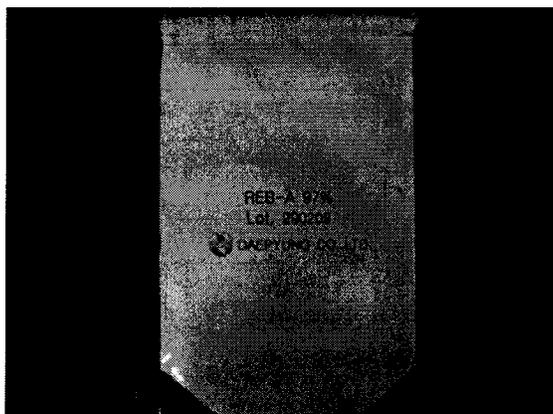
1) Test temperature: 1~30□

## 5. Target Product

5.1 name of Product: Reb-A 97%

5.2 Product Lot No. : 290209

5,3 Photos:



Reb-A 97%

## 6. Sampling

6.1 Korea Food & Drug Administration Food Code Chapter 9. Based on the sampling and handling methods to perform the experiments.

## 7. Testing procedures

### 7.1 Real life study

#### 1) Procedures

- By sampling the Reb-A 97% is placed in a plastic bag, seal and should be kept at room temperature.
- 24 months to maintain the temperature 1-30°C.
- Being kept for three months to sample is analyzed for purity and moisture.
- 24 months while still retaining the samples are analyzed every 3 months.
- Purity and content analysis should be recorded.

### 7.2 Testing and acceptance criteria

Item	Method	Criteria
Purity	See below	more than Rebaudioside-A 97%
Moisture	105°C, 2hr	Not more than 6%

## 8. Analysis conditions

### 8.1 Purity analysis conditions

#### Mobile phase

Mix HPLC-grade acetonitrile and water(80:20). Adjust the to pH3.0 with phosphoric acid(98% reagent grade).  
Filter through 0.22 µm Millipore filter or equivalent.

#### Standard solutions

- (a) Accurately weigh 50 mg of dried (105°C, 2 h) stevioside standard into a 100-mL volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase
- (b) Repeat with previously dried Rebaudioside A standard.

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- Stevioside : approx 20, 40, 80, 120 mg/100ml Wako Stevioside solutions in Diluent
- Rebaudioside A: approx 20, 40, 80, 120 mg/100ml Wako Rebaudioside A solutions in Diluent

**Sample solution**

Accurately weigh 60~120 mg of dried(105□, 2 h) sample into a 100-mL volumetric flask. Dissolve with mobile phase and dilute to volume with the mobile phase.

**Chromatogram Conditions**

Column: TSKgel™ column Amide-80 (length: 25 cm; inner diameter: 4.6 mm)  
 Mobile phase: (80:20) mixture of acetonitrile and water(pH3.0, phosphoric acid)  
 Flow rate: Adjust so that the retention time of rebaudioside A is about 21 min  
 Injection volume: 20 µL  
 Detector: UV at 210 nm  
 Column temperature: 25 □ ± 5□

**Procedure**

Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample solution and of the standard solutions.

The retention times relative to Rebaudioside A(1.00) are:

- 0.45~0.48 for Stevioside
- 0.12-0.16 for Rubusoside
- 0.25~0.30 for Dulcoside A
- 0.35~0.41 for Steviolbioside
- 0.63~0.69 for Rebaudioside C
- 0.73~0.79 for Rebaudioside B

Measure the peak areas for the seven Steviol glycosides from the sample solution(the minor components might not be detected). Measure the peak area for stevioside for the standard solution.

Calculate the percentage of each of the seven steviol glycoside, X, in the sample from the formula:

$$\%X = [W_s/W] \times [f_x A_x / A_s] \times 100$$

Where

WS is the amount (mg) of stevioside in the standard solution

W is the amount(mg) of sample in the sample solution

AS is the peak area for stevioside from the standard solution

AX is the ratio of the formula weight of X to the formula weight of stevioside: 1.00(stevioside), 0.98(dulcoside A), 1.20(rebaudioside A), 1.18(rebaudioside C), 0.80(rubusoside), 0.80(steviolbioside), 1.00(rebaudioside B).

**8.2 Moisture analysis conditions**

2 hours at 105 □ for storage calculations evaporated moisture.

**9. Results**

	Purity(%)	Moisture(%)	Remarks
2009. 02. 10	97.43	2.6	
2009. 05. 10	97.44	2.6	
2009. 08. 10	97.42	2.7	
2009. 11. 10	97.40	2.5	
2010. 02. 10	97.42	2.7	

2010. 05. 10	97.39	2.8	
2010. 08. 10	97.40	2.6	
2010. 11. 10	97.38	2.6	
2010. 02. 10	97.41	2.6	

Purity is Rebaudioside-A

### Summary

Test results showed no change in the purity and moisture, the powder remains on the state of the self-life period of two years, say it is considered set.

### 10. Testing Lab

Location: Daepyeong Co., Ltd.

Address: #417, Odong-ri, Hamchang-eup, Sangju-si, Gyeongsangbuk-do, Rep of Korea

Tel : +82(54)541-9005 Fax : +82(54)541-9016

Internet Address: [www.daepyeong.co.kr](http://www.daepyeong.co.kr)

## **APPENDIX E**

### **Summary Evaluation of Stevioside Subchronic Study**

### Summary of Study on Stevioside by Awney et al., 2010<sup>19</sup>

In a recently published exploratory subchronic toxicity study, Awney et al. (2010) investigated the effects of 97% pure stevioside on bodyweight, organ relative weight, hematological and biochemical parameters and enzyme activities in Sprague Dawley. In this 12-week toxicity study, groups of male rats (8/group) were given drinking water containing stevioside. The groups were assigned to drink distilled water (control), low-dose stevioside solution (15 mg/kg/day), high-dose stevioside solution (1500 mg/kg/day) or low-dose stevioside (15 mg/kg/day) plus inulin solution for 12 weeks as the sole source of liquid. Fluid intake was recorded daily and levels of test articles were adjusted weekly to receive the appropriate target concentration. Low dose stevioside (15 mg/kg bw/day) administration without or with inulin for 12 weeks did not reveal any adverse effects on body weight, organs relative weight, hematological and biochemical parameters or enzymes activities. However, treatment with high dose stevioside caused significant changes in several investigated toxicological parameters. Among the hematological parameters, significant changes were noted in all except WBCs, RBCs, and PCV% and in all clinical chemistry parameters except proteins, total lipids, serum alanine aminotransferase (ATL) and aspartate aminotransferase (AST). These data suggest the NOEL of 15mg/kg/day. However, critical review of the publication reveals that the study was poorly designed and implemented. Design deficiencies include: insufficient numbers of animals, group-housing with the potential for stress-related changes, unreliable access to steviol via drinking water resulting in suspect dosing calculations in group-housed cages, no indication of fasting prior to blood collection which affects many chemistry and hematological values, no urine collection and no histopathological evaluations for confirmation of findings beyond the controls. In addition to these study design deficiencies, the report fails to adequately present mean or individual organ weight data and, in general, there appears to be inadequate comparison of study findings against laboratory historical control data. Any one of these oversights could have adversely affected the results and/or interpretation of the hematological and chemistry data.

In addition to the above described parameters, tartrate-resistant alkaline phosphatase (TRAP) levels were measured and found to be significantly decreased (Awney et al., 2010). TRAP is an enzyme that is expressed by bone-resorbing osteoclasts, inflammatory macrophages and dendritic cells. This enzyme was not measured in any previous steviol glycoside studies nor has it been adequately vetted for application in toxicological studies. These investigators did not identify the specific TRAP isomer measured, the methodology employed, the handling of the samples, or any historical data on TRAP levels. The significance and relevance of this poorly documented toxicological endpoint which lacks histopathological confirmation does not appear to have a distinct role in determining the toxicological profile of a material in a test animal. The data presented by Awney et al (2010) are probably not representative of changes due to the subchronic dietary administration of steviol glycoside because of overall poor study design and reliance on the findings of the untested enzyme TRAP. The preponderance of the data from several well designed studies on steviol glycoside suggests that differences noted in hematological and chemistry data are probably random, nonspecific and not toxicologically significant.

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<sup>19</sup> Awney, H.A., Massoud, M.I. El-Maghrabi, S., 2010. Long-term feeding effect of stevioside sweetener on some toxicological parameters of growing male rats. *Journal of Applied Toxicology*, Online Publication: 19 NOV 2010; DOI: 10.1002/jat. 1604.

SUBMISSION END

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