APPLICATION TO AMEND AUSTRALIA/ NEW ZEALAND FOOD STANDARDS CODE TO REVISE THE CURRENT USE-LEVELS FOR STEVIOL GLYCOSIDES

Submitted to:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
Australia

Submitted by:

Cargill, Incorporated
15407 McGinty Rd W
Wayzata, MN 55391
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September 25, 2009

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EXECUTIVE SUMMARY

This application requests an increase to 200 mg/kg steviol equivalents for the permitted use level for ice cream, water based beverages, brewed soft drinks, formulated beverages, flavoured soy beverages and to 100 mg/kg steviol equivalents for plain soy beverages. . This request would require an amendment to Schedule 1 of Standard 1.3.1 of the Food standards Code. The new use levels are supported by sensory testing of prepared formulations. The dietary exposures were determined using survey data of consumption of intense sweeteners that were adjusted based on a comparison of sweetening potency of aspartame and steviol glycosides preparation that were both 200 times as sweet as sugar. The resulting exposures were approximately the same as calculated using the DIAMOND food consumption base by Food Standards Australia/New Zealand (FSANZ). Both exposure determinations were below the current Acceptable Daily Intake (ADI) of 0 to 4 mg/kg body weight/day as steviol equivalents.

The use of steviol glycosides preparation in the food categories and at the Maximum Permitted Levels (MPL) requested in this Application would not result in any adverse dietary implications. These levels will be consistent with existing practice and regulatory status in other jurisdictions including the United States where Cargill‟s steviol glycoside preparation is permitted at levels equivalent to current Good Manufacturing Practices (cGMP).

Steviol glycosides (steviol conjugated with glucose, xylose, and/or rhamnose) are natural constituents of the *Stevia rebaudiana* (Bertoni) plant leaves. Rebaudioside A and stevioside (closely related structural analogues) are typically identified as the principal sweetening constituents within *S. rebaudiana* and are accompanied by smaller amounts of other steviol glycosides. The steviol glycoside preparation that is the subject of this application is a chemically defined simple mixture that comprises not less than 95% of nine steviol glycosides including stevioside, rebaudioside A, rebaudioside C, dulcoside A, rubusoside, steviolbioside, rebaudioside B, rebaudioside D and rebaudioside F similar to the current Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2007) specification for steviol glycosides. The inclusion of rebaudiosides D and F in the definition of steviol glycosides will be addressed by JECFA in 2010. It is produced as a white to off-white powder, with a characteristic sweet taste. It has a sweetening potency of approximately 200 to 300 times that of sucrose.

As mentioned previously, rebaudioside A and stevioside are the primary constituents within *S. rebaudiana.* Rebaudioside A, has the chemical name 13-[(2-O-β-D glucopyranosyl-3-O-β-Dglucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester (CAS No. 58543-16-1), while stevioside, has the chemical name: 13-[(2-O-β-D-glucopyranosyl-β-Dglucopyranosyl)oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester.

All other minor steviol glycosides have the structural backbone or aglycone structure of steviol with varying number of glycoside linkages.

Subsequent to the steviol glycoside approval within Australia/New Zealand a number of pre-clinical and clinical studies have been reported within the literature supporting the safety of steviol glycosides. A review of the most recently conducted pre-clinical and clinical studies which were a key component of the recent JECFA steviol glycoside re-evaluation are provided for completeness in this application.

Studies conducted to evaluate the metabolism and pharmacokinetic profile for steviol glycosides have been established. Rat and human metabolism studies carried out by Cargill using high-purity rebaudioside A and stevioside affirm their metabolic equivalence. Because of this equivalence, safety studies conducted with various steviol glycoside preparations including high purity rebaudioside A or stevioside meeting the JECFA specification may be used to support the safety assessment of steviol glycosides in general.

Steviol glycosides are not readily absorbed from the upper small intestine of the rat or human following oral administration. Human digestive enzymes are not capable of hydrolysing β-glycosidic bonds and thus steviol glycosides are expected to escape digestion in the upper gastrointestinal tract. Microbes of the *Bacteroidaceae* family (predominantly Bacteroides) transform rebaudioside A and stevioside to steviol in the large intestine of the rat and human. Following absorption, steviol enters the hepatic circulation and undergoes conjugation with glucuronic acid to form steviol glucuronide. In rats, steviol, administered as steviol or available following cleavage of glycosides in the gut, has been shown to be primarily excreted in the faeces *via* the bile (generally within 48 hours), with smaller amounts in the urine (less than 3%) (Roberts and Renwick, 2008). In an additional human metabolism study, the major excretory route was confirmed to be urinary (Wheeler *et al*., 2008) due to the lower molecular weight threshold for biliary excretion in rats (325) as compared to humans (500 to 600) (Renwick, 2007).

Additional subchronic feeding studies of 4 to 13 weeks in duration in rats with high-purity rebaudioside A have reported no evidence of systemic toxicity (Curry and Roberts, 2008; Nikiforov and Eapen, 2008). In a study designed to meet FDA Redbook Guidelines and conducted according to Good Laboratory Practices (GLP)-compliance standards, Cargill"s steviol glycosides preparation was reported not to present any evidence of systemic toxicity when provided to both sexes of Han Wistar rats at dietary concentrations of up to 100,000 ppm (9,938 and 11,728 mg/kg body weight/day for males and females, respectively) for 4 weeks or 50,000 ppm (4,161 and 4,645 mg/kg body weight/day for males and females, respectively) for 13 weeks (Curry and Roberts, 2008). The results of the 13-week toxicity study of Cargill"s steviol glycosides preparation (Curry and Roberts, 2008) closely reflect those of a similar study in Sprague-Dawley rats using test material of similar purity (Nikiforov and Eapen, 2008). Nikiforov and Eapen (2008) reported that feeding of rebaudioside A in the diet *ad libitum* to produce target doses 500, 1,000, or 2,000 mg/kg body weight/day was without adverse effect on body weight gain, terminal body weights, clinical and functional observational battery observations, or on the results of the haematology, serum chemistry, or urinalysis evaluations. Treatment was reportedly not associated with any organ weight or macroscopic or microscopic tissue changes.

Multi-generation reproductive and developmental studies conducted with high-purity rebaudioside A (Curry *et al*., 2008) have shown a lack of reproductive or developmental toxicity in rats.

The existing data pertaining to the genotoxicity of rebaudioside A and stevioside, as reviewed by Brusick (2008), demonstrate a lack of genotoxic activity for both compounds *in vitro* and *in vivo*. Furthermore, rebaudioside A, in the presence and in the absence of an exogenous metabolic activation system (rat S9), was not mutagenic in *Salmonella*

typhimurium (*S. typhimurium*) strains TA98, TA100, TA1535, or TA1537 as well as *Escherichia coli* (*E. coli*) WP2 uvrA at concentrations of up to 5 mg/mL (Williams and Burdock, 2009). In Chinese hamster lung fibroblasts and human lymphoma cells, rebaudioside A at concentrations up to 5 mg/mL failed to induce chromosomal aberrations and rebaudioside A did not produce any gene mutations at concentrations of up to 5 mg/mL, with or without metabolic activation, in mouse lymphoma L5178YTk$^{+/-}$ cells (Williams and Burdock, 2009). Moreover, rebaudioside A (95.6% purity) did not elicit a genotoxic response in a mouse micronucleus assay when administered intraperitoneally at doses of up to 750 mg/kg body weight or in an unscheduled DNA synthesis assay when administered by gavage at 2,000 mg/kg body weight (Williams and Burdock, 2009).

One comet assay conducted with stevioside (88.6% purity) indicated a positive result, but this study was subsequently shown to be uninterpretable (Geuns, 2007; Williams, 2007). Another comet assay (Sekihashi *et al*., 2002) demonstrated no evidence of genotoxicity of *Stevia* extract comprising 52% stevioside and 22% rebaudioside A when orally administered to mice at up to 2,000 mg/kg body weight/day. The lack of genotoxic potential is confirmed by the results of 2 carcinogenicity studies on stevioside which show no oncogenic or toxicological effects (Xili *et al*., 1992; Toyoda *et al*., 1997).

Human studies specifically on Cargill"s steviol glycosides preparation showed no effects on glucose homeostasis or blood pressure at doses of up to 1,000 mg/day (approximately 16 mg/kg body weight/day), a dose more than 3-fold greater than the predicted intake of steviol glycosides in adults and children with diabetes (4.5 mg/kg body weight/day).

The latest pre-clinical and clinical steviol glycoside publications fully support the safety of the material and corroborate the FSANZ conclusion that steviol glycosides are safe for human consumption within specified food categories at defined use levels. The revision of the food categories and use levels outlined within the application is fully supported by the steviol glycoside safety database.

PART A. GENERAL INFORMATION

1.0 APPLICATION DETAILS

(A) Applicant's Name

Amy Boileau, PhD, RD
Manager, Regulatory and Scientific Affairs

(B) Company/Organisation Name

Cargill, Incorporated

(C) Address (Street and Postal)

15407 McGinty Rd W
Wayzata, MN 55391
USA

(D) Telephone and Facsimile Numbers

Telephone: (952) 742-4262
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(E) Email Address

Email: amy_boileau@cargill.com

(F) Nature of Applicant's Business

Cargill is an international producer and marketer of food, agricultural, financial and industrial products and services. As part of our business, we are a supplier to the food industry of steviol glycosides.

(G) Details of Other Individuals, Companies or Organisations Associated with the Application

David Bennett of Mimda Enterprises Pty Ltd is our local contact for this application. He can be contacted as follows:
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2.0 PURPOSE OF APPLICATION

Cargill, Inc. is submitting this application concerning steviol glycosides to Food Standards Australia/New Zealand (FSANZ) seeking to amend Schedule 1 of Standard 1.3.1, to increase the permitted use levels of steviol glycosides in the following food categories:

- Increase use level of steviol glycosides in ice cream from 64 mg/kg steviol equivalents to 200 mg/kg steviol equivalents;
- Increase use level of steviol glycosides in water based flavoured drinks from 160 mg/kg steviol equivalents to 200 mg/kg steviol equivalents;
- Increase use level of steviol glycosides in brewed soft drinks from 160 mg/kg steviol equivalents to 200 mg/kg steviol equivalents;
- Increase use level of steviol glycosides in formulated beverages from 160 mg/kg steviol equivalents to 200 mg/kg steviol equivalents;
- Increase use level of steviol glycosides in flavoured soy beverages from 175 mg/kg steviol equivalents to 200 mg/kg steviol equivalents;
- Increase use level of steviol glycosides in plain soy beverages from 65 mg/kg steviol equivalents to 100 mg/kg steviol equivalents;

The increase in permitted use levels in ice cream, water based flavoured drinks, brewed soft drinks, formulated beverages and plain and flavoured soy beverages is comparable to the levels requested for the same food categories within the European Union. Likewise, these levels would be acceptable within the US as a consequence of the GRAS determination which supports levels related to current good manufacturing practices (cGMP).

3.0 JUSTIFICATION FOR THE APPLICATION

3.1 Technological Function for the Food Additive

The application to revise the current use levels for steviol glycosides within Schedule 1 of Standard 1.3.1 of the Food standards Code is supported by sensory testing of prepared formulations (See section D part 3 of the application).

In support of the proposed change to the current use levels, steviol glycosides are known to represent the first natural plant extracts that have the potential to be commercially successful high-potency sweeteners with a wide range of applications. The flavour and sweetness qualities of steviol glycosides, coupled with their high stability, mean these extracts are capable of wide use and can function as multi-purpose, low-calorie sweeteners.

3.1.1 Benefits of Steviol Glycosides to Consumers

Steviol glycosides fulfil all the key requirements of effective low-calorie sweeteners. Benefits of using steviol glycosides as sweeteners include:

- Low levels of use (high sweetness potency);
- Low calorie;
- Good taste quality;
- Good chemical stability under conditions of use in food;
- Suitable for use by individuals with diabetes;
- No adverse effects on oral hygiene;
- Can be blended with other high-potency and bulk sweeteners.

According to the literature, the sweetness intensity of steviol glycosides is in the range of 100 to 400 times that of sucrose, weight-for-weight. In beverage applications, steviol glycoside preparations have the same sweetness potency as aspartame and can replace aspartame on a 1:1 basis. Thus, the amount required to give equal sweetness intensity to foods and beverages is considerably smaller than the amount of sugar.

As discussed in detail in later sections, orally ingested steviol glycosides pass through the gastrointestinal tract unabsorbed and therefore are not metabolized *via* energy-generating physiological pathways. Instead, steviol glycosides are hydrolysed by the microflora of the large intestine to release steviol and glucose. Since glucose is released in the lower segment of the gut (colon in humans/caecum in rodents), and due to the low levels used, it is not expected to be a significant source of energy. Given their metabolic fate, steviol glycosides are considered to be of low caloric value. Consequently, steviol glycosides may be used to sweeten foods and beverages without providing appreciable incremental energy.

Steviol glycosides have been determined to be stable under various conditions of use in foods and beverages. Following their review of the data pertaining to the stability of steviol glycosides, The Joint FAO/WHO Expert Committee on Food Additives (JECFA) “concluded that steviol glycosides are sufficiently thermally and hydrolytically stable for use in foods, including acidic beverages, under normal conditions of processing and storage” (JECFA, 2007).

Human studies conducted with Cargill"s steviol glycoside preparation showed no adverse effects on glucose homeostasis at dose levels of up to 1 g/day (16 mg/kg body weight/day). Since special studies failed to show any impact on glucose homeostasis, steviol glycosides can be a valuable dietary asset as a sweetener for use by the diabetic population and others who follow a low-glycaemic diet.

No cariogenic effects were observed in *Streptococcus sobrinus*-inoculated rats administered 0.5% rebaudioside A or stevioside (purity not reported) for 5 weeks in the drinking water (Das *et al*., 1992). Steviol glycosides can therefore be used in sugar-free products for dental hygiene purposes.

Steviol glycosides can be blended freely with other high-potency and bulk, non- or low-calorie, sweeteners. Both stevioside and rebaudioside A have been reported to be widely synergistic with other sweeteners (Schiffman *et al.*, 1995). This indicates that blends could be developed where, through the action of quantitative synergy, the overall sweetener content could be lowered.

3.2 Safety of the Food Additive

Steviol glycosides are currently permitted in the Code in a wide range of foods supported by a safety assessment conducted by FSANZ. This application proposes to increase the levels of use of steviol glycosides in ice cream, water based flavoured drinks, brewed soft drinks, formulated beverages, and plain and flavoured soy beverages. The increased use levels do not impact significantly on the exposure estimates. New exposure estimates determined from post marketing surveillance studies of other intense sweeteners indicate that the dietary exposures are still below the current Acceptable Daily Intake (ADI) of 0 to 4 mg/kg body weight/day (steviol equivalents). New additional animal safety studies and human studies that were not reviewed by FSANZ in the previous safety assessment were also submitted to support safety. The new safety studies do not change the current ADI but address issues related to potential pharmacological effects previously attributed to impure steviol glycoside preparations. These studies were assessed by JECFA (2008) and led to the re-classification from a temporary to a full ADI.

3.3 Costs and Benefits for Industry, Consumers and Government Associated With Use of the Food Additive

Manufacturers and/or importers of steviol glycosides and foods containing steviol glycosides would benefit from the opportunities within the market for sale both domestically and internationally, as well as product innovation. Since steviol glycosides are already approved for many food uses within Australia and New Zealand, there is no perceived benefit or added cost to government.

4.0 SUPPORT FOR THE APPLICATION

Following release of the approval for steviol glycosides by FSANZ in 2008, a number of scientific and regulatory decisions have been published including the JECFA opinion amending the temporary ADI to a full ADI and several FDA “no objection” letters following formal GRAS notifications to the Agency.

To support these regulatory opinions a number of pre-clinical and clinical studies were conducted which further corroborated the safety and lack of pharmacological activity of steviol glycosides when administered orally at levels supportive of likely human exposures from use as a sweetening agent. A review of the pre-clinical and clinical studies is provided in Section C.

5.0 ASSESSMENT PROCEDURE

Consistent with current policy, the request for changes to use levels in specified food products would be considered a General Procedure (level 1) endeavour.

6.0 CONFIDENTIAL COMMERCIAL INFORMATION (CCI)

None provided.

7.0 EXCLUSIVE CAPTURABLE COMMERCIAL BENEFIT (ECCB)

The applicant does not claim any exclusive commercial benefit. Although the manufacturers of steviol glycoside preparations will derive some economic benefit from approval of the application, other manufacturers who use the steviol glycoside preparation will also derive some economic benefit. In addition, there are several different manufacturers of steviol glycoside preparations and therefore the economic benefit will not be exclusive.

8.0 INTERNATIONAL AND OTHER NATIONAL STANDARDS

8.1 International Standards

At the 69th meeting of JECFA (2008), the Committee evaluated further data regarding steviol glycosides and re-classified the status of the ADI from temporary to a full ADI of 0 to 4 mg/kg based on steviol equivalents since only free steviol is absorbed from the gastrointestinal tract. The Committee also established specifications for steviol glycosides that require purity levels of greater than 95%. Proposed food categories of use and use levels were used to evaluate estimated consumption based on replacement of sugar in various diets and based on replacement of other intense sweeteners.

8.2 Other National Standards or Regulations

8.2.1 United States

Rebiana was the subject of a GRAS Notification (GRN 000253 – U.S. FDA, 2008a) submitted by Cargill on the rebaudioside A material discussed in this dossier "where the FDA did not have any safety questions concerning the use of Rebiana for general purpose uses, not to exceed GMP". Another GRAS notice (GRN 000252 – U.S. FDA, 2008b) from a different manufacturer of steviol glycosides has been accepted. Additional GRAS Notices (GRN 000275 – U.S. FDA, 2009a for purified steviol glycosides with rebaudioside A as the principal component; GRN 000278, U.S. FDA, 2009b for purified rebaudioside A for use as a sweetener in foods; GRN 000282, U.S. FDA, 2009c for purified rebaudioside A for use as a sweetener in foods; and GRN 000287, U.S. FDA, 2009d for purified steviol glycosides for use as a sweetener in foods) have also been recently accepted by the U.S. FDA.

8.2.2 European Union

Several food additive petitions for steviol glycosides are currently being reviewed by the European Food Safety Agency (EFSA) including one for Cargill's steviol glycoside preparation. The food categories and use levels requested within the EU are similar to those revisions to be considered by FSANZ within the application.

8.2.3 Japan

In Japan, 3 forms of purified stevioside (*i.e.*, crude extract, 50% pure, and ≥90% pure) and *S. rebaudiana* leaf extracts are accepted for general use as sweeteners in a variety of foods and beverages including pickling gum, pickles, dried seafood, meat, fish, soy sauce, bean pastes, sugarless chewing gums, juices, cola, table-top sweeteners, and ice cream (Marie, 1991; Das *et al.*, 1992; Ferlow, 2005). This approval for general use is under the pretence that stevioside is a naturally occurring sweetener and its use is believed to be safe (Bertorelli and Czarnowski-Hill, 1990).

8.2.4 Korea

Stevioside is 1 of the 4 most widely-used high intensity sweeteners in Korea (the other 3 sweeteners being saccharin, D-sorbitol, and aspartame) and has been approved for use as a sweetener with some limitations (*i.e*., stevioside is not added to some food categories) (Kinghorn *et al.*, 1998; Chung *et al*., 2005). The main food usages of stevioside in Korea include cookies, sugar products, beverages, seasonings soy sauce, honey, and *so-ju* (a traditional liquor made of starch).

8.2.5 Central/South America

Stevioside, *S. rebaudiana* leaves, and highly refined extracts are permitted for use as low-calorie sweeteners in Brazil, Argentina, Paraguay, Uruguay, Mexico, Peru, and Colombia.

8.2.6 Other Jurisdictions

Other countries permitting the use of steviol glycosides include China, Malaysia, Russia, Switzerland, Taiwan, Turkey, and Ukraine.

9.0 STATUTORY DECLARATION

9.1 Statutory Declaration – Australia

Statutory Declarations Act 1959

I, Amy Boileau, PhD, RD, at 15407 McGinty Rd W , Wayzata, MN 55391 USA
as Manager, Regulatory and Scientific Affairs, Cargill, Inc.

Make the following declaration under the Statutory Declarations Act 1959.

1. the information provided in this application fully sets out the matters required

2. the information provided in this application is true to the best of my knowledge and belief

3. no information has been withheld that might prejudice this application, to the best of my knowledge and belief.

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

Amy Boileau, PhD, RD
15407 McGinty Rd W.
Wayzata, MN 55391 USA
Manager, Regulatory and Scientific Affairs, Cargill, Inc.

State Of: Minnesota, USA

County Of: Hennepin

This instrument was acknowledged before me on 8/14/09 (Date) by Amy Boileau.

Brenda J. Doubler
(signature of notarial officer)

BRENDA J DOUBLER
Notary Public
Minnesota
My Commission Expires January 31, 2010

Administrative Assistant
(Title)

My commission expires: January 31, 2010

7

9.2 Statutory Declaration- New Zealand

Oaths and Declarations Act 1957

I, Amy Boileau, PhD, RD at 15407 McGinty Rd W , Wayzata, MN 55391 USA as Manager, Regulatory and Scientific Affairs, Cargill, Inc. solemnly and sincerely declare that:

1. the information provided in this application fully sets out the matters required; and

2. the information is true to the best of my knowledge and belief; and

3. no information has been withheld which might prejudice this application to the best of my knowledge and belief.

And I make this solemn declaration conscientiously believing the same to be true and by virtue of the *Oaths and Declarations Act 1957.*

Amy Boileau, PhD, RD
15407 McGinty Rd W.
Wayzata, MN 55391 USA
Manager, Regulatory and Scientific Affairs, Cargill, Inc.

State Of: Minnesota, USA

County Of: Hennepin

This instrument was acknowledged before me on 8/14/09 (Date) by Amy Boileau.

Brenda J. Doubler
(signature of notarial officer)

BRENDA J DOUBLER
Notary Public
Minnesota
My Commission Expires January 31, 2010

Administrative Assistant
(Title)

My commission expires: January 31, 2010

10.0 CHECKLIST

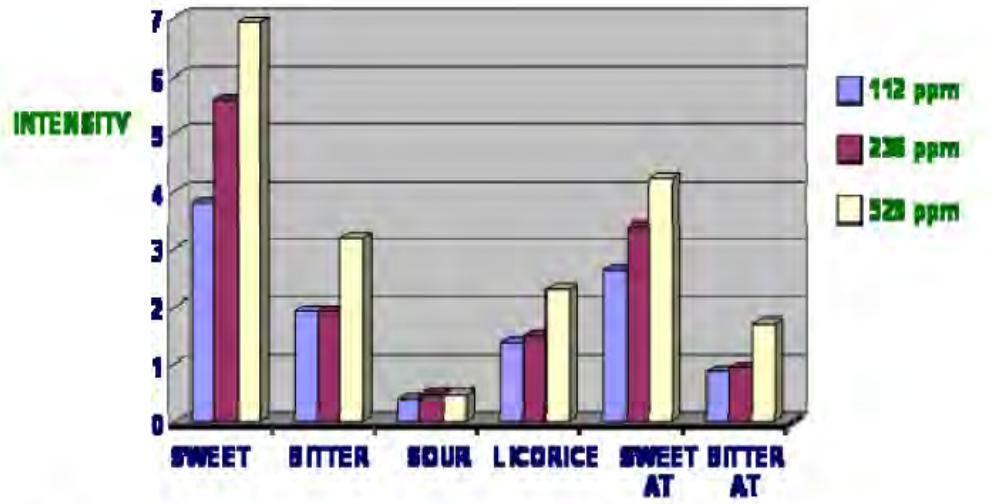
Checklist Parameters	Data Provided	Data Not Provided	Omission Justified
General Requirements			
Form of application	X		
Applicant details	X		
Purpose of application	X		
Justification for the application	X		
Information to support the application	X		
Assessment procedure	X		
Confidential Commercial Information	X		
Exclusive Capturable Commercial Benefit	X		
International standards	X		
Statutory Declaration	X		
Food Additives			
Support for the application	X		
Nature and technological function information	X		
Identification information	X		
Chemical and physical properties	X		
Impurity profile	X		
Manufacturing process	X		
Specifications	X		
Food labelling	X		
Analytical detection method	X		
Toxicokinetics and metabolism information	X		
Toxicity information	X		
Safety assessments from international agencies	X		
List of foods likely to contain the food additive	X		
Proposed levels in foods	X		
Percentage of food group to contain the food additive	X		
Use in other countries (if applicable)	X		

PART B. TECHNICAL INFORMATION ON THE FOOD ADDITIVE

1.0 NATURE AND TECHNOLOGICAL FUNCTION OF STEVIOL GLYCOSIDES

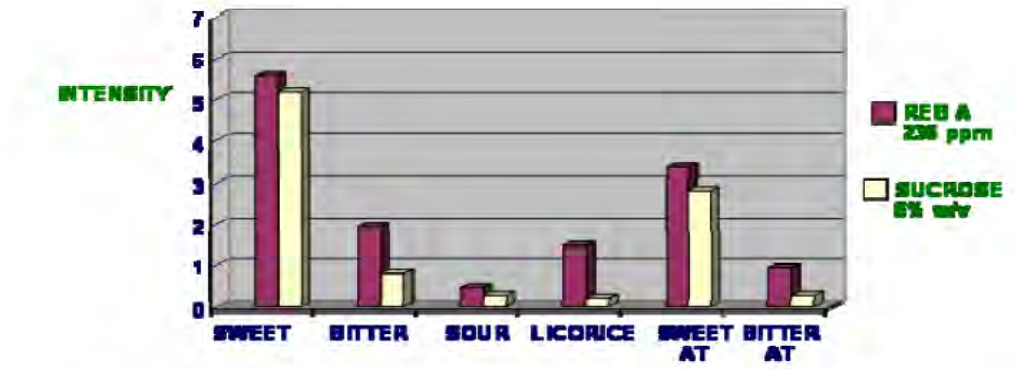
The descriptive taste characteristics (*i.e*., sweetness, bitterness, sourness, black liquorice flavour, sweetness aftertaste, and bitterness aftertaste) of steviol glycoside preparation at concentrations of 112, 236, or 529 mg/L were evaluated and compared to those of aspartame at concentrations of 155, 249, or 358 mg/L and to sucrose at concentrations of 3, 4.5, or 6%.

The results show that, in common with a number of permitted sweeteners, steviol glycosides have other tastes in addition to sweetness. Specifically, steviol glycoside preparation was characterized by sweetness, bitterness, black liquorice flavour, and sweetness and bitterness aftertastes, with the intensity of these attributes increasing with the concentration (see Figure 1-1). In comparison, both aspartame and sucrose elicited perceptions of sweetness and a sweetness aftertaste, with the intensity of these sensations also increasing in a concentration dependent manner. However, these additional tastes are not significant at the concentrations in which the sweetener would be perceived in the mouth as a result of the uses proposed. Direct comparison of the taste characteristics of sucrose and steviol glycoside preparation reveals a similar taste profile for both sweeteners at levels of comparable sweetness (see Figure 1-2).



AT = Aftertaste

Figure 1-1 Quantitative Descriptive Scores for 3 Concentrations of Steviol Glycoside Preparation in Room-Temperature Water



AT = Aftertaste

Figure 1-2 Comparison of Quantitative Descriptive Scores of Steviol Glycoside Preparation and Sucrose in Room-Temperature Water

2.0 INFORMATION TO ENABLE IDENTIFICATION OF STEVIOL GLYCOSIDES

2.1 Identity of Substance

JECFA has concluded that that the most appropriate name to be used for this natural extract was „steviol glycosides". Steviol glycosides have the food additive number INS 960. Steviol glycosides (steviol conjugated with glucose, xylose, and/or rhamnose) are natural constituents of the *Stevia rebaudiana* (Bertoni) plant leaves. Rebaudioside A and stevioside (closely related structural analogues) are typically identified as the principal sweetening constituents within *S. rebaudiana* and are accompanied by smaller amounts of other steviol glycosides. JECFA has defined steviol glycosides as a mixture comprising not less than 95% of steviol glycosides including rebaudioside A, stevioside, rebaudioside C, dulcoside A, rubusoisde, steviolbioside and rebaudioside B. The inclusion of rebaudiosides D and F in the definition of steviol glycosides will be addressed by JECFA in 2010. It is produced as a white to off-white powder, with a characteristic sweet taste.

2.1.1 Chemical Name and Chemical Abstract Service (CAS) Number

2.1.1.1 Rebaudioside A

Chemical Name: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-Dglucopyranosyl) oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester

CAS No.: 58543-16-1

2.1.1.2 Stevioside

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

CAS No: 57817-89-7

2.1.1.3 Rebaudioside C

Chemical Name: 13-[(2-O-6-deoxy-β-L-mannopyransoyl-3-O-β-D-glucopyranosyl-β-Dglucopyranosyl) oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester

CAS No: 63550-99-2

2.1.1.4 Dulcoside A

Chemical Name: 13-[(2-O-6-deoxy-β-L-mannopyransoyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester

CAS No: 64432-06-0

2.1.1.5 Rubusoside

Chemical Name: 13-[β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester

CAS No: 64849-39-4

2.1.1.6 Steviolbioside

Chemical Name: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid

CAS No: 41093-60-1

2.1.1.7 Rebaudioside B

Chemical Name: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-Dglucopyranosyl) oxy] kaur-16-en-18-oic acid

CAS No.: 58543-17-2

2.1.1.8 Rebaudioside D

Chemical Name: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-Dglucopyranosyl) oxy] kaur-16-en-18-oic acid-2-O-β-D-glucopyranosyl-β-Dglucopyranosyl ester

CAS No: 64849-39-4

2.1.1.9 Rebaudioside F

Chemical Name: 13-[(2-O-β-D-xylopyranosyl-3-O-β-D-glucopyranosyl-β-Dglucopyranosyl) oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester

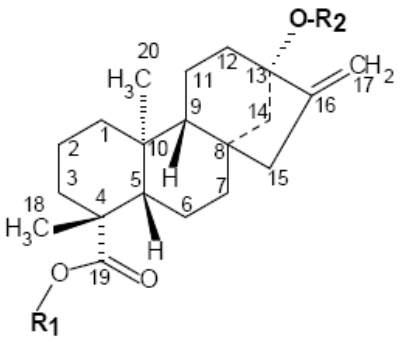
CAS No.: 438045-89-7

2.1.2 Synonyms, Trade Names, and Abbreviations

steviol glycosides, rebiana, rebaudioside A, Truvia™

2.1.3 Molecular and Structural Formulae, and Molecular Weights of the Components of the Mixture

Stevioside, rebaudioside A, rebaudioside C, dulcoside A, rubusoside, steviolbioside, rebaudioside B, rebaudioside D and rebaudioside F share the same general steviol aglycone structure (see Figure 2.1.3-1).

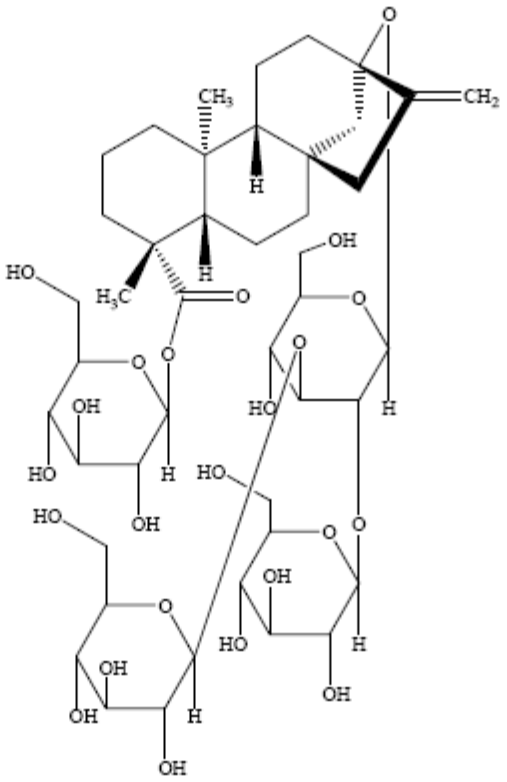


Steviol: R_1 and R_2 = H

Figure 2.1.3-1 Steviol Aglycone Structure

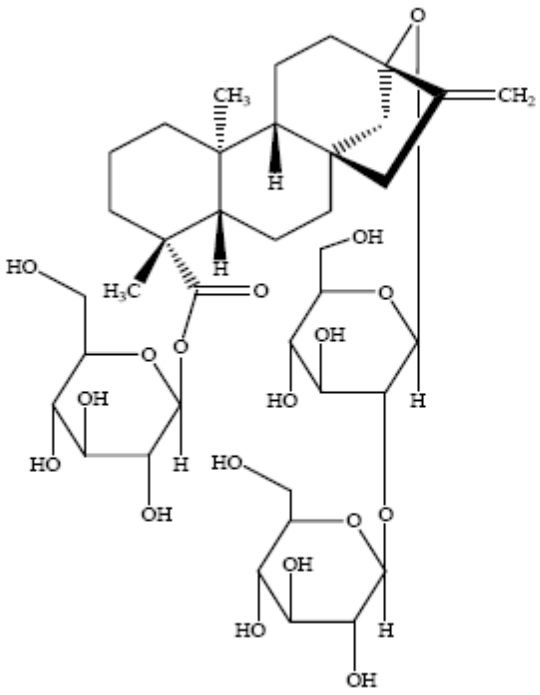
2.1.3.1 Rebaudioside A (CC-00201)

Molecular Formula: $C_{44}H_{70}O_{23}$ Molecular Weight: 967.014 g/mol



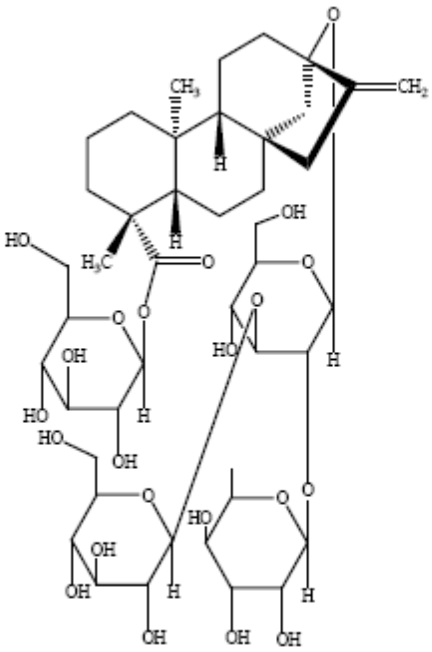
2.1.3.2 Stevioside

Molecular Formula: $C_{38}H_{60}O_{18}$ Molecular Weight: 804.87



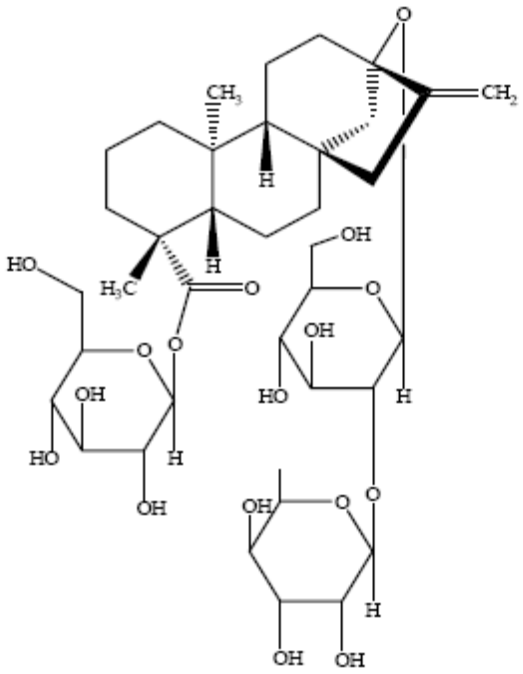
2.1.3.3 Rebaudioside C

Molecular Formula: $C_{44}H_{70}O_{22}$ Molecular Weight: 951.01



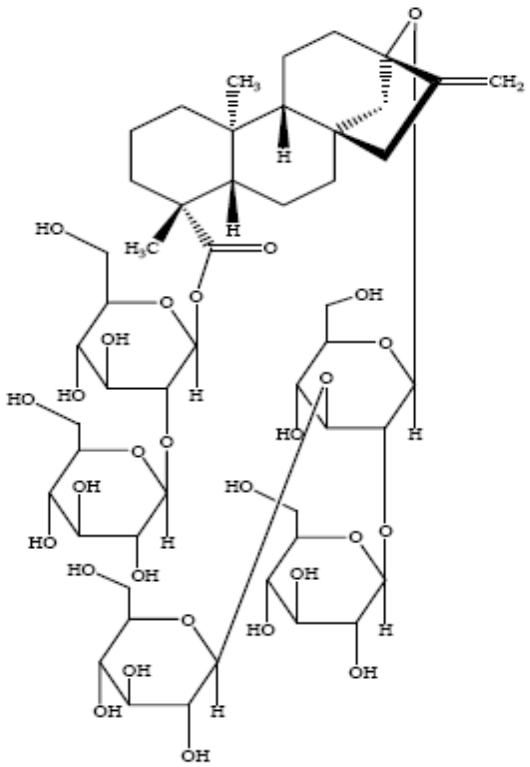
2.1.3.4 Dulcoside A

Molecular Formula: $C_{38}H_{60}O_{17}$ Molecular Weight 788.87



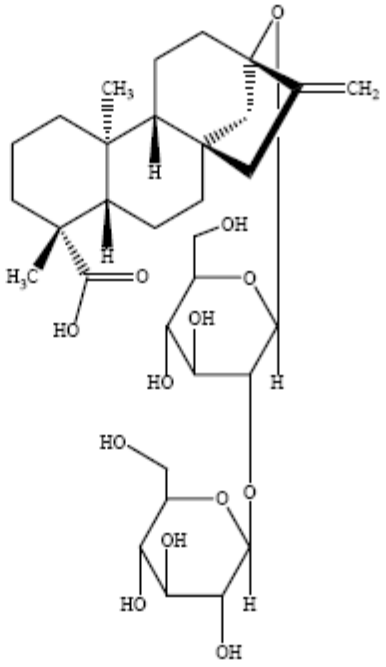
2.1.3.5 Rubuside

Molecular Formula: $C_{32}H_{50}O_{13}$ Molecular Weight 642.73



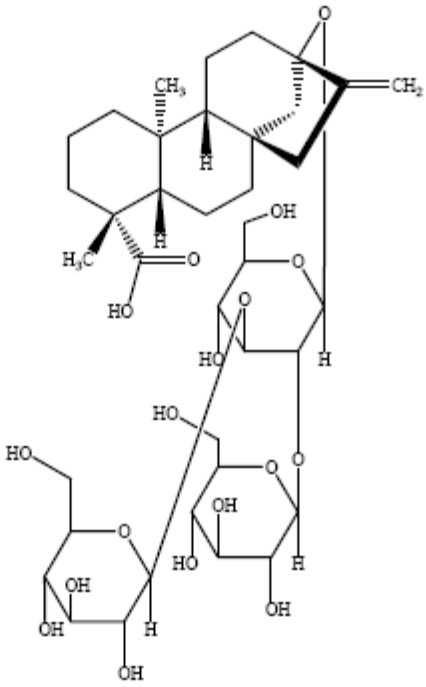
2.1.3.6 Steviolbioside

Molecular Formula: $C_{32}H_{50}O_{13}$ Molecular Weight 642.73



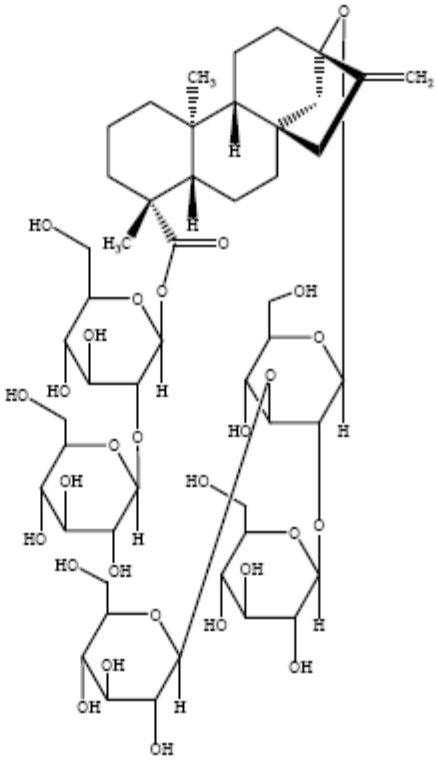
2.1.3.7 Rebaudioside B

Molecular Formula: $C_{38}H_{60}O_{18}$ Molecular Weight: 804.88



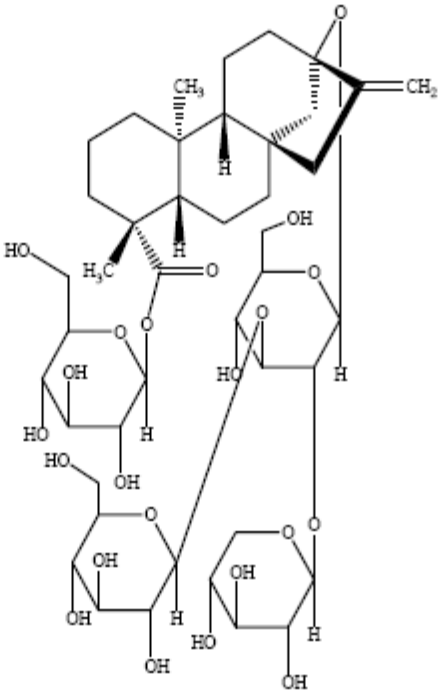
2.1.3. 8 Rebaudoside D

Molecular Formula: $C_{50}H_{80}O_{28}$ Molecular Weight: 1128



2.1.3.9 Rebaudioside F

Molecular Formula: $C_{43}H_{68}O_{22}$ Molecular Weight: 936.99



2.1.4 Purity in Percentage; Method of Determination; Data Printout (Chromatograms, spectra, *etc*.)

The steviol glycoside preparation contains not less than 95% of stevioside, rebaudioside A, rebaudioside C, dulcoside A, rubusoside, steviolbioside, rebaudioside B, rebaudioside D and rebaudioside F. The product is assayed for steviol glycoside content by high-performance liquid chromatography (HPLC). HPLC analysis of 3 production lots of Cargill's steviol glycoside preparation indicated rebaudioside A levels of 96.7 to 97.8%, rebaudioside B levels of 1.1 to 1.2% and rebaudioside F levels of 0.20 to 0.27% (see Table 2.1.4-1) For the HPLC chromatograms for each sample lot, please refer to Appendix A.

Table 2.1.4-1 Levels of Steviol Glycosides (% wt/wt) in Cargill's Steviol Glycoside Product in 3 Production Lots

Related Steviol Glycosides	Lot Numbers		
	08F161SB201	08U091DR201	08U092DR201
Rebaudioside A	97.80	96.7	97.4
Stevioside	0.56	0.40	0.40
Rebaudioside C	0.31	0.20	0.20
Dulcoside A	ND	ND	ND
Rubusoside	Not tested	Not tested	Not tested
Steviolbioside	ND	ND	ND
Rebaudioside B	ND	1.1	1.20
Rebaudioside D	1.11	1.60	1.60
Rebaudioside F	0.27	0.20	0.20

ND = not detected

3.0 INFORMATION ON THE CHEMICAL AND PHYSICAL PROPERTIES OF STEVIOL GLYCOSIDE PREPARATION

As per the original application.

4.0 INFORMATION ON IMPURITY PROFILE

Composition discussed in Section 2.1.

5.0 MANUFACTURING PROCESS

Manufacturers use the same basic steps to extract steviol glycosides from the leaves of the stevia plant, although there is some variation in the later stages of purification and separation of glycosides. The process generally involves:

- Extraction from the leaves by dissolving the steviol glycosides in warm/hot water in a batch system 3 – 5 times or by a continuous reverse flow system
- Flocculation and precipitation of suspended matter
- Filtration
- Concentration by vacuum assisted evaporation
- Adsorption (and release by alcohol) in a resin exchange process
- Ion-exchange purification
- Further filtration and concentration
- Spray drying or crystallisation.

Further processing to concentrate and separate a high rebaudioside A product is often undertaken (especially in Japan) and may involve patented procedures, such as some enzymatic modification.

6.0 SPECIFICATION FOR IDENTITY AND PURITY

6.1 Current Official JECFA Chemical Specifications for Steviol Glycosides

JECFA has assessed the safety of stevioside specifically and steviol glycosides in general on several previous occasions. Specifications for steviol glycosides were adopted by the Committee at the 69th meeting and are listed in Table 6.1-1 (JECFA, 2008).

Table 6.1-1 Current Chemical Specifications for Steviol Glycosides as Adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the 69th Meeting	
Parameter	**Limit**
Assay	Not less than 95% of the total of the seven named steviol glycosides[1] on the dried basis
Identification	
Solubility	Freely soluble in water
Stevioside and rebaudioside A	The main peak in the chromatogram obtained by the following procedure in Method of Assay corresponds to either stevioside or rebaudioside A
pH	Between 4.5 and 7.0 (1 in 100 solution)
Purity	
Total ash	Not more than 1%
Loss on drying	Not more than 6% (105°, 2h)
Residual solvents	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol
Arsenic	Not more than 1 mg/kg
Lead	Not more than 1 mg/kg

[1] Stevioside, rebaudioside A, rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B.

6.2 Proposed Minor Change to Chemical Specifications for Steviol Glycoside Preparation

A number of chemical Lots of steviol glycosides contain detectable levels of two additional steviol glycosides, rebaudioside D and rebaudioside F. This is the only proposed change to the current official JECFA specifications that the applicant is aware of which was discussed at the Codex Committee on Food Additives in March, 2009. It will be also discussed at JECFA in 2010. Details regarding the proposed chemical specifications are provided in Table 6.2-1.

Table 6.2-1 Proposed Chemical Specifications for Steviol Glycosides	
Parameter	**Limit**
Assay	Not less than 95% of the total of the nine named steviol glycosides[1] on the dried basis
Identification	
Solubility	Freely soluble in water
Stevioside and rebaudioside A	The main peak in the chromatogram obtained by the following procedure in Method of Assay corresponds to either stevioside or rebaudioside A
pH	Between 4.5 and 7.0 (1 in 100 solution)
Purity	
Total ash	Not more than 1%
Loss on drying	Not more than 6% (105°, 2h)
Residual solvents	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol
Arsenic	Not more than 1 mg/kg
Lead	Not more than 1 mg/kg

[1] Stevioside, rebaudioside A, rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B, rebaudioside D and rebaudioside F.

6.3 Chemical Analysis Data to Support the Proposed Specifications

Chemical analysis of three manufactured Lots of Cargill"s steviol glycoside preparation (see Table 6.3-1) indicated that the manufacturing process resulted in a steviol glycoside preparation that has a consistent chemical composition.

Table 6.3-1 Chemical Analysis Data for Three Manufacturing Lots of Cargill's Steviol Glycosides Preparation				
Parameter	**JECFA Specification Limit**	**Manufacturing Lots**		
		07C281MN201	**07C291MN201**	**07C301MN201**
Assay[1]	Not less than 95%	101.6	101.2	100.5
Identification				
Solubility	Freely soluble in water	Freely soluble in water	Freely soluble in water	Freely soluble in water
Stevioside and Rebaudioside A	Main HPLC peaks	Positive	Positive	Positive
pH	Between 4.5 and 7.0	5.5	5.5	5.5
Purity				
Total ash	Not more than 1%	0.036	0.025	0.034
Loss on drying	Nor more than 6%	0.31	0.13	0.20
Residual solvents				
Methanol	Not more than 200 mg/kg	<50	<50	<50
Ethanol	Not more than 5000 mg/kg	3000	2300	4500
Arsenic	Not more than 1 mg/kg	<1	<1	<1
Lead	Not more than 1 mg/kg	<1	<1	<1

[1] Total of nine steviol glycosides including stevioside, rebaudioside A, rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B, rebaudioside D and rebaudioside F.

7.0 STABILITY

Stability aspects of steviol glycosides have already been reviewed by FSANZ.

8.0 INFORMATION FOR FOOD LABELLING

Steviol glycosides are considered to be intense sweeteners and flavour enhancers when added to various food products. Steviol glycosides have been assigned the INS number of 960.

9.0 ANALYTICAL METHOD FOR DETECTION

HPLC methods were developed by Cargill for the identification of steviol glycosides in food and beverage matrices.

PART C. INFORMATION RELATED TO THE SAFETY OF STEVIOL GLYCOSIDE

FSANZ and JECFA have previously conducted thorough and independent safety analyses of steviol glycosides and have set an ADI of between 0 and 4 mg/kg body weight/day based on steviol equivalents. Considering the previous safety evaluation conducted by FSANZ, the following safety information on steviol glycoside only includes information that has previously not been reviewed by FSANZ; however, all information presented has previously been evaluated by JECFA.

1.0 METABOLISM/TOXICOKINETICS

1.1 Microbial Degradation

The hydrolysis of steviol glycosides by the gut microflora has recently been reviewed by Renwick and Tarka (2008). As the majority of the data discussed by Renwick and Tarka (2008) previously have been reviewed by FSANZ, no further discussion on the microbial degradation of steviol glycosides is presented in this section.

1.2 Absorption and Distribution

Most recently, several Cargill-sponsored studies have been completed to allow for direct comparison of the metabolic fate of rebaudioside A, stevioside, and steviol. In a preliminary study designed to provide concentration-time data for use in selecting appropriate sampling time points for subsequent investigations, levels of total plasma radioactivity were assessed following administration of single oral doses of ^{14}C-rebaudioside A (5 mg/kg body weight), ^{14}C-stevioside (4.2 mg/kg body weight), or ^{14}C-steviol (1.6 mg/kg body weight), all >97% radiochemical purity, to groups of male and female Sprague-Dawley [Crl:CD(SD)] rats by gavage (Roberts and Renwick, 2008). Blood samples (3 animals/sex per substance per time point) were taken at 0.5, 1, 4, 8, 12, and 24 hours post-dosing from the tail vein and at time of death (72 hours postdosing) by cardiac puncture. Following administration of ^{14}C-steviol, total plasma radioactivity peaked at 142 and 82 ng steviol equivalents/g in males and females, respectively, 30 minutes after dosing. In males and females administered ^{14}C-rebaudioside A, peak total plasma radioactivity of 47 and 74 ng steviol equivalents/g was detected at 8 hours, respectively. Peak plasma levels of total radioactivity following administration of ^{14}C-stevioside were attained at 4 hours in males (33 ng steviol equivalents/g) and 8 hours in females (55 ng steviol equivalents/g).

The pharmacokinetic profiles of rebaudioside A, stevioside, and steviol were determined in groups of male and female Sprague-Dawley [Crl:CD(SD)] rats (27 animals/sex per substance) (Roberts and Renwick, 2008). As in the preliminary study, animals were administered single oral doses of ^{14}C-rebaudioside A (5 mg/kg body weight), ^{14}C-stevioside

(4.2 mg/kg body weight), or ^{14}C-steviol (1.6 mg/kg body weight), all >97% radiochemical purity, by gavage. Blood samples were collected from 3 animals/sex per substance per time point by cardiac puncture at 0.25, 0.5, 1, 2, 4, 8, 24, 28, and 72 hours post-dosing. Following administration of either ^{14}C-rebaudioside A or ^{14}C-stevioside, an initial rise and decrease in plasma radioactivity in the 1st hour was followed by another increase, with radioactivity peaking at 2 to 8 hours, before declining again. Conversely, levels of radioactivity following administration of ^{14}C-steviol declined rapidly between 15 minutes (time point at which maximum levels were attained) and 1 hour and continued to decrease at all other time points, only to increase slightly at 2 hours. The maximum plasma concentration based on total radioactivity (C_{max}), the time at which maximum plasma concentration was observed (T_{max}), the area under the curve (AUC_{72}), and the half-life ($T_{1/2}$) for males and females administered ^{14}C-rebaudioside A, ^{14}C-stevioside, and ^{14}C-steviol are summarized in Table 1.2-1. Female rats administered either rebaudioside A or stevioside exhibited higher C_{max} values (approximately 2- to 3-fold), greater AUC_{72} (3- to 5-fold), and longer T_{max} (2- to 4-fold) and $T_{1/2}$ (2-fold) compared to males. With the exception of C_{max} (2.3-fold higher in females than males), pharmacokinetic values were comparable between males and females following treatment of rats with steviol. The pharmacokinetic data indicate that maximum plasma concentrations of radioactivity were attained faster following oral administration of the aglycone, steviol, than following administration of the steviol glycosides, stevioside or rebaudioside A, which must undergo hydrolysis in the gut to steviol prior to absorption. Rebaudioside A and stevioside attained comparable peak plasma levels at similar time points.

Table 1.2-1 Summary of Pharmacokinetic Parameters for Rebaudioside A, Stevioside, and Steviol (Roberts and Renwick, 2008)

Parameter (total radioactivity)	Rebaudioside A		Stevioside		Steviol	
	Males	Females	Males	Females	Males	Females
C_{max} (ng steviol equiv./g)	90	177	101	279	114	264
T_{max} (h)	2	8	4	8	0.25	0.25
AUC_{72} (ng steviol equiv.·h/g)	645	3329	1617	4287	1251	1604
$T_{1/2}$ (h)	5	10	9	15	16	16

C_{max} = peak plasma concentration; equiv. = equivalents; T_{max} = time to reach maximum concentration; AUC = area under the plasma concentration-time curve; $T_{1/2}$ = half-life.

1.3 Metabolism and Excretion

Rats

Although total plasma radioactivity was measured in the main study to determine the pharmacokinetic profiles following gavage administration of ^{14}C-stevioside, ^{14}C-rebaudioside A, and ^{14}C-steviol (Roberts and Renwick, 2008), concentrations of steviol and steviol glucuronide were determined in the plasma of rats at the same time points. In rebaudioside

A-treated rats, steviol was identified only in the plasma of females at levels above the limit of detection (19 and 29 ng/mL at 4 and 8 hours, respectively). Plasma steviol glucuronide was not identified in either sex of rebaudioside A-treated rats. Following treatment of rats with stevioside, steviol was detected at concentrations of 20 and 18 ng/mL at 4 and 8 hours in males, respectively. Steviol concentrations were slightly greater in stevioside-treated females (40, 46, and 80 ng/mL at 2, 4,and 8 hours, respectively). At 8 hours, steviol glucuronide was identified in the plasma of males and females at levels of 12 and 22 ng/mL, respectively. When steviol was administered to rats, steviol glucuronide was detected in the plasma between 15 minutes and 4 hours after dosing, but not thereafter, with similar concentrations reported in both males and females. Generally, metabolites were not identified in all plasma samples at all time points and when detected, were present at low levels, indicating that these were rapidly cleared in rats.

In a subsequent evaluation of plasma metabolite levels following single gavage administrations of ^{14}C-rebaudioside A (5 mg/kg body weight), ^{14}C-stevioside (4.2 mg/kg body weight) or ^{14}C-steviol (1.6 mg/kg body weight), all >97% radiochemical purity, to male and female Sprague-Dawley [Crl:CD(SD)] rats (4 animals/sex per substance), blood samples were collected from 2 animals/sex per substance at 2 different time points that were selected based on peak plasma levels of total radioactivity in the preliminary pharmacokinetic study (Roberts and Renwick, 2008). Blood samples from both sexes of rebaudioside A-treated animals werecollected at 8 hours (T_{max} in preliminary study) and 24 hours post-dosing, and at 30 minutes (T_{max} in preliminary study) and 8 hours post-dosing for both males and females administered steviol. For stevioside-treated males, blood samples were collected at 4 hours (T_{max} in preliminary study) and 8 hours post-dosing, whereas for stevioside-treated females, samples were obtained at 8 hours (T_{max} in preliminary study) and 12 hours post-dosing.

Males and females treated with rebaudioside A or stevioside exhibited similar plasma metabolic profiles, with steviol, steviol glucuronide, and an unidentified metabolite (P2) detected at all timepoints. Approximately 65% of the administered radioactivity was recovered in plasma. The authors noted that steviol, being sparingly soluble, likely represented the majority of the unrecovered radioactivity. In females, a further unidentified metabolite, P1, was detected 8 hours post-dosing in both rebaudioside A- and stevioside-treated groups. Eight (8) hours following administration of ^{14}C-rebaudioside, 51% (6, 3, and 42% steviol glucuronide, P2, and steviol, respectively) and 63% (6, 1, 2, and 54% steviol glucuronide, P1, P2, and steviol, respectively) of the plasma radioactivity was accounted for by the metabolites in males and females, respectively. At 24 hours, the percentage of radioactivity accounted by the metabolites declined to 32 and 60% in males and females, respectively. Following treatment of rats with ^{14}C-stevioside, metabolites comprised 76 and 72% of the plasma radioactivity in males at 4 hours and females at 8 hours, respectively. Steviol accounted for the majority of the plasma radioactivity in males and females at these time points (60 and 55%, respectively), followed by steviol glucuronide (9%) in females and P2 (8%) and steviol glucuronide (7%) in males. Plasma radioactivity related to the metabolites , increased to 87% in males by 8 hours, but remained relatively unchanged (69%) in females at 12 hours.

Following treatment with ^{14}C-steviol, steviol accounted for the majority of the radioactive components in plasma (35 and 50% at 30 minutes and 44 and 54% at 8 hours in males and females, respectively). Male and female rats treated with steviol had relatively comparable metabolic profiles, with steviol glucuronide identified as the principal metabolite (17 and 18% at 30 minutes and 16 and 10% at 8 hours in males and females, respectively). In males, P2 comprised 8 and 6% of the total radioactivity at 30 minutes and 8 hours, respectively, whereas in females 2 and 3% of the total radioactivity was accounted by P2 at 0.5 and 8 hours, respectively. With the exception of males at 8 hours, P1 was identified in both males and females at 30 minutes (1 and 2%, respectively) and in females at 8 hours (1%). Chromatographic analysis revealed that the 2 previously unidentified metabolites (P1 and P2) were of slightly higher polarity than steviol.

Metabolites of rebaudioside A, stevioside, and steviol were examined in the faeces of uncannulated (intact) rats and in the bile and faeces of cannulated rats (for study description see Roberts and Renwick, 2008). Among intact rats administered rebaudioside A, steviol was the major metabolite identified in the faeces, representing 44 and 57% of the dose for male and female rats, respectively. Unchanged rebaudioside A was the next most prevalent compound, representing 29 and 19% of the dose for male and female rats respectively. Stevioside and steviol glucuronide also were detected, representing approximately 2 to 4% of the dose for both male and female rats, respectively. Steviol also was the major metabolite detected in the faeces of rats administered stevioside, with 56 and 72% of the dose in male and female rats, respectively. Steviol glucuronide accounted for 14 and 10% of the dose in male and female rats, respectively. Males and females eliminated 12 and 2% of the administered dose unchanged. In male and female intact rats administered steviol, the majority of the dose was excreted unchanged in the faeces (69 and 74% as steviol, respectively). In all intact, uncannulated rats administered rebaudioside A, stevioside, or steviol, between 9 and 15% of the administered doses were recovered as other unidentified metabolites. In bile ductcannulated rats administered rebaudioside A or stevioside, the major metabolite detected in the faeces was steviol, with 25 and 18% of the dose detected in males, respectively, and 16 and 16% of the dose detected in females, respectively. Small amounts of steviol glucuronide (0.4 to 1%) also were detected. Since levels of radioactivity identified in the faecal samples of stevioltreated rats were not sufficiently high, they were not analyzed further. In the bile, steviol glucuronide was consistently identified as the major metabolite in rebaudioside A-, stevioside-, and steviol-treated males and females (60 and 72%, 69 and 69%, and 91 and 91%, respectively).

To determine the excretion profiles for rebaudioside A, stevioside, and steviol, groups of 10 male and 10 female Sprague-Dawley [Crl:CD(SD)] rats were administered single oral doses of ^{14}C-rebaudioside A (5 mg/kg body weight), ^{14}C-stevioside (4.2 mg/kg body weight), or ^{14}C-steviol (1.6 mg/kg body weight), all >97% radiochemical purity, and the total radioactivity was measured in urine, cagewash, faeces, and gastrointestinal tracts (Roberts and Renwick, 2008). The rats were equally divided into 2 groups (5 animals/sex per group), where 1 group underwent bile duct cannulation and the other group was left uncannulated. Only 3 animals/sex per group were included in the final analysis. Urine, cage wash, bile, and faecal samples, as well as the gastrointestinal tract and contents were collected from all rats. The livers from bile duct-cannulated rats also were collected. The faecal samples from uncannulated and cannulated animals were analysed for metabolites (see above). In the uncannulated rats, the majority of radioactivity was detected in the faeces within the first 24 hours after administration. The total amount of radioactivity detected in the faeces of male and female rats administered rebaudioside A was 97 and 98% of the dose, respectively. In the faeces of rats administered stevioside, the total amount of radioactivity was 98% of the dose in both males and females. In males and females administered steviol, 93 and 87% of the dose was recovered in the faeces. The amount of radioactivity in the urine accounted for approximately 1, 1, and 3% of the administered rebaudioside A, stevioside, and steviol doses, respectively. In the gastrointestinal tract, radioactivity was identified at not more than 1%. In rebaudioside A-treated rats, no detectable levels of radioactivity were identified in the carcasses, whereas in the 2 other treatment groups, levels ranged from 0.01 to 0.1% of the dose. Total radioactivity recovered from males was 98, 99, and 97% for rebaudioside A, stevioside, and steviol, respectively. Total radioactivity recovered from females was 99, 100, and 90% for rebaudioside A, stevioside, and steviol, respectively.

In bile duct-cannulated rebaudioside A- or stevioside-treated rats, the majority of radioactivity was detected in the bile within 24 hours, with smaller amounts recovered in the faeces and the urine (Roberts and Renwick, 2008). The total amount of radioactivity detected in the bile of male and female rats administered rebaudioside A was 69 and 80% of the dose, respectively. In the bile of male and female rats administered stevioside, the total amount of radioactivity was 77 and 79% of the dose, respectively. In male and female rats administered steviol, 97 and 98% of the dose, respectively, was detected in the bile. Steviol appeared in the bile more rapidly than when rats were administered the glycosides, with the majority of the radioactivity excreted in the first 3 hours. The amount of radioactivity in the faeces in male and female rats administered rebaudioside A was 30 and 21% of the dose, respectively. Approximately 23 and 24% of the administered dose of stevioside and 2 and 1% of the administered steviol dose were recovered in the faeces of cannulated male and female rats, respectively. With all 3 test substances, only 0.5 to 2% of radioactivity was collected in the urine from the cannulated rats. The gastrointestinal tract and livers contained levels of radioactivity in the range of 0.01 to 0.03% of the dose, with no radioactivity detected in the gastrointestinal tract of steviol-treated males. Radioactivity was not detected in the carcasses of any group of cannulated rats. In total, 101 and 103%, 101 and 104%, and 99 and 101% of the administered doses were recovered in males and females following treatment with rebaudioside A, stevioside, and steviol, respectively.

Based on the addition of radioactivity recovered in the bile, urine, liver, and remaining carcass of bile duct-cannulated rats, approximately 71 and 82% of ^{14}C-rebaudioside A, 78 and 81% of ^{14}C-stevioside, and 97 and 99% of ^{14}C-steviol were estimated to have been absorbed by males and females, respectively.

Humans

Pharmacokinetic parameters of the major metabolites (steviol and steviol glucuronide) of a steviol glycoside preparation (98.7% rebaudioside A) and stevioside (96.6% purity) were examined in a randomized, double-blind, 2-way crossover single dose trial in 8 healthy adult males between the ages of 21 and 42 years and a mean body mass index of 23.8 kg/m$_2$ (Wheeler *et al*., 2008). The subjects were administered either a single oral dose of 5 mg/kg body weight of the preparation or 4.2 mg/kg body weight of stevioside as aqueous solutions in a randomized sequence. Following a 2-week wash-out period, the subjects were provided the other steviol glycoside. Blood, urine, and faecal samples were collected pre-dose and through 72 hours post-dose and were assayed for steviol and steviol glucuronide. The pharmacokinetic parameters for steviol and steviol glucuronide following administration of the preparation or stevioside are presented in Table 1.3-1. Following administration of the preparation, steviol was detected in the plasma of a single subject at the 72-hour time point. Following administration of stevioside, steviol was detected in the plasma of a single subject 6 hours following dose administration. Steviol was not detected above the lower limit of quantitation in any other plasma samples. Steviol glucuronide appeared in plasma of all subjects after administration of the preparation and stevioside with peak concentrations noted at 12 and 8 hours post-dose, respectively. Administration of the preparation resulted in slightly lower steviol glucuronide C_{max} and AUC values than administration of stevioside. Steviol was mainly excreted in the faeces, with a very small amount excreted in the urine after administration of the preparation or stevioside. The amount of steviol excreted in the urine or faeces after oral administration of the preparation or stevioside was similar. Very low levels of steviol were detected in the urine of the subjects after administration of the preparation or stevioside. Steviol glucuronide, in comparison, was completely excreted in the urine after oral administration of the preparation or stevioside. In general, the detection of steviol or steviol glucuronide in plasma after administration of the preparation was delayed in comparison to stevioside. The delay in detection was reported to be caused by increased time for rebaudioside A to be metabolized to steviol by the colonic microflora in comparison to stevioside, as rebaudioside A has 4 glucose units and stevioside has 3 glucose units. There was only one adverse event reported during the study and this was not considered to be related to study treatment. The authors concluded that rebaudioside A and stevioside are metabolized and excreted by similar pathways in humans.

Table 1.3-1 Summary of Pharmacokinetic Parameters of Steviol and Steviol Glucuronide Following Oral Administration of A Steviol Glycoside Preparation and Stevioside (Wheeler *et al.*, 2008)		
Parameter	**A Steviol Glycoside Preparation**	**Stevioside**
Steviol		
C_{max} (ng steviol equiv/mL)	227	121
T_{max} (h)	72.0	6.0
AUC_{0-t} (ng steviol equiv·h/mL)	N/A[a]	N/A
AUC_{0-inf} (ng steviol equiv·h/mL)	N/A	N/A
$T_{1/2}$	N/A	N/A
λ_z	N/A	N/A
A_{eu}(0-72) (mg)	0.0510±0.0877	0.0238±0.0675
A_{ef}(0-72) (mg)	5.88±6.95	6.50±7.08
Steviol glucuronide		
C_{max} (ng steviol equiv/mL)	1,588±700	2,222±1,078
T_{max} (h)[b]	12.0 (6.02, 24.0)	8.0 (6.0, 12.0)
AUC_{0-t} (ng steviol equiv·h/mL)	33,904±15,139	39,928±20,129
AUC_{0-inf} (ng steviol equiv·h/mL)	46,197±18,604	53,211±23,782
$T_{1/2}$	14.8±3.32	14.0±5.61
λ_z	0.0483±0.00908	0.0551±0.0221
A_{eu} (0-72) (mg)	106±24	112±36.8
A_{ef} (0-72) (mg)	0	0

C_{max}, peak concentration; T_{max}, time to reach maximum concentration; AUC, area under the plasma concentration-time curve; $T_{1/2}$, half-life; λ_z, terminal elimination constant; A_{eu}, amount excreted in urine; A_{ef}, amount excreted in faeces

[a] Not applicable. The majority of steviol plasma concentrations were below the limit of quantification after administration of rebaudioside A or stevioside.

[b] T_{max} is present as median (min, max)

2.0 SUBCHRONIC TOXICITY

In a dose-range finding study, a steviol glycoside preparation (97% rebaudioside A) was administered *via* the diet to HsdBR1 Han:Wist (Han Wistar) rats (10/sex/group) at levels of 0 (control), 25,000, 50,000, 75,000, or 100,000 ppm for a period of 4 weeks (Curry and Roberts, 2008). Based on data reported by the authors, these levels were equivalent to mean intakes of the preparation of 0 (control), 2,367, 4,842, 7,143, and 9,938 mg/kg body weight/day, respectively, for males and 0 (control), 2,616, 5,422, 8,190, and 11,728 mg/kg body weight/day, respectively, for females (approximately 0, 781, 1,598, 2,357, and 3,280 mg steviol equivalents/kg body weight/day, respectively, for males and 0, 863, 1,789, 2,703, and 3,870 mg steviol equivalents/kg body weight/day, respectively, for females). Rats were examined twice daily for signs of clinical toxicity and detailed physical examinations were performed weekly. Body weights were recorded 3 days before the start of treatment, on the day treatment began, twice weekly throughout the treatment period, and before necropsy. Food consumption was measured daily. Blood samples for clinical chemistry and haematology and urine samples for urinalysis were taken during week 4 or at necropsy. At necropsy, all animals were subjected to a macroscopic examination, selected organs were weighed, and the testes and epididymides of all males were preserved for microscopic examination.

The authors reported no deaths or clinical signs of toxicity. Mean body weight gains of rats of both sexes receiving the preparation at dietary levels of 50,000 ppm or higher were significantly decreased compared to controls during the first 4 days of treatment. However, with the exception of female rats at the 100,000 ppm level, there were no significant differences in body weight gain in any of the preparation groups over the entire study period compared to controls. Food consumption was significantly decreased compared to controls on day 1 in males consuming the 3 highest concentrations and in females in the 75,000 and 100,000 ppm groups; however, beginning on day 6, food consumption in males was significantly increased at the 3 highest concentration groups and remained higher than controls particularly in the high-dose group on most days of the study until termination. When the entire treatment period was considered, food consumption was significantly higher only in males in the 100,000 ppm group. Apart from the initial decrease noted at study beginning, female food consumption was generally comparable to that of controls for the remainder of the study. Overall, there were no significant differences in food conversion efficiency.

Haematological analysis revealed a few statistically significant results between the control and treatment groups, but the differences were considered to be biologically insignificant as the differences were small in magnitude and did not occur in a dose-dependent manner. Small but statistically significant increases in plasma creatinine levels were reported in all treated males and in females in the 2 highest treatment groups (75,000 and 100,000 ppm) compared to the control group. The increases in plasma creatinine also were associated with significant increases in urine specific gravity in the males of the 2 highest treatment groups and all treated females. Reductions in total bile acids in females were observed in all test groups compared to controls; however, the reductions in bile acids did not follow a dose-response pattern. Bile acids also were reduced in males at the 2 highest concentrations (75,000 and 100,000 ppm). When atypically high bile acid values from the control males and control females were excluded from the analysis, no clear treatment-related effect was observed. The study authors considered changes in serum bile acids to be the result of metabolism and/or excretion of large amounts of the test article in the bile (Roberts and Renwick, 2008). Organ weight changes included significantly decreased relative adrenal weights in females at concentrations of 50,000 ppm and above and relative heart weights in males at 75,000 and 100,000 ppm; however, the differences were not dose-dependent. In males, reductions also were observed in absolute testes weights at the highest concentration. Macroscopic examination did not reveal any abnormalities and microscopy of the testes, epididymides, and seminiferous tubules also was unremarkable. The no-observed-adverse-effect level (NOAEL) was determined by the authors to be 100,000 ppm for a steviol glycoside preparation, equivalent to 9,938 and 11,728 mg/kg body weight/day for males and females, respectively (approximately 3,280 and 3,870 mg steviol equivalents for males and females, respectively).

In a 13-week toxicity study, HsdRcc Han:Wist rats (20 rats/sex/group) were administered a steviol glycoside preparation (97% rebaudioside A) in the diet at concentrations of 0 (control), 12,500, 25,000, or 50,000 ppm (Curry and Roberts, 2008). The dietary concentrations were established on the basis of the 4-week study. At each of the 3 dietary concentrations, males consumed daily doses of the preparation of 1,506, 3,040, and 5,828 mg/kg body weight/day, respectively, during the first week of the study, and 698, 1,473, and 3,147 mg/kg body weight/day, respectively, during the last week. In females, doses of the preparation of 1,410, 2,841, and 5,512 mg/kg body weight/day were achieved at each of the 3 dietary concentrations, respectively, during the first week and 980, 1,914, and 3,704 mg/kg body weight/day during week 13. Calculated as steviol equivalents, these doses are equal to approximately 497, 1,003, and 1,923 mg/kg body weight/day, respectively, during the first week of the study and 230, 486, and 1,039 mg/kg body weight/day, respectively, during the last week for males, and approximately 465, 938, and 1,819 mg/kg body weight/day, respectively, during the first week and 323, 632, and 1,222 mg/kg body weight/day, respectively during week 13 for females. The animals were examined twice daily for signs of clinical toxicity and more detailed physical examinations were performed weekly. Body weight and food consumption were recorded 7 days before the start of treatment, on the first day of treatment, twice weekly for the first 2 weeks of treatment, and weekly for the remainder of the treatment period. Sensory reactivity, grip strength, and motor activity were assessed before treatment and during weeks 4, 8, and 12. Ophthalmic examinations were conducted on all animals before treatment and during week 12 on animals in the control and high-dose (50,000 ppm) groups. Blood and urine samples were collected from 10 animals/ sex/ group on days 10, 46, and 89 of treatment for the evaluation of routine haematological, clinical chemistry, and urinalysis parameters. All animals were subjected to necropsy and the adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus, thyroid with parathyroids, and uterus with cervix were weighed and examined microscopically.

No compound-related changes in general appearance, behaviour, and sensory reactivity were noted between test and control animals. Significant changes in fore- and hindlimb grip strength in males and females, and activity scores in females were reported at various time points and dose levels; however, the authors reported that there were no clear compound-related effects.

During the first 4 days of treatment, body weight gains were significantly lower in males and females receiving 25,000 or 50,000 ppm of the preparation. Following the first 4 days of treatment to study termination, body weight gains were significantly lower in all male test groups compared to the controls. Overall, body weight gains were significantly lower in treated males. Also, in females treated at 25,000 and 50,000 ppm overall body weight gains were slightly, but significantly lower than the controls. During the first 3 days of the study, food intake was significantly lower in treated-males and in mid- and high-dose females compared to the control groups.

Rebaudioside A is known to resist digestion and absorption in the gut, but is degraded in the colon to the aglycone steviol prior to absorption (Koyama *et al*., 2003a,b); therefore, rebaudioside A is assumed to provide little to no nutritional value. To account for this, the caloric density of the rebaudioside A-containing diets were considered lower by 1.25%, 2.5%, and 5.0% in the low- through high-dose groups, respectively, in comparison to the control diet. When corrected for caloric density, overall food consumption was found to be 94.8%, 97.5%, and 95.0% of the controls in the low- through high-dose males, respectively, and 104.7%, 97.5%, and 95.0% of the controls in low- through high-dose females, respectively.

Food conversion efficiency was significantly decreased at various times in treated males early in the study (days 1 to 14). Sporadic decreases in food conversion efficiency in males also were noted later on in the study. In females, food conversion efficiency was unaffected by treatment, except for a significant decrease during days 1 to 3 in the high-dose group and a significant increase in all dose groups during days 8 to 10. Over the course of the entire study (weeks 0 to 13), the food conversion efficiency values were slightly lower in some treated groups, with statistical significance achieved in the high-dose males. No statistically significant effects on food conversion efficiency for weeks 0 to 13 were reported in females. The initial decrease in food consumption noted in the 13-week study also is consistent with the results of the 2- generation reproductive toxicity study on the steviol glycoside preparation (Curry *et al*., 2008) where body weight gain decrements in the high-dose groups in comparison to controls occurred during the first 2 weeks of the weaning period.
The interpretation of the effects of the steviol glycoside preparation on body weight gain can be guided by previous evaluations as described by Flamm *et al*. (2003). Flamm and associates have presented a procedure for assessing palatability/body weight gain issues and have described a number of criteria to establish that decreases in body weight gain and/or food consumption are not adverse. These include:

a) Treatment does not affect food conversion efficiency during the phase of rapid growth (*i.e*., first 13 weeks);

b) The test substance affects palatability at concentrations that cause reductions in body weight and/or food consumption;

c) There is consistency between effects of palatability and patterns of reduced food consumption;

d) Changes in body weight gain occur without a dose-response over a wide-range of doses with no other signs of toxicity.

As stated, the overall effects of the preparation treatment on food conversion efficiency are minimal, with the most notable effects occurring in the first 2 weeks of the study. Furthermore, the preparation has effects on food consumption and body weight that are associated in a fairly consistent manner with palatability in the first days of the study. Food consumption (adjusted for caloric density of the diet) of the treated-males and in the mid- and high-dose females was consistently lower than controls and can substantively explain the decrements in body weight gain noted in the study. Based on the procedure outlined by Flamm *et al*. (2003), the calculated 5% decrease in food consumption related to the decreased caloric density in high-dose males could explain up to a 15% decrease in weight gain in a 13-week study. Finally, despite the observations of reduced food consumption and body weight gain, no toxicity was observed over the dose-range in the study.

Based on WHO guidance (WHO, 1987) in regard to the interpretation of lower body weight gain in the absence of other toxicity due to consumption of a test material with known nutritive and palatability effects, the body weight effects observed in both studies were not considered as an adverse effect of the preparation. Similar effects have been reported in safety studies with other high intensity sweeteners (HIS) dosed at high levels which were likewise determined to be of no toxicological significance.

The ophthalmological examination of high-dose animals and controls was unremarkable. A few statistically significant haematological changes were observed between the control and treatment groups, but the differences were considered to be biologically irrelevant as they were reported in only 1 sex and did not follow a temporal or dose-dependent pattern.

Statistically significant increases in plasma urea and decreased in bilirubin, glucose, and triglycerides were detected in all treated males compared to controls on Day 89 of the study, as well as significant increases in plasma creatinine in males administered 50,000 ppm of the preparation. In female rats, significantly decreased plasma alanine aminotransferase (ALT) and cholesterol in the 25,000 and 50,000 ppm treatment groups, and bilirubin in the 50,000 ppm treatment group were observed compared to the control group on day 89 of the study. Significantly increased plasma urea and creatinine were observed in female rats administered 25,000 and 50,000 ppm and 12,500 to 50,000 ppm of the preparation, respectively. The various statistically significant increases and decreases in clinical chemistry parameters between the control group and treatment groups on day 89 were within the historical control values for HanWistar rats and were considered to be of no biological significance. Significantly decreased levels of total bile acids on days 10, 46, and 89 were observed in males in all of the preparation groups and on day 46 in females of all of the preparation groups; however, the decreases in females did not follow a dose-dependent pattern. As in the 4-week study, the study authors considered changes in serum bile acids to be the result of an alteration in enterohepatic recirculation of bile acids due to the presence of excessive amounts of test article metabolites in the bile and intestinal tract. This is consistent with the results of the metabolism study (Roberts and Renwick, 2008) demonstrating that the main route of excretion of the steviol glycoside preparation metabolites is *via* the bile in rats.

High-dose males and females were reported to have significantly decreased urine volumes compared to controls on day 89. Urine specific gravity was reported to be significantly increased in all treated-female groups on day 89 compared to control values. Since changes observed in urinalysis parameters were not accompanied by any gross or histopathological changes in the kidneys and were within the historical control values for Han Wistar rats, they were not considered to be toxicologically significant. Absolute epididymal weights of the highdose males and the absolute weight of the ovaries and the relative weights of the heart and kidneys of the high-dose females were significantly decreased compared to the controls. Macroscopic examination was reported to reveal a higher incidence of pale areas on the lungs and bronchi of treated males and females compared to the controls, but a dose-response was not observed in either sex, and as a result, these findings were not considered to be treatmentrelated. All histopathology findings were reported to be within the normal limits for rats of this age and findings were considered to be unrelated to treatment. As a result, the sporadic organ weight findings in the high-dose males and females were determined to be of no toxicological significance. The NOAEL for the 13-week oral toxicity study in rats was determined by the authors to be 50,000 ppm, the highest dietary concentration tested. Based on food intake measurements, this equates to 4,161 mg/kg body weight/day in male rats and 4,645 mg/kg body weight/day in female rats (approximately 1,373 and 1,533 mg steviol equivalents/kg body weight/day for male and female rats, respectively).

In a 13-week Redbook- and OECD-compliant toxicity study, Crl:CD(SD) rats (20/sex/group) were administered rebaudioside A (99.5% purity) in the diet to reach target doses of 0 (control), 500, 1,000, or 2,000 mg/kg body weight/day (Nikiforov and Eapen, 2008). The animals were observed twice daily for mortality and moribundity and clinical examinations were performed daily. Detailed physical examinations, as well as body weight and food consumption, were recorded weekly. Functional observational parameters (*i.e.*, home cage, handling, open field, sensory, neuromuscular, and physiological observations) and locomotor activity data were recorded during week 12 for 10 animals/sex/group. Ophthalmic examinations were conducted 1 week prior to study initiation and during week 12. Blood samples for haematology and serum chemistry evaluations were collected from 10 animals/ sex/group during weeks 2, 5, and 13. Urinalysis also was performed on the same 10 animals/ sex/group prior to necropsy (week 13). At the end of the treatment period, all animals were necropsied, selected organs were weighed, and selected tissues were examined microscopically.

The actual doses of the test article achieved were calculated to be 0 (control), 517, 1,035, and 2,055 mg/kg body weight/day for males and 0 (control), 511, 1,019, and 2,050 mg/kg body weight/day for females (approximately 0, 171, 342, and 678 mg steviol equivalents/kg body weight/day for males and 0, 169, 336, and 677 mg steviol equivalents/kg body

weight/day for females). No treatment-related clinical signs or symptoms of toxicity or effects on food consumption, functional observational parameters, and locomotor activity data were noted in the animals. All animals survived until necropsy. As compared to the control group, significantly lower body weight gains were observed in the high-dose males (*i.e.*, 2,000 mg/kg body weight/day) throughout the study period, which cumulated to a decrease in terminal body weights in this dose group. The authors, however, considered these decreases were not adverse as they were small in magnitude when compared to the control group. Furthermore, the authors noted that the decreases in body weights may have been the result of the treatment diets not being isocaloric with the diet of the control group. A similar trend in body weights was not observed in female rats; however, the authors noted that this may have been the result of the lower overall inclusion rates of rebaudioside A in the diet of females in comparison to males.

Throughout the study, sporadic significant increases and decreases in haematology and serum chemistry were observed compared to the control group (*i.e.*, increased mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, chloride, and sodium; decreased basophil counts, red blood cell counts, haemoglobin values, total protein, cholesterol, calcium, phosphorous, triglyceride levels, and serum bile acids). Furthermore, no clear treatment-related effects on urinalysis parameters were observed, although urine volume tended to be lower in the low- and mid-dose males (*i.e.*, 500 and 1,000 mg/kg body weight/day, respectively). No significant differences in absolute organ weights or organ-to-brain weights were observed in rebaudioside A-treated males or females in comparison to the control group, with the exception of increased absolute liver weights in highdose males. Relative to body weight, significantly increased adrenal gland weights in the midand high-dose group males, heart weights in the mid-dose group males, spleen weights in the low-dose group males, and testes weights in the high-dose group males were observed compared to the control group. No significant differences as compared to the control group in the relative organ weights of females administered rebaudioside A were reported. During necropsy, no treatment-related observations were noted; however, among females administered rebaudioside A, uterine clear fluid was observed more frequently in comparison to the control females. Microscopic examination of the uterine tissues did not reveal any alterations inconsistent with normal oestrus cycle-related physiological changes. No other treatmentrelated histopathological alterations were reported. The authors concluded, however, that the above differences in haematology, serum chemistry, organ weights, and histopathology were of no toxicological concern as they were either within the range of historical control values, did not occur in a dose- or time-dependent manner, or were limited to one sex. Based on the results of this study, Nikiforov and Eapen (2008) determined the NOAEL of rebaudioside A to be 2,055 and 2,050 mg/kg body weight/day for males and females, respectively, the highest dose tested in this study (approximately 678 and 677 mg steviol equivalents/kg body weight/day for males and females, respectively).

3.0 GENOTOXICITY

In a review of the available data on the potential *in vitro* and *in vivo* genotoxicity of steviol glycosides and the aglycone, steviol, Brusick (2008) concluded that steviol glycosides do not pose a genotoxic/mutagenic risk following human consumption. Furthermore, rebaudioside A, in the presence and in the absence of an exogenous metabolic activation system (rat S9), was not mutagenic in *Salmonella typhimurium* (*S. typhimurium*) strains TA98, TA100, TA1535, or TA1537 as well as *Escherichia coli* (*E. coli*) WP2 uvrA at concentrations of up to 5 mg/mL (Williams and Burdock, 2009). In Chinese hamster lung fibroblasts and human lymphoma cells, rebaudioside A at concentrations up to 5 mg/mL failed to induce chromosomal aberrations and rebaudioside A did not product any gene mutations at concentrations of up to 5 mg/mL, with or without metabolic activation, in mouse lymphoma L5178YTk$^{+/-}$ cells (Williams and Burdock, 2009). Moreover, rebaudioside A (95.6% purity) did not elicit a genotoxic response in a mouse micronucleus assay when administered intraperitoneally at doses of up to 750 mg/kg body weight or unscheduled DNA synthesis assay when administered by gavage at 2,000 mg/kg body weight (Williams and Burdock, 2009). These conclusions corroborate the findings of FSANZ, who also determined that steviol glycosides are unlikely to pose a genotoxic/mutagenic risk to human health.

4.0 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In the preliminary reproduction study, a steviol glycoside preparation (>97% rebaudioside A) was administered to adult F_0 female HsdBrl:Han Wistar rats and their litters (juvenile F_1 HsdBrl:Han Wistar rats) at dietary concentrations of 0 (control), 25,000, 37,500, or 50,000 ppm. Food and water were provided *ad libitum* and fresh diets were prepared weekly (Curry *et al*., 2008). The F_0 females (6/dose level) were administered the diets from the 14th to the 21st day of lactation, resulting in the offspring being exposed to the preparation from day 14 post-partum. A total of 10 rats/sex were selected from each treatment level (a maximum of 2 rats/sex selected from each litter) for continuation of dietary treatment from weaning (day 21) until the 35th day post-partum.

Clinical observations were conducted at least twice daily with more detailed physical examinations performed on a weekly basis for all F_0 females and on selected weaned F_1 juveniles. Body weight measurements were conducted on F_0 females on lactation days 11, 14, 18, and 21, while juvenile F_1 rats were weighed on post-partum days 11, 14, 17, and 21, as well as on post-partum days 24, 27, 31, and 35 for F_1 rats selected to continue past weaning. Food consumption of F_0 females was measured prior to the initiation of treatment (day 11 to 13 of lactation) and following the initiation of treatment (day 14 to 17 and 18 to 21 of lactation). Food consumption of juvenile F_1 rats was measured only for those rats selected to continue in the study past weaning (*i.e.*, on post-partum days 21, 24, 27, 31, and 35).

Litter sizes and mortality of the F_1 rats were recorded daily with litters culled to 8 rats (4/sex) on post-partum day 11. All F_0 females were killed on lactation day 21, along with unselected F_1 rats and a detailed necropsy was conducted on each F_0 and F_1 rat killed (Curry *et al*., 2008). The testes from the high-dose F_1 males were fixed in Bouins's solution and examined for the presence of retained spermatids, missing germ cell layers or types, multinucleated giant cells, or sloughing of epithelial cells into the lumen. The intertubular cell compartment of the testes was assessed histopathologically.

At each of the dietary concentrations of the preparation (0, 25,000, 37,500, or 50,000 ppm), female F_0 rats were reported to achieve calculated doses of 0 (control), 4,711, 8,021, and 9,484 mg/kg body weight/day, respectively (approximately 0, 1,555, and 2,647, 3,130 mg steviol equivalents/kg body weight/day, respectively). The doses achieved in female F_0 rats from day 17 to 20 of lactation were reported to be 0, 6,291, 10,045, and 11,386 mg/kg body weight/day, respectively (approximately 0, 2,076, 3,315, and 3,757 mg steviol equivalents/kg body weight/day, respectively). The general condition of the F_0 animals, body weight, body weight gain, food consumption, and the incidence of macroscopic abnormalities noted at necropsy were reported to be unaffected by the consumption of the preparation. In light of the specific concerns on the male reproductive system raised by the Scientific Committee on Food (SCF) (1999), the male reproductive organs were examined specifically. No effects were reported on testicular morphology based on results of gross and histopathologic examinations of the testes from high-dose (*i.e.*, 50,000 ppm) F_1 rats.

A marked reduction in body weight gain was reported in male and female F_1 rats from 14 to 21 days of age in the 50,000 ppm group, as well as from 14 to 17 days of age in the 25,000 and 37,500 ppm dose groups compared to the control group. The reduction in body weight gain in the 37,500 and 50,000 ppm dose groups compared to the control group was reported to continue in both sexes until rats were killed (*i.e.*, day 35 post-partum). The reduction in body weight gain among rats selected to continue past weaning was reported to be most notable during days 21 to 24 post-partum. The consumption of food during this period was reported to be marginally lower in the 37,500 and 50,000 ppm dose groups compared to the control rats; however, no effect related to the preparation treatment was reported on the body weight gain or food consumption of rats in the 25,000 ppm dose group post-weaning. The occurrence of enlarged parotid salivary glands was reported in 10/10 F_1 male and 8/10 F_1 female rats in the high-dose group, as well as in one male F_1 rat in the 37,500 ppm dose group at necropsy on post-partum day 35. In order to minimize effects of maternal body weight change on reproductive outcomes, caused by diet palatability that resulted in decreased food intake when high concentrations were used, and due to the very high exposure on a mg/kg body weight/day basis likely achieved during lactation and in the young rat pups during pre-weaning, the 25,000 ppm dietary concentration was considered suitable as the top dose for use in the main 2-generation reproductive toxicity study.

In the definitive 2-generation study, a steviol glycoside preparation (97% rebaudioside A) was administered *via* the diet to male and female HsdRcc:Han Wistar rats (30/sex/group) at concentrations of 0 (control), 7,500, 12,500, or 25,000 ppm for 2 generations to determine its potential reproductive and developmental effects (Curry *et al*., 2008). Male and female F_0 rats received the respective test diets for a period of 10 weeks prior to mating. Mating occurred within the same treatment group and lasted for a period of up to 3 weeks. Dams continued to receive the test diets for the duration of gestation and lactation. Females were monitored for evidence of mating. Once mating was confirmed, the females were observed for parturition beginning on day 20 after mating. During parturition, dams were monitored for any signs of difficulties and at completion, the numbers of live and dead offspring, litter size, offspring survival, and sex ratio were recorded.

Rats were monitored daily for the occurrence of any clinical symptoms of toxicity and more detailed physical examinations were conducted on a weekly basis until mating. Females continued to be examined weekly through gestation and lactation. Food consumption was monitored weekly for both males and females until mating and daily for females thereafter. Male body weights were measured on the day treatment was initiated, at weekly intervals during treatment, and before necropsy. For females, body weights were measured on the first day of treatment, at weekly intervals until mating was detected, at approximately weekly intervals during gestation and lactation, and before necropsy. Male rats were killed when the majority of litters had been weaned (approximately 17 weeks of treatments), whereas females that littered and reared offspring to weaning were killed on post-partum day 28.

Females that failed to mate, produce a viable litter, or rear a litter also were killed and necropsied. At necropsy, animals were examined macroscopically and the adrenals, brain, kidneys, liver, pituitary, spleen, ovaries, uterus with cervix, and oviducts or epididymides, ventral prostate, testes, and seminal vesicles with coagulation gland were removed, weighed, and preserved along with any gross lesions for further microscopic examination. Additionally, the number of implantation sites was counted in females and for those females whose litter died before weaning, presence of mammary tissues was noted. Sperm samples were collected from males shortly after death for determination of count, motility, and morphology (control and highdose groups only). The same procedures were repeated for selected first generation parental (F_{1P}) animals.

First generation (F_1) offspring were observed daily for any symptoms of ill-health. Litter size and mortality also were recorded daily. On day 4, litter sizes were randomly reduced to 8 pups (equal numbers of males and females whenever possible). Grossly abnormal pups and pups found dead prior to weaning were necropsied and examined macroscopically. Pup body weights were recorded on days 1, 4, 7, 14, 21, and 25. Several pre-weaning reflex developmental tests (surface righting, air righting, auditory startle response, and pupil closure response) were conducted. Offspring were weaned on day 21 and on day 25 a minimum of 1 male and 1 female were randomly selected from as many litters as possible within each group, after grossly abnormal offspring were excluded, until the required number of F_{1P} animals for breeding of the second generation (F_2) offspring was attained (30/sex/group). Pups not selected for the F_{1P} generation were necropsied on post-natal day 30. The brain, spleen, and thymus of 4 randomly selected rats from each litter (2/sex/litter) were weighed and examined. A similar treatment protocol was applied to the pups of the F_2 generation.

The average doses of the preparation achieved during different phases of the study are summarized in Table 4-1.

Table 4-1	A Steviol Glycoside Preparation Dose Levels Achieved at Different Dietary Concentrations in Rats during Different Phases of the Reproductive/ Developmental Study (Curry *et al.*, 2008)					
Group	**F_0**			**F_{1P}**		
	Concentrations (ppm)			**Concentrations (ppm)**		
	7,500	**12,500**	**25,000**	**7,500**	**12,500**	**25,000**
Males	586[1] (193)[2]	975 (322)	2,048 (676)	734 (242)	1,254 (414)	2,567 (847)
Females						
Pre-mating	669 (221)	1,115 (368)	2,273 (750)	798 (263)	1,364 (450)	2,768 (913)
Gestation	648-713 (214-235)	1,119-1,169 (369-386)	2,263-2,381 (747-786)	562-625 (186-206)	911-1,058 (301-349)	2,036-2,212 (672-730)
Lactation	715-1,379 (236-455)	1,204-2,388 (397-788)	2,602-5,019 (859-1,656)	976-1,406 (322-464)	1,752-2,394 (578-790)	3,289-4,893 (1,085-1,616)

[1] Values expressed as mg/kg body weight/day.
[2] Values in parentheses represent doses calculated as mg steviol equivalents/kg body weight/day.

No compound-related deaths or clinical signs of toxicity were reported among the F_0 or F_{1P} rats; however, 2 F_0 (1 control and 1 low-dose) males and 1 F_{1P} (mid-dose) male, and 2 F_0 and 2 F_{1P} females (all low-dose), were killed during the study for humane reasons.

In the F_0 generation, significant increases in food consumption were observed in high-dose males and females and sporadically in the other test groups during most of the 10-week pre-mating period as compared to controls. Likewise, F_{1P} generation mid- and high-dose animals also exhibited sporadic increases in food consumption. Compared to controls, food intakes of low- (F_{1P} only), mid-, and high-dose females of both parental generations during lactation also were significantly increased.

In mid- and high-dose male and female F_0 animals, transient reductions were observed in body weights and weight gains during the early stages of treatment (up to week 3 pre-mating) compared to controls. Similar reductions in body weight gains early in the study were observed in F_{1P} animals, including low-dose females. In the F_{1P} generation, lower body weights, in comparison to controls, were related to decreased growth of the F_1 offspring (see below). However, terminal body weights of all parental animals in all test groups did not differ significantly from controls. During lactation, body weights of high-dose F_0 females were significantly increased relative to controls.

The relative adrenal weights of F_0 females in all of the preparation-treated groups were significantly higher compared to the controls; however, no differences were reported in the absolute weights of the adrenals, and the increase was not accompanied by any microscopic abnormalities. Furthermore, no differences in relative or absolute adrenal weights were noted in F_0 males or in either sex of the F_{1P} animals. As such, the authors concluded that the relative adrenal weight increase observed in F_0 females was of no toxicological significance. Relative liver weights were significantly greater in mid- and high-dose F_0 and F_1 females, and in F_0 males. Although no histopathology was performed on the livers of the animals, microscopic examination of hepatic tissue in the 13-week toxicity study conducted with the same strain of rats was unremarkable (Curry *et al.*, 2008).

No significant effects on mating performance, fertility, oestrus cycles, or sperm motility, concentration, or morphology were reported in either the F_0 or F_1 generations. Length of gestation of the high-dose F_0 females, but not F_{1P} females at any treatment level, was significantly shorter than that of controls, but remained within the normal expected range. Times of sexual maturation of F_{1P} test and control animals were comparable. Administration of the preparation in the diet did not affect the ability of the F_0 or F_1 females to successfully produce litters and rear their offspring to weaning. No significant effects were observed in the number of implantations per litter, total litter size, pre- and post-natal survival of the offspring and the offspring sex ratio in either parental generation. With the exception of 1 control, 1 lowdose F_0, and 1 mid-dose F_{1P} female which did not become pregnant, all other females produced live litters. One instance of total post-natal litter loss was noted in the F_{1P} low-dose group. However, considering that this was not observed at higher dose levels, and the fact that milk was absent in the stomachs of the pups at the time of necropsy, this likely occurred due to inadequate milk intake by the pups and therefore, was not considered to be treatment-related.

No treatment-related clinical signs were observed in the F_1 and F_2 offspring. Compared to controls, male and female high-dose F_1 offspring exhibited significantly lower body weights on post-natal days 14 (females only), 21, and 25, as did mid-dose females on day 25. Likewise in the F_2 male and female high-dose offspring, significant reductions also were observed in body weights on day 25. Significant reductions in post-natal body weight gains were observed in both treated male and female F_1 (all dose levels) and F_2 [mid- (females only) and high-dose groups] offspring compared to controls. Given the absence of any effects on survival or general condition of the offspring, the reductions in growth were not considered to be biologically significant. Furthermore, reductions in body weights coincided with the time period when offspring would begin to consume their respective diets in addition to maternal milk, thus likely resulting in a period of dietary adaptation due to intense sweetness. The pre-weaning reflex development of F_1 and F_2 offspring in the preparation-treated groups was comparable to those of corresponding controls.

On post-natal day 30, significantly lower body weights were observed in all groups of unselected F_1 offspring. Unselected F_1 offspring males and females at all dose levels exhibited significantly increased relative brain weights compared to controls. In mid- and high-dose females, significant reductions in absolute brain weights also were observed. Both absolute and relative spleen weights were decreased in all groups of treated unselected F_1 offspring males and in high-dose unselected F_1 offspring females. Significant reductions in absolute spleen weights also were observed in mid-dose females. Absolute thymus weights were significantly decreased in all male test groups and in mid- and high-dose females. Since there were no changes in the relative weight of the thymus, the absolute weight change is indicative of being correlated to the decreased body weight gain of these animals during the rearing period, a period of dietary acclimation in which caloric consumption was likely decreased in comparison to the controls.

In F_2 offspring, significant decreases in terminal body weights were observed in males and females in the high-dose group compared to controls. Relative brain weights in high-dose males and females were significantly increased, whereas absolute weights were decreased in males in the high-dose group. Both relative and absolute spleen weights were reduced in high-dose males compared to controls, as were absolute thymus weights in both high-dose males and females. The lack of biological significance of either the spleen or thymic organ weight observations is further supported by the absence of any findings in the 13-week toxicity study suggesting a lack of treatment-related effects on immune system function (Curry *et al*., 2008). Specifically, the preparation-treatment in the 13-week study at up to 50,000 ppm in the diet had no effect on the absolute or relative weights of the spleen or thymus, did not have any clinically significant effects on differential blood counts, and was

not associated with any histopathological changes in the spleen or thymus, or, for that matter, in any other organ associated with the immune system (*i.e.*, lymphoid tissue). Macroscopic and microscopic examinations of unselected F_1 and F_2 offspring were unremarkable.

Under the conditions of the present study, the authors reported a NOAEL of 25,000 ppm (see Table 4-1 for dosage calculated on a mg/kg body weight/day basis) for reproductive performance in the F_0 and F_1 adult rats, for survival, growth, and general condition of the F_1 and F_2 offspring, and for sexual maturation of the F_1 offspring (Curry *et al*., 2008).

5.0 HUMAN STUDIES

5.1 Studies on Glucose Homeostasis

To examine the effects of longer-term consumption of a steviol glycoside preparation (>97% rebaudioside A) in subjects with type-2 diabetes mellitus, a total of 122 male and female subjects (from 33 to 75 years of age) were randomized to receive 4 capsules daily containing 250 mg of the preparation, for a total dose of 1,000 mg/day (approximately 330 mg steviol equivalents/day); 32 males and 28 females (mean age 59±1) or placebo (powdered cellulose) 30 males and 32 females (mean age 61±1) for 16 weeks in a double-blind clinical trial (Maki *et al*., 2008a). The study was designed to have at least 90% power to detect a change in glycosylated haemoglobin (HbA_{1C}) of 0.5 % between placebo and preparation-treated subjects. The investigation included only subjects with type-2 diabetes since the purported mechanism of action for steviol glycosides involves enhanced secretion of insulin from the pancreas when there is impaired response to glucose stimulation. In order to evaluate baseline glycaemic control and to assure compliance prior to the 16-week treatment phase, a 2-week single-blind placebo lead-in phase was included.

There was no significant difference between the groups provided the steviol glycoside preparation and placebo in the change from baseline to week 16 in HbA_{1C} levels, the primary variable. Likewise, no significant differences between the groups provided the preparation and placebo were observed in the changes from baseline to end of treatment (average of weeks 12 and 16) in fasting insulin, C-peptide, glucose, and lipid [triglycerides, total cholesterol, lowdensity lipoprotein (LDL), high-density lipoprotein (HDL), and non-HDL cholesterol] levels, body weights, and systolic and diastolic blood pressure.

The lack of an effect of the preparation on HbA_{1c} also was supported by the measurements of other indicators of glycaemic control, including no differences between the preparation and placebo in the index of changes in dosages of diabetes medications or new diabetes medications, and hypoglycaemic episodes reported in a daily diary. Results of serum clinical chemistry, revealed small but statistically significant differences in the change from baseline to study-end in ALT and γ-glutamyl transferase (GGT) levels between the placebo and treatment group (*i.e*., enzyme levels increased in test subjects and decreased in the placebo group); however, the mean post-treatment values remained within the normal reference ranges in both groups and were not considered by the investigators to be clinically significant. A small but statistically significant increase also was noted in the change from baseline in the basophil count in the preparation group compared to the placebo group, but as no other haematological parameters were altered and there was no evidence for an increase in the incidence of anaemia in preparation-treated subjects and no other indicators of disease associated with basophilia, this result was deemed to be due to random occurrence.

A total of 50 adverse events were reported during the study (27 and 23 in the preparation and placebo groups, respectively); however, no differences in the occurrence of the adverse events were reported between groups. The overall incidence of adverse events was similar in both groups. There was one serious adverse event; gastrointestinal haemorrhage in a subject receiving the preparation. There was a similar incidence of adverse events categorized as "severe" in each treatment group with 4 (6.7%) patients in the preparation group and 3 (4.8%) subjects in the placebo experiencing a severe event. The severe events reported during the steviol glycoside preparation treatment were astroenteritis, influenza-like symptoms, a cyst on the back, and a gastrointestinal haemorrhage. Severe events reported during treatment with placebo were a fracture of the 11th thoracic vertebrae, gastroenteritis, and bronchitis. The only adverse events that were reported in 3 or more subjects per group (correlating to 5% in the preparation group and 4.8% in the placebo group) were gastroenteritis in 3 (5.0%) of the preparation subjects and 3 (4.8%) of placebo subjects, upper respiratory tract infection in 6 (10.0%) of the preparation subjects and 4 (6.5%) of the placebo subjects. Overall, there was no evidence of a treatment-related increase in any individual adverse event or in the incidence of events in any 1 particular body system. Daily consumption of the preparation for up to 16 weeks was well-tolerated, had no significant effects on laboratory indices of safety (clinical chemistry, haematology, and urinalysis), and did not cause any pharmacological actions (hypoglycaemia or blood pressure changes) in subjects with type-2 diabetes mellitus.

5.2 Studies on Blood Pressure

The effects of a steviol glycoside preparation (>97% rebaudioside A) on haemodynamic parameters (blood pressure and heart rate) were investigated in a randomized, double-blind, placebo-controlled clinical trial in healthy adults with normal or low-normal blood pressure (Maki *et al*., 2008b). After a 2-week single-blind lead-in period to assess baseline haemodynamic parameters and to assure subject compliance with treatment, 100 healthy male and female volunteers (18 to 73 years of age) were randomized into 2 groups to receive 4 capsules per day of either placebo (powdered cellulose) (9 males and 41 females; mean age 41±2) or 250 mg of the preparation, providing a total daily dose of 1,000 mg (approximately 330 mg steviol equivalents/day; 12 males and 38 females; mean age 42±2), taken as 2 capsules with the first meal of the day and 2 capsules with the evening meal for 4 weeks. The study was designed to provide at least 80% power to detect a 4.5 mm Hg difference in resting, seated systolic blood pressure (SBP) for the preparation *vs.* placebo. As a result of lower than anticipated intersubject variability, the study had >90% power and was, therefore, very sensitive.

Compared with placebo, the preparation did not significantly alter resting, seated SBP, the primary variable. Likewise, there were no significant effects of the preparation treatment on resting, seated diastolic blood pressure (DBP), mean arterial pressure (MAP), or heart rate (HR). Furthermore, no significant differences were observed in the changes from baseline in ambulatory blood pressure monitor readings taken in the morning, during the day, at night, or over 24 hours between the preparation-treated subjects and the placebo-control group.

Measurements of supine and standing blood pressure and heart rate were measured before and for 2 hours after consumption of a standard breakfast meal with two 250 mg capsules of the preparation or placebo. At week 0 (baseline), all subjects were challenged with placebo, and at week 4 (end of study), subjects were challenged with their assigned study product. At week 0, there were no significant differences between groups in the changes from pre-meal to post-meal values for supine SBP, DBP, MAP, and HR. At week 4, there was a small post-meal increase in supine SBP in the preparation group (1.52 mm Hg) compared with a small decrease in the placebo group (-0.8 mm Hg). This difference did not reach statistical significance. Similar small pre- to post-meal increases in supine DBP and MAP noted at

week 4 in the preparation group contrasted with similar small decreases in the placebo group. These differences did reach statistical significance, but were not considered by the investigators to be clinically meaningful from a safety perspective.

At week 0, there were no significant differences between the preparation and placebo groups in the changes from pre-meal to post-meal values for standing SBP, DBP, and MAP. At week 4, pre-meal standing DBP, MAP, and HR were slightly lower in the preparation group. Post-meal, there were small increases in standing SBP noted in both the preparation group (1.13 mm Hg) and the placebo group (0.38 mm Hg). For standing DBP and MAP, similarly small post-meal increases in the preparation group compared with a small decrease in the placebo group were noted. These differences reached statistical significance for DBP and MAP, but were not considered by the investigators to be clinically meaningful from a safety perspective.

A pre-specified statistical evaluation of the subgroup of subjects with baseline SBP below the sex-specific median was conducted (baseline SBP <108 mm Hg for females and <117 mm Hg for males in this study). This analysis was included in order to address the potential effects of the preparation in subjects with lower than average blood pressure. Mean baseline SBP for this subgroup of subjects (n = 48) was 104 mm Hg and 103 mm Hg for the preparation and placebo groups, respectively. According to data from the National Health and Nutrition Examination Survey (NHANES), mean values for Americans aged 40 to 59 years are 124 mm Hg and 75 mm Hg for SBP and DBP, respectively (Ong *et al.*, 2007). Therefore, the lower SBP subgroup included individuals whose values were well below the U.S. population average. Likewise, the analysis of subjects with lower than normal blood pressure values was considered to be applicable to the European population. In the lower baseline SBP group, there was no significant difference between the preparation and placebo groups for change in resting, seatedSBP, the primary outcome variable, or for change in resting, seated DBP. However, a slight, but statistically significant reduction from baseline to treatment for the preparation *vs.* placebo in resting, seated MAP (-0.3 *vs.* 1.5 mm Hg, p = 0.036) was noted. Twenty-four (24)-hour SBP and DBP responses in the lower baseline SBP group were not statistically different between the preparation and placebo. In subjects with baseline SBP above the median, no significant differences were observed between the preparation and placebo for the changes in resting,
seated SBP, DBP and MAP; or for changes in 24-hour SBP or DBP values.

Results of clinical chemistry, haematology, and urinalysis (conducted 1 week prior to study initiation and at week 4) were unremarkable and no statistically significant differences were identified between the preparation-treated and placebo group. The preparation was reported to be well-tolerated and side effects reported by study participants were equally distributed between the test and placebo group. No serious adverse events were reported by the volunteers in the preparation group, including absence of any signs or symptoms of hypotension. In conclusion, the detailed assessment conducted over a 4-week treatment period demonstrated that the preparation did not cause haemodynamic changes in volunteers with normal or low-normal blood pressure.

PART D. INFORMATION RELATED TO THE DIETARY EXPOSURE TO STEVIOL GLYCOSIDES

1.0 CURRENT PERMITTED FOOD USES AND USE LEVELS OF STEVIOL GLYCOSIDES

Based on the Final Assessment Report of Application A540 on Steviol Glycosides As Intense Sweeteners (Food Standards Australia/New Zealand - FSANZ, 2008a), the Food Standards Code was amended to allow the following uses and use levels of steviol glycosides (Food Standards Australian/New Zealand - FSANZ, 2008b)

Table 1-1 Summary of Currently Permitted Uses and Use Levels for Steviol Glycosides

Category No	Food Description	Steviol Glycoside Concentration (mg/kg) as Steviol Equivalents
1.1.2	Liquid milk products and flavoured milk	115
1.2.2	Fermented milk products and rennetted milk products	176
3	Ice cream and edible ices	64
	Ice confection sold in liquid form	115
	Reduced and low fat ice cream and edible ices	208
4.3.2	Fruits and vegetables in vinegar, oil, brine, or alcohol	160
4.3.4	Fruit and vegetable spreads including jams, chutneys, and related products	-
	Low joule chutneys, low joule jams, and low joule spreads	450
4.3.6	Fruit and vegetable preparations including pulp	208
5	Confectionary	-
5.1	Chocolate and cocoa products	550
5.2	Sugar confectionary	1100
	Low joule chewing gum	1100
6.3	Processed cereal and meal products	250
7	Breads and bakery products	-
7.1	Breads and related products	-
	Fancy breads	160
7.2	Biscuits, cakes, and pastries	160
11.4	Tabletop sweeteners	GMP
11.4.1	Tabletop sweeteners - liquid preparation	GMP
11.4.2	Tabletop sweeteners – tablets or powder or granules packed in portion sized packages	GMP
13.3	Formula meal replacements and formulated supplementary foods	175
13.4	Formulated supplementary sports foods	175

Table 1-1 Summary of Currently Permitted Uses and Use Levels for Steviol Glycosides		
Category No	**Food Description**	**Steviol Glycoside Concentration (mg/kg) as Steviol Equivalents**
14.1.2.1	Fruit and vegetable juices	50
14.1.2.2	Fruit and vegetable juice products	-
	Soybean beverage (plain)	65 (plain)
	Soybean beverage (flavoured)	175 (flavoured)
	Low joule fruit and vegetable juice products	125
14.1.3	Water based flavoured drinks	160
14.1.3.1	Brewed soft drink	160
14.1.4	Formulated beverages	160
14.1.5	Coffee, coffee substitutes, tea, herbal infusions, and similar products	100
20.2	Food other than beverages	-
	Custard mix, custard powder, and blanc mange powder	80
	Jelly	260
	Dairy and fat based desserts, dips, and snacks	150
	Sauces and toppings (including mayonnaises and salad dressings)	320

Based on food consumption data from the 1995 Australian and 1997 New Zealand National Nutrition Surveys and the proposed use levels of steviol glycosides provided by the original applicant, the dietary exposure of steviol glycosides was estimated previously by FSANZ. Additionally, 2 scenarios, a 100% sugar replacement and a 30% market share, were used to estimate the market penetration of steviol glycosides. The resulting mean and heavy consumer intakes for both scenarios are presented in Table 1-2. Australian children between the ages of 2 and 6 years are predicted to have the highest intakes of steviol glycosides based on the proposed use levels presented in Application A540 and the 100% sugar replacement scenario. The mean intake was calculated to be 40 mg/day (55% of the ADI) and the heavy consumer intake was calculated to be 74 mg/day (100% of the ADI). When the 30% market share scenario was used to estimate the exposures of steviol glycosides, Australian children between the ages of 2 and 6 years also were demonstrated to have the highest levels of intake, with 24 mg/day (35% of the ADI) and 41 mg/day (55% of the ADI) calculated as the mean and heavy consumers of steviol glycosides, respectively.

Table 1-2 Estimated Dietary Exposure of Steviol Glycosides using DIAMOND				
Population group	**Scenario 1: Sugar replacement scenario**		**Scenario 2: 30% market share scenario**	
	Mean Intake (mg/d)[a]	**Heavy Consumer (mg/d)[a]**	**Mean Intake (mg/d)[a]**	**Heavy Consumer (mg/d)[a]**
2 years and above (Australia)	57 (25)	107 (50)	37 (15)	63 (30)
2 to 6 years (Australia)	40 (55)	74 (100)	24 (35)	41 (55)
15 years and above (New Zealand)	40 (15)	74 (25)	29 (10)	49 (15)

[a] Numbers in parentheses ( ) represent percentage of the estimated exposures compared to the Acceptable Daily Intake established for steviol glycosides (up to 4 mg/kg body weight/day)

FSANZ, however, noted several limitations to using DIAMOND to calculate the intake of steviol glycosides. These limitations included:

- The age of the data;
- Changes in eating patterns that may have occurred since the data were collected;
- An uncertainty associated with the consumption of foods that may have changed in consumption since 1995/1997, or that have been introduced to the market since 1995/1997;
- Only 24-hour dietary survey data were available that tend to over-estimate habitual food consumption amounts for high consumers;
- Data from occasionally consumed foods would be higher than daily food consumption amounts for those foods based on a longer period of time – this specifically affects food groups such as sauces, toppings, mayonnaises, and salad dressings;
- Maori and Pacific Islanders were over-sampled in the 1997 New Zealand data;
- Difficult to predict the concentrations of steviol glycosides used in foods, and the proportion of food groups containing steviol glycosides as they were not permitted to be added to foods at the time of the dietary exposure assessment.

Based on the results presented in Table 1-2, the above mentioned limitations of dietary intake modelling, and the available scientific data establishing the safety of steviol glycosides, FSANZ determined that the resulting predicted intakes were highly conservative and that “there are no public health and safety concerns for steviol glycosides when used as a food additive at the maximum levels proposed by the Applicant.”

2.0 PROPOSED NEW FOOD USE LEVELS OF STEVIOL GLYCOSIDES

As a result of formulation taste testing of carbonated soft drinks and ice cream indicating that higher levels of steviol glycosides than permitted were required to formulate an acceptable product, a number of changes to the permitted use levels of specific beverage products, and ice cream are requested. These changes in use levels are summarized in Table 2-1 and the requested changes are highlighted.

Table 2-1 Summary of Permitted Food Categories and Use Levels of Aspartame and Steviol Glycosides

Food Category		Permitted Level		Proposed levels for Steviol Glycosides (mg/kg) (Steviol Equivalents)
		Aspartame	Steviol glycosides (mg/kg) (Steviol Equivalents)	
1.1.2	Liquid milk products and flavoured milk	GMP	115	115
1.2.2	Fermented milk products and rennetted milk products	GMP	176	176
3	**Ice cream**	**GMP**	**64**	**200**
	Edible ices	GMP	64	64
	Ice confection sold in liquid form	-	115	115
	Reduced and low fat ice cream and edible ices	-	208	208
4.3.2	Fruits and vegetables in vinegar, oil, brine, or alcohol	GMP	160	160
4.3.4	Fruit and vegetable spreads including jams, chutneys, and related products	GMP	-	-
	Low joule chutneys, low joule jams, and low joule spreads	-	450	450
4.3.6	Fruit and vegetable preparations including pulp	GMP	208	208
5	Confectionary	10,000	-	-
5.1	Chocolate and cocoa products	-	550	550
5.2	Sugar confectionary	GMP	1100	1100
	Low joule chewing gum	-	1100	1100
6.3	Processed cereal and meal products	GMP	250	250
7	Breads and bakery products	GMP	-	-
7.1	Breads and related products	GMP	-	-
	Fancy breads	-	160	160
7.2	Biscuits, cakes, and pastries	GMP	160	160
11.4	Tabletop sweeteners	GMP	GMP	GMP

Table 2-1 Summary of Permitted Food Categories and Use Levels of Aspartame and Steviol Glycosides				
Food Category		**Permitted Level**		**Proposed levels for Steviol Glycosides (mg/kg) (Steviol Equivalents)**
		Aspartame	**Steviol glycosides (mg/kg) (Steviol Equivalents)**	
11.4.1	Tabletop sweeteners - liquid preparation	GMP	GMP	GMP
11.4.2	Tabletop sweeteners - tablets or powder or granules packed in portion sized packages	GMP	GMP	GMP
13.3	Formula meal replacements and formulated supplementary foods	GMP	175	175
13.4	Formulated supplementary sports foods	GMP	175	175
14.1.2.1	Fruit and vegetable juices	-	50	50
14.1.2.2	Fruit and vegetable juice products	GMP	-	-
	Soybean beverage (plain)	-	**65**	**100**
	Soybean beverage (flavoured)	-	**175 (flavoured)**	**200 (flavoured)**
	Low joule fruit and vegetable juice products	-	125	125
14.1.3	**Water based flavoured drinks**	**GMP**	**160**	**200**
14.1.3.1	**Brewed soft drink**	**1000**	**160**	**200**
14.1.4	**Formulated beverages**	**GMP**	**160**	**200**
14.1.5	Coffee, coffee substitutes, tea, herbal infusions, and similar products	GMP	100	100
20.2	Food other than beverages	GMP	-	-
	Custard mix, custard powder, and blanc mange powder	-	80	80
	Jelly	-	260	260
	Dairy and fat based desserts, dips, and snacks	-	150	150
	Sauces and toppings (including mayonnaises and salad dressings)	-	320	320

3.0 TECHNICAL JUSTIFICATION FOR REVISED USE LEVELS

3.1 Water Based Flavoured Drinks

A taste test involving different formulations of either sucrose or steviol glycosides was conducted in 80 untrained panellists. The panellists were asked to specify which of the two samples was perceptually sweeter. Lemon-lime and cola beverages were evaluated and sucrose and steviol glycosides were tested at various levels in each formulation. The H_3PO4 (pH2.5) and citric acid buffer (pH 3.1) systems, which are commonly used in cola and lemon-lime were used in this analysis. Given that concentration/response (C/R) function determines the relationship between response (R) in sweetness equivalence (SE) and Concentration (C) in mg/L (ppm w/v), the following equation can be generated based on the results of the sweetness matching experiments:

R = 18.7C/(561 + C) cola sparkling soft drinks

R = 18.1C/(474 + C) fruit flavoured sparkling soft drinks

where R = response in units of sucrose equivalence and C is the concentration of steviol glycosides in ppm (mg/L).

The relationship between response in sucrose equivalents and concentration of steviol glycosides is hyperbolic in nature (see Figure 4.1-2). In effect for cola sparkling soft drinks, 480 ppm steviol glycosides is equivalent to 8.6% sucrose equivalents however, these types of products generally require 10% sucrose equivalents which can only be obtained at approximately 600 ppm steviol glycosides. Similarly for fruit flavoured sparkling soft drinks, 480 ppm steviol glycosides is equivalent to 9.1% sucrose equivalents but these types of drinks normally require 10% sucrose equivalents to reach an acceptable sweetness level. A concentration of 600 ppm steviol glycosides (or 200 mg/kg steviol equivalents) is generally equivalent to 10.1% sucrose equivalents. Since most of the beverages (cola, lemon-lime flavoured) have ~10% SE, which cannot be obtained from 480 ppm steviol glycosides, it is therefore necessary to increase the use level of steviol glycosides from 480 ppm (160 mg/kg steviol equivalents) to 600 ppm (200 mg/kg steviol equivalents).

Another study was conducted to explore the concentration-dependent effects of steviol glycosides on the consumer acceptability of lemon-lime sparkling beverages. The steviol glycoside concentrations ranged from 0 ppm to 1500 ppm (500 mg/kg steviol equivalents). One hundred seventeen (117) consumers assessed the taste profile of several formulations using a 9-point scale to rate overall liking as well as the intensity of several diagnostic attributes: flavour, sweetness, bitterness, sourness and mouth feel. The results of this study indicate that “Overall Liking” and Sweetness both increase as the concentration of the steviol glycoside increases between 480 ppm (160 mg/kg steviol equivalents) and 600 ppm (200 mg/kg steviol equivalents). There were no significant differences in “Overall Liking” at concentrations higher than 600 ppm. In addition, bitterness intensity increases at higher levels. Based on these sensory results for bitterness intensity and overall likeability, 600 ppm (200 mg/kg steviol equivalents) is considered to be the maximum required level for beverages.

A full descriptive analysis profile of steviol glycoside was obtained from a panel of trained assessors. Steviol glycosides were evaluated at different concentrations up to 529 ppm (176 mg/kg steviol equivalents). All samples were evaluated at room temperature (21-23°C). Six attributes (sweetness, bitterness, sourness, black licorice flavor, sweetness aftertaste, and bitterness aftertaste) were evaluated. The results indicate that steviol glycosides were characterized by sweetness, bitterness, black licorice, sweetness aftertaste, and bitterness aftertaste. As the concentration increased, the attribute intensities increased as well. Interestingly, “bitterness”, “bitterness aftertaste”, and “black licorice” intensities increased as the concentration increased and was significant at the highest level tested. Thus, as the steviol glycoside sweetness intensity level increased from lowest to highest intensity, bitterness increased as did black liquorice flavor, sweetness aftertaste, and bitterness aftertaste. These findings suggest that use levels higher than 600 ppm in beverages is unlikely given the increase in bitterness profile.

Not all beverage matrices will require the maximum usage levels requested in this application. As consumer preference tends towards a lighter style of beverages, it is envisaged that beverages sweetened with steviol glycoside will be marketed with a range of usage levels. This is already seen in sugar based beverages where iced tea beverages and sports beverages are generally marketed with up to 40% less sugar than regular soft drinks.

Although other beverages types such as brewed soft drinks, formulated beverages, and flavoured soy beverages have not undergone taste testing formulation trials, since all these beverage types generally require 10% sucrose equivalents, or require 10 Brix (flavoured soy beverages) they will all require 200 mg/kg steviol equivalents to prepare products which are acceptable to the consumer.

3.2 Ice Cream

Comparative taste testing of vanilla ice creams prepared using the current permitted use level of steviol equivalents of 64 mg/kg with equivalent products prepared with higher levels of steviol equivalents of 165 and 200 mg/kg was assessed by an untrained panel (n= 70-73) for overall liking and overall sweetness. (See Appendix B for further details).
It was concluded based on comparisons of the 64 mg/kg formulation with the 165 mg/kg and 200 mg/kg formulations that the current permitted level of 64 mg/kg was too low to achieve optimal sweetness. The highest level of 200 mg/kg steviol equivalents was significantly preferred and produced a more balanced distribution of “just about right” responses.

4.0 PERCENT MARKET PENETRATION AND DIETARY EXPOSURE CALCULATIONS

4.1 Relative Sweetness Comparison Between Aspartame and Steviol Glycosides

Since there have been numerous dietary surveillance studies in the U.S., Canada, Australia/New Zealand, and countries in the EU that identify the intakes of aspartame and other HIS through post-market surveillance data, a more realistic, but equally conservative approach to determining consumption estimates is to estimate intake of steviol glycosides based on the intake figures reported in these published studies and the relative sweetness intensity of aspartame and steviol glycosides. This approach does not require an estimate of percent market penetration as it assumes that the market penetration will reach the market penetration of other intense sweeteners.

According to the literature, the sweetness intensity of steviol glycosides is in the range of 100 to 400 times that of sucrose, weight-for-weight. Rebaudioside A specifically is 200 to 300 times more potent than sucrose. Two (2) factors contribute to this rather broad range of sweetness potencies. First, different glycosides do exhibit some variability in their sweetening properties. Secondly, like all high-intensity sweeteners (HIS), the actual potency depends on the concentration and the matrix in which it is used. For accuracy, it is usual to state the sucrose equivalence (SE) at which a particular level of sweetness has been measured, as well as the measuring medium or matrix. For comparison of different HIS, the most common medium is water and, where the medium is not specified, it is always presumed to be water. However, there is no industry-wide agreement on a common sucrose equivalency at which to quote sweetness potencies. Realistic use-levels of HIS are generally in the range where the sweetener replaces 4 to 8% added sucrose. Consequently, 6% SE represents a reasonable average value at which to compare HIS. At 6% SE, the potency of rebaudioside A is 200 times that of sucrose (DuBois *et al*., 1991). The concentration-dependence of the sweetness potency is illustrated by the concentration/response curves for rebaudioside A and stevioside are shown in Figure 4.1-1.

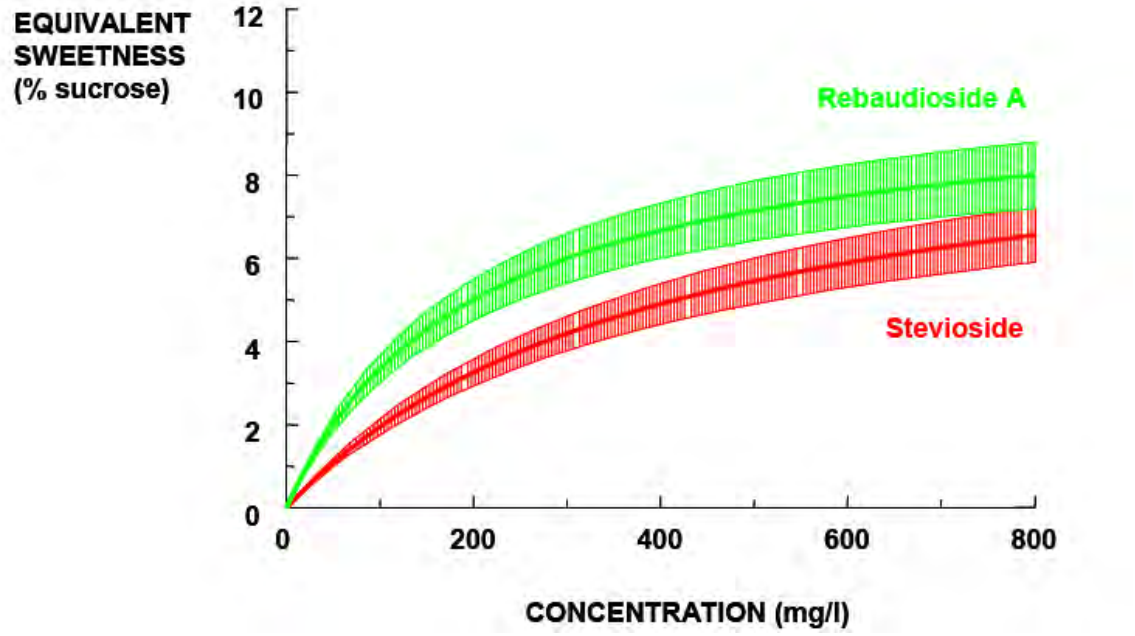


Figure 4.1-1 Dependence of Sweetness on Concentration for Rebaudioside A and Stevioside in Room-Temperature Water (Mean±10%) (from the data of DuBois *et al*., 1991)

These same investigators who measured the relative sweetness of rebaudioside A at various concentrations also examined the sweetness potency of a variety of other HIS. The sweetness of rebaudioside A was compared to that of other previously approved sweeteners including aspartame. The sweetness potency of rebaudioside A was shown to be similar to the sweetness of aspartame, a HIS that is widely marketed and approved for addition to numerous foods and beverages in several countries. In comparison to sucrose, aspartame is 180 times as sweet at 6% SE (DuBois *et al*., 1991). Although the precise concentration-response curves for rebaudioside A and aspartame differ slightly, the 2 are similar in sweetness over a wide range of sucrose equivalency values (see Figure 4.1-2).

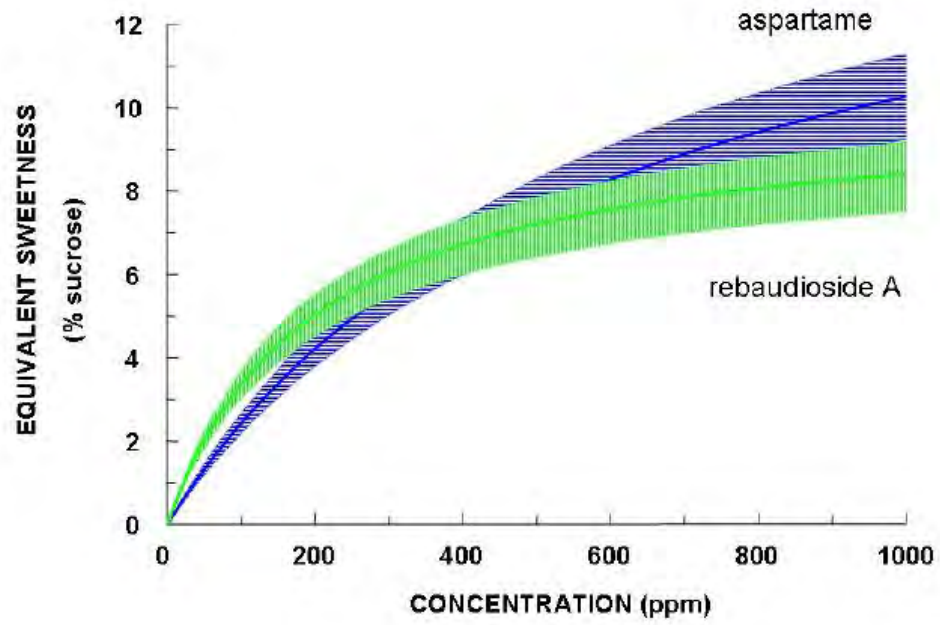


Figure 4.1-2 Comparisons of Concentration-Response Curves for Rebaudioside A and Aspartame (Mean±10%) (from the data of DuBois *et al*., 1991)

4.2 Predicted Intakes Using Post-Market Surveillance Data

The intake of rebaudioside A was estimated by Renwick (2008) using published data on dietary exposures to approved intense sweeteners, such as aspartame from post-market surveillance studies, with adjustment for their relative sweetness intensities, assuming a relative sweetness for rebaudioside A of 200 times that of sucrose (Renwick, 2008). Renwick (2008) noted that post-market surveillance data for HIS has a major advantage over using intake modellingscenarios, such as DIAMOND, in that the resulting estimated exposures are based on actual intakes of HIS. Moreover, post-market surveillance data are considered to be conservative for the exposure estimates of steviol glycosides because:

- It is assumed that the novel compound achieves the same market penetration as currently available for HIS;
- The analysis focuses on those studies and sweeteners that show the highest sucrose replacement;
- The analysis of the dietary exposures of children used data for the age group with the highest intakes;
- The analysis provides data for high consumers for whom the intake would reflect brand loyalty;
- Data are available for groups with higher than average intakes (*i.e*., diabetics and children); and
- Data for Australia and New Zealand, which contribute significantly to the exposures, are for subjects selected from a preliminary screen as likely to have higher than average intakes.

For the purposes of this assessment, it was assumed that the composition of the steviol glycoside preparation is 100% rebaudioside A. The data used in these analyses were primarily from studies that used specifically designed food diaries combined with actual use levels or approved levels in different foods and beverages (Renwick, 2008). These data were pooled in order to provide a realistic, but conservative estimate of potential consumption of steviol glycosides.

The calculated intakes of intense sweeteners (as sucrose equivalents) based on published data, and the corresponding predicted intake of steviol glycosides preparation, assuming complete replacement of other intense sweeteners and a relative sweetness of the steviol glycosides of 200 times that of sucrose, are presented in Table 4.2-1. The predicted intakes of steviol glycosides preparation are all below the current ADI defined by the JECFA for steviol glycosides (JECFA, 2007) of 0 to 4 mg/kg body weight/day as steviol. Based on the post-market surveillance data, diabetic children are predicted to have the highest mean intake of steviol glycosides (1.12 mg/kg body weight/day or 28% of the ADI), whereas non-diabetic children are predicted to have the highest intake of heavy consumers (1.64 mg/kg body weight/day or 41% of the ADI).

Table 4.2-1 Intakes of Intense Sweeteners and Predicted Intakes of Cargill's Steviol Glycoside Preparation (Renwick, 2008)

Population Group	Intakes of Intense Sweeteners (as sucrose equivalents)		Predicted Intakes of Steviol Glycosides		Predicted Intakes of Steviol Glycosides (as steviol equivalents)	
	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)	Mean Intake (mg/kg bw/d)	Heavy Consumer (mg/kg bw/d)	Mean Intake (mg/kg bw/d)[a]	Heavy Consumer (mg/kg bw/d)[a]
Non-diabetic Adults	255	675	1.3	3.4	0.43 (11)	1.12 (28)
Diabetic Adults	280	897	1.4	4.5	0.46 (12)	1.48 (37)
Non-diabetic Children	425	990	2.1	5.0	0.69 (17)	1.64 (41)
Diabetic Children	672	908	3.4	4.5	1.12 (28)	1.48 (37)

[a] Numbers in parentheses ( ) represent percentage of the estimated exposures compared to the Acceptable Daily Intake established for steviol glycosides (up to 4 mg/kg body weight/day)

5.0 PERMITTED FOOD USES AND USE LEVELS IN OTHER COUNTRIES OF STEVIOL GLYCOSIDES

5.1 United States

As noted in Part A, Section 8.2.1, steviol glycosides have been the subject of several GRAS Notifications to the U.S. Food and Drug Administration (FDA) where FDA had no questions about their respective GRAS determination conclusions. Cargill"s steviol glycosides preparation was the subject of a GRAS Notification (GRN 000253 – U.S. FDA, 2008a) where FDA did not have any questions about (the notifier"s) conclusions that Cargill"s steviol glycosides preparation is GRAS under the intended conditions of use in the same food and beverage products that are currently marketed with aspartame. Although Cargill"s steviol glycoside preparation is GRAS for levels based upon cGMP a table outlining the estimated proposed uses and use levels of steviol glycosides in the United States is presented in Table 5.1-1.

Table 5.1-1 Summary of the Individual Food-Uses and Use-Levels for Aspartame in the U.S. Prior to Regulatory Approval as a General Purpose Sweetener and Proposed Food-Uses and Use-Levels for Cargill's Steviol Glycosides Preparation

Food Category	Proposed Food Uses	Use-Level (%)	
		Aspartame	Steviol Glycosides
Alcoholic Beverages	Aromatized Alcoholic Beverages (Excluding Beer)	0.06	0.06
Baked Goods and Baking Mixes	Cakes	0.17	0.1
	Cookies	0.17	0.1
	French Toast, Pancakes, and Waffles	0.17	0.1
	Muffins, Scones and Doughnuts	0.17	0.1
	Pastries and Pie Crust	0.17	0.1
	Sweet Breads and Rolls	0.17	0.1
Beverages and Beverage Bases	Carbonated Beverages	0.06	0.06
	Coffee and Tea Drinks	0.06	0.06
	Fruit Flavoured Drinks	0.06	0.06
	Energy, Sport, and Electrolyte Drinks	0.06	0.06
	Meal Replacements (non-milk based), Not for Weight Reduction	0.06	0.06
	Meal Replacements (non-milk based) for Weight Reduction	0.08	0.08
Breakfast Cereals	Ready to Eat Breakfast Cereals	0.1	0.1
	Instant and Regular Hot Breakfast Cereals	0.1	0.1
Chewing Gum	Chewing Gum	0.55	1.5
Condiments and Relishes	Mustard	0.035	0.035
	Ketchup	0.035	0.035

Table 5.1-1 Summary of the Individual Food-Uses and Use-Levels for Aspartame in the U.S. Prior to Regulatory Approval as a General Purpose Sweetener and Proposed Food-Uses and Use-Levels for Cargill's Steviol Glycosides Preparation

Food Category	Proposed Food Uses	Use-Level (%)	
		Aspartame	Steviol Glycosides
Confections and Frostings	Cocoa Mixes	0.1	0.1
	Cocoa-Based Spreads and Fillings	0.1	0.1
	Frostings, Icings, and Coatings	0.1	0.1
Dairy Product Analogs	Soybean-Based Beverages		0.06
Fats and Oils	Emulsified Sauces	0.035	0.035
	Fat-Based Desserts	0.1	0.1
Frozen Dairy Desserts and Mixes	Ice Cream, Novelties, and Frozen Milk Desserts	0.1	0.1
	Frozen Yogurt	0.1	0.1
Fruit and Water Ices	Edible Ices, Sherbet, and Sorbet	0.08	0.08
Gelatines, Puddings, and Fillings	Puddings and Other Milk-Based Desserts	0.1	0.1
	Flans, Custards, and Other Egg-Based Desserts	0.1	0.1
Grain Products and Pastas	Cereal and Granola Bars	0.17	0.10
	Energy, Meal Replacement, and Fortified Bars	0.2	0.2
Gravies and Sauces	Water and Milk-Based Sauces, Gravies, and Dressings, Including Mixes	0.035	0.035
	Clear Sauces	0.035	0.035
Hard Candy	Breath-Freshening Micro Mints with No Added Sugar	0.6	1.0
	Hard Candy	0.1	0.1
	Freshening Throat Pastilles with No Added Sugar	0.2	0.2
Jams and Jellies	Jams, Jellies, Preserves, and Marmalades	0.1	0.1
Milk Products	Fermented Milks, Plain	0.1	0.1
	Flavoured Milk, Milk Drinks, and Mixes (not cocoa)	0.06	0.06
	Milk-Based Meal Replacements, Not for Weight Reduction	0.06	0.06
	Milk-Based Meal Replacements, For Weight Reduction	0.08	0.08
	Yogurt	0.1	0.1
	Yogurt Drinks	0.06	0.06
Nut and Nut Products	Nut Spreads	0.1	0.1
	Processed Whole Nuts, Coated Nuts, and Mixtures	0.05	0.05

Table 5.1-1 Summary of the Individual Food-Uses and Use-Levels for Aspartame in the U.S. Prior to Regulatory Approval as a General Purpose Sweetener and Proposed Food-Uses and Use-Levels for Cargill's Steviol Glycosides Preparation

Food Category	Proposed Food Uses	Use-Level (%)	
		Aspartame	Steviol Glycosides
Processed Fruits and Fruit Juices	Canned or Bottled Fruit	0.1	0.1
	Coconut Milk and Coconut Cream	0.1	0.1
	Fruit Fillings For Pastries	0.1	0.1
	Fruit Puree	0.1	0.1
	Fruit-Based Desserts	0.1	0.1
Processed Vegetables and Vegetable Juices	Vegetable Purees	0.1	0.1
Soft Candy	Cocoa and Chocolate Products	0.2	0.2
	Soft Candy, Nougats, and Marzipans	0.1	0.15
Sugar Substitutes	Table Top Sugar Substitutes	GMP	GMP
Sweet Sauces, Toppings, and Syrups	Cocoa Syrups	0.1	0.1
	Fruit Sauces, Syrups, and Toppings	0.1	0.1
	Sweet Sauces and Toppings (not fruit, not syrups)	0.1	0.1

5.2 European Union

A petition for Cargill"s steviol glycosides preparation has been filed in the European Union for the uses and use levels similar to those outlined in the table above.

5.3 Asia

In Japan, 3 forms of purified stevioside (*i.e*., crude extract, 50% pure, and ≥90% pure) and *S. rebaudiana* leaf extracts are accepted for general use as sweeteners in a variety of foods and beverages including pickling gum, pickles, dried seafood, meat, fish, soy sauce, bean pastes, sugarless chewing gums, juices, cola, table-top sweeteners, and ice cream (Marie, 1991; Das *et al.*, 1992; Ferlow, 2005). This approval for general use is under the pretence that steviol is a naturally occurring sweetener and its use is believed to be safe (Bertorelli and Czarnowski-Hill, 1990).

Stevioside is 1 of the 4 most widely-used high intensity sweeteners in Korea (the other 3 sweeteners being saccharin, D-sorbitol, and aspartame) and has been approved for use as a sweetener with some limitations (*i.e*., stevioside is not added to some food categories) (Kinghorn *et al.*, 1998; Chung *et al*., 2005). The main food usages of stevioside in Korea include cookies, sugar products, beverages, seasonings soy sauce, honey, and *so-ju* (a traditional liquor made of starch).

5.4 Central/South America

Stevioside, *S. rebaudiana* leaves, and highly refined extracts are permitted for use as lowcalorie sweeteners in Argentina, Paraguay, Brazil, Uruguay, Mexico, Peru and Colombia.

5.5 Other Jurisdictions

Other countries permitting the use of steviol glycosides include China, Malaysia, Russia, Switzerland, Taiwan, Turkey, and Ukraine.

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