FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Supporting Document 1

RISK AND TECHNICAL ASSESSMENT REPORT

Executive Summary

The term steviol glycosides refers to mixtures of compounds extracted from the leaves of the plant *Stevia rebaudiana.* Several of these glycosides have the property of intense sweetness and steviol glycosides are permitted as a non-caloric sweetener in a range of foods and beverages. Stevioside and rebaudioside A are typically identified as the principal sweetening compounds of *S. rebaudiana* extracts and are accompanied by smaller amounts of other steviol glycosides.

Maximum permitted levels of steviol glycosides are prescribed in Standard 1.3.1 - Food Additives. These levels are expressed in terms of steviol, the precursor compound from which the various glycoside compounds are derived. The current Application requests an increase to 200 mg/kg steviol equivalents for the maximum permitted 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.

The Applicant provided results of taste trials to support their claim that higher steviol glycoside levels are required for the above food groups to give products which consumers will consider to be acceptably sweet. The food technology assessment concluded that the requested increased levels were technologically justified.

An acceptable daily intake (ADI) of 0-4 mg/kg bodyweight, expressed as steviol, was established by FSANZ in 2008, JECFA in 2009 and EFSA in 2010. For the present Application, toxicological and other relevant data published subsequent to the original FSANZ assessment have been considered. These additional published data raise no concerns regarding the safety of steviol glycosides and do not indicate a need to change the ADI.

The dietary exposure assessment, based on a 30% market uptake scenario for broad food groups at maximum levels specified, indicated that estimated dietary exposures to steviol glycosides were less than 60% of the ADI for both mean and 90th percentile exposures for all population groups assessed, including children. The assumptions made in the assessment are likely to lead to considerable over-estimates of dietary exposure to steviol glycosides. For example, apart from two food groups, it was assumed that where permission for steviol glycosides was given to a food group, every food in that group contained steviol glycosides. It is also likely that the assumption of a 30% market uptake of steviol glycosides is an overestimation.

Using a scenario to represent 'brand loyal' consumers of water based flavoured drinks, 90th percentile estimated dietary exposures were 110% of the ADI for Australian children aged 2-6 years and 100% of the ADI for New Zealand children aged 5-14 years. A further scenario considered 'brand loyal' consumers of flavoured milk products (including yoghurt) which are the highest contributor to steviol glycosides exposure for Australian children aged 2-6 years.

This scenario predicted that estimated mean and 90th percentile dietary exposures for Australian children aged 2-6 years were approximately 55% and 100% of the ADI, respectively. For these scenarios, it was assumed that 'brand loyal' consumers would always choose the same product within a food category containing steviol glycosides up to the maximum permitted level. However, as for the 30% market uptake scenario, the 'brand loyal' consumer scenarios are based on broadly conservative assumptions, in particular the assumption that the concentration in all relevant food categories is at the level of the MPLs – these are likely to lead to a considerable overestimation of dietary exposure. On this basis, there are no public health and safety concerns associated with the proposed increases in the maximum permitted levels in ice cream and certain beverages.

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1. Introduction

1.1 Background

On 28 October 2009, Food Standards Australia New Zealand (FSANZ) received an Application from Cargill Inc. seeking an amendment to Standard 1.3.1 of the *Australia New Zealand Food Standards Code* (the Code). The Application seeks to increase the maximum permitted levels of the sweetener steviol glycosides in ice cream and a range of beverages.

1.2 Risk Assessment Questions & Scope

For this Application, the risk assessment questions were developed in the context of the Section 18 Objectives of the *Food Standards Australia New Zealand Act 1991*, having regard to the Ministerial Policy Guidelines for the addition of substances other than vitamins and minerals.

The following risk assessment questions are addressed in this Risk and Technical Assessment Report:

- Are the proposed increases in maximum permitted levels in selected foods consistent with achieving the stated purpose?
- Is there a need to change the acceptable daily intake (ADI) of 0-4 mg/kg bodyweight established previously by FSANZ?
- If the maximum permitted levels of steviol glycosides are increased in the proposed foods, would the resulting exposure for all consumers pose an unacceptable risk for public health and safety?

This Risk and Technical Assessment Report is structured to address the above questions and comprises the following components:

(1) Food Technology Assessment, which reviewed the chemical properties of the substance, its technological function in the final food, and assessed whether the proposed increases in maximum permitted levels in selected foods are consistent with achieving the stated purpose.

(2) Hazard Assessment, which considered whether new toxicological or other data indicate a need to change the existing ADI.

(3) Dietary Exposure Assessment (DEA), which estimated the levels of dietary intake of steviol glycosides in Australia and New Zealand.

(4) Risk Characterisation, which compared the estimated levels of intake of steviol glycosides with the ADI.

2. Food Technology Assessment

2.1 Introduction

Steviol glycosides are a non-caloric intense sweetener and are natural components of the leaves of *Stevia Rebaudiana* (Bertoni). Water extracts of *S. rebaudiana* have been used as a sweetener in some Asian and South American countries for many years (FAO 2007). The commercially used purified extract of the leaves of *S. rebaudiana* contains ten sweetening substances (at various levels) which are glycosides of steviol, including the two dominant components stevioside and rebaudioside A.

The common names used for the purified extract of the leaves of *S. rebaudiana* have included stevia, stevioside and various other names. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2004 concluded that the most appropriate name to be used for this extract was “steviol glycosides”, to reflect that the extract contained a mixture of steviol glycosides. Steviol glycosides was given the food additive number INS 960 in 2005 by the Codex Alimentarius.

The main purpose of using steviol glycosides in foods is to enhance the taste and sweetness without needing to use mono- and disaccharide sweeteners (such as sucrose, glucose, fructose, honey) or man-made chemical intense sweeteners. Steviol glycosides are claimed to have wide use in a range of foods due to their flavour and sweetness profile, along with high stability.

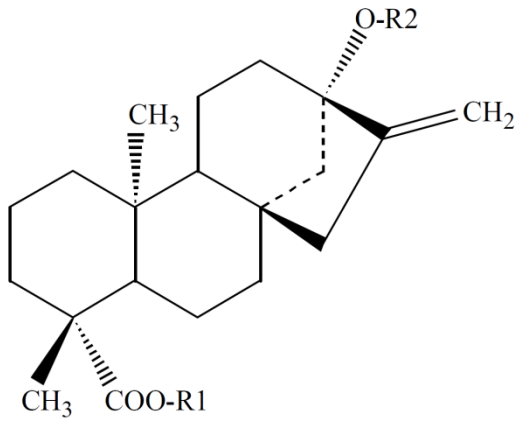
2.2 Chemical Structure and Specification

2.2.1 Chemical structure

The purified extract of the leaves of *S. rebaudiana* contain ten different glycosides of steviol, referred to as steviol glycosides. Each of the glycosides contains steviol as a common central component of its molecular structure. There are four main steviol glycosides: stevioside, rebaudioside A, rebaudioside C and dulcoside A, with stevioside and rebaudioside A generally comprising around 80% of the extract. The other six minor glycosides present generally constitute less than 5% of the total extract. Figure 1 below illustrates the chemical structure of the steviol skeleton and includes the structures of the related R group compounds of each steviol glycoside.

The Applicant’s steviol glycoside preparation is a chemically defined mixture that comprises not less than 95% of nine steviol glycosides, which are stevioside, rebaudioside A, rebaudioside C, dulcoside A, rubusoside, steviolbioside, rebaudioside B, rebaudioside D and rebaudioside F. The prime sweetening glycoside in the Applicant’s preparation is rebaudioside A, which the Applicant reports from analyses of three preparations, corresponds to greater than 95% of the glycosides. The Applicant states that their preparation has a sweetening potency that is approximately 200 to 300 times that of sucrose.

Figure 1: Steviol skeleton and R groups



Compound name	R1	R2
Steviol	H	H
Steviolbioside	H	*β*-Glc-*β*-Glc(2→1)
Stevioside	*β*-Glc	*β*-Glc-*β*-Glc(2→1)
Rubusoside	*β*-Glc	*β*-Glc
Rebaudioside A	*β*-Glc	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)
Rebaudioside B	H	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)
Rebaudioside C (dulcoside B)	*β*-Glc	*β*-Glc-*α*-Rha(2→1) | *β*-Glc(3→1)
Rebaudioside D	*β*-Glc-*β*-Glc(2→1)	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)
Rebaudioside E	*β*-Glc-*β*-Glc(2→1)	*β*-Glc-*β*-Glc(2→1)
Rebaudioside F	*β*-Glc	*β*-Glc-*β*-Xyl(2→1) | *β*-Glc(3→1)
Dulcoside A	*β*-Glc	*β*-Glc-*α*-Rha(2→1)

Glc, Xyl and Rha represent, respectively, glucose, xylose and rhamnose sugar moieties
[taken from the JECFA Chemical and Technical Assessment for steviol glycosides (FAO 2007)].

2.2.2 Quantification of steviol glycosides

The ratio of the various glycosides in steviol glycosides will differ according to the soil conditions and climate where the raw material (leaves) are harvested and on the extraction and purification processes used to produce the extract. Therefore, JECFA considered that for accurate and consistent quantification of steviol glycosides they should be expressed as their steviol content. Each of the glycosides contains steviol as a common central component of its molecular structure – one molecule of each of the different glycosides contains one unit of steviol (molecular weight 318). For the various steviol glycosides the ratio of steviol glycosides to steviol equivalents is approximately three, which is used for the various calculations for permissions in the Code.

2.2.3 Specification

An updated specification for steviol glycosides was prepared by JECFA in 2010, which superseded the 2008 specification. This specification is published in FAO JECFA Monographs 10 (2010). Currently the Code references the earlier JECFA monographs up to monograph 5 (2008) as a primary source of specifications for substances added to food. This reference is contained in clause 2 of Standard 1.3.4 – Identity and Purity. The title as referenced is the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs. The JECFA specification reference needs to be updated in Standard 1.3.4 (either via this Application or by some other mechanism). The online specification for steviol glycosides from the Combined Compendium of Food Additive Specifications can be found at the following URL: http://www.fao.org/ag/agn/jecfa-additives/details.html?id=898.

The Application provided chemical analysis of three production lots of their commercial steviol glycoside production that showed that their preparation meets and is consistent with the JECFA specification for steviol glycosides.

The major component of the Applicant's steviol glycoside preparation is rebaudioside A, which is over 95% of the steviol glycosides in the preparation. The other minor components are stevioside, rebaudioside C, rebaudioside D and rebaudioside F.

2.3 Technological justification

Steviol glycosides are 200 to 300 times sweeter than sucrose and have been used for many years in a number of countries as non-caloric sweeteners for a range of food products. The relative sweetness of individual glycosides is different. Rebaudioside A is sweeter than stevioside (300 times compared with 250 times sucrose respectively) and is associated with a more palatable taste profile, which is very relevant for the Applicant's commercial product as it is predominantly composed of rebaudioside A. Stevioside and rebaudioside A are the dominant components of steviol glycosides and the ratio of these two is the main determinant of taste 'quality'. Where stevioside is more than 50% of the total glycosides the taste is 'common/traditional', with a 'metallic' or 'liquorice' after-taste. Where rebaudioside A makes up more than 50%, the taste is 'improved' with a reduced after-taste.

Steviol glycosides can be used in conjunction with sugar or other sweeteners and it is claimed could replace some (or all) of the sweetener now used in various food products. They could be used at concentrations that are 250 times less than the rate of sugar currently used in food products.

Steviol glycosides are heat and acid stable and are therefore suitable for use in a wide range of food products, including baked and cooked products. Steviol glycosides also have a flavour enhancing effect when used in association with other sweeteners or flavours.

Therefore, if steviol glycosides are added to a food, other flavours and sweeteners may be used at lower rates than required without the inclusion of steviol glycosides.

2.4 Manufacture

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.

2.5 Analytical method

The Application states that the Applicant has developed their own in-house analytical methods for the identification and quantification of steviol glycosides in food and beverage matrices by using High Performance Liquid Chromatography (HPLC).

The European Food Safety Authority (EFSA) scientific opinion on steviol glycosides (EFSA 2010) contains a relevant section dealing with methods of analyses in food. Again these methods are based on using HPLC. Two recent validated HPLC analytical methods have been published (Geuns et al. 2008; Gardana et al. 2010).

2.6 Technological justification for increasing addition to some food

The Application has requested an increase in the maximum permitted amounts of steviol glycosides (in steviol equivalents) in some foods and beverages above those currently permitted in the Code (from an original earlier steviol glycoside Application, A540).

The justification from the Applicant is that increased levels of steviol glycosides are required to ensure an appropriate sweetness of the foods with added steviol glycosides to provide a commercially acceptable product. The Applicant further argues that the increased levels for steviol glycosides in these food categories would be comparable to the levels requested for the same food categories in the European Union. The Applicant claims that the tasting analysis they have performed for a number of foods using the current limits of added steviol glycosides in the Code produce a product that tasters (as surrogates for consumers) commented on was not sweet enough. The Applicant performed some taste test analyses on the taste (essentially sweetness) perceptions of foods with various levels of added steviol glycosides, with untrained taste panellists. These consumer taste trials were performed to support the Applicant's claim that higher permitted levels of steviol glycosides are needed to produce consumer acceptable products.

In particular the Applicant performed taste test analyses on two types of food; being soft drinks (lemon-lime and cola flavoured) and ice-cream. The taste panel evaluation report performed for the Applicant on ice cream sweetened with steviol glycoside was provided in the Application. The soft drink studies were not provided but the summary of the results was provided and discussed.

Ice cream studies

For the ice-cream taste test trial an external company performed the testing and compiled the report for the Applicant. Vanilla ice cream (with no initial added sugar) was produced with three levels of added steviol glycosides. One preparation was the control with the current maximum permitted level of steviol glycosides as permitted in the Code, and two others at higher levels. The three preparations were:

1. The current permitted maximum steviol glycosides use level for ice cream, being 64 mg/kg steviol equivalents (control);
2. 165 mg/kg steviol equivalents;
3. 200 mg/kg steviol equivalents.

The ice creams were tasted and assessed by an untrained consumer panel for sweetness on a scale they called 'just-about-right' and for overall liking.

The panel's conclusions of the control ice cream compared to the preparation 2 (165 mg/kg steviol equivalents) product were that it was:

- Not significantly differently liked;
- Not deemed sweet enough by a very significant margin.

Similar conclusions were noted when the control product was compared to preparation 3 (200 mg/kg steviol equivalents). Then the control ice cream was concluded to be:

- Very significantly less liked;
- Not deemed sweet enough by a very significant margin.

The conclusion of the consulting company that conducted the taste trials was that the current maximum use level for steviol glycosides in ice cream is too low to achieve optimum sweetness.

The preferred steviol glycoside level for ice cream is 200 steviol equivalents (approximately 600 mg/kg steviol glycosides), which produces a highly preferred product with a more balanced distribution of sweetness ('just about right') than the current maximum Code limit.

Beverage studies

The Application contains some discussion and summary statements of trials that have been conducted on adding steviol glycosides to provide acceptable sweetness to various beverages.

The Applicant provides two equivalence equations determined for steviol glycosides compared to sucrose equivalence (sweetness) for soft drinks (specifically cola and fruit flavoured, they refer to lemon-lime flavoured drinks). The Applicant concludes that both types of flavoured soft drinks require a 10% sucrose equivalent to produce acceptable sweetness, which is obtained using 600 mg/kg steviol glycosides (approximately 200 mg/kg

steviol equivalents).

Currently the Code permits water based flavoured drinks (which encompasses soft drinks) to contain a maximum permitted level of steviol equivalents of 160 mg/kg (equivalent to approximately 480 mg/kg of steviol glycosides).

The Applicant performed other taste evaluations of lemon-lime flavoured soft drinks comparing products flavoured with 160 mg/kg (control, current permission) and 200 mg/kg steviol equivalents for various flavour attributes; being flavour, sweetness, bitterness, sourness and mouth feel. There was an overall increase in overall liking and sweetness when the concentrations were increased. However there was no significant increase in overall liking at concentrations greater than 200 mg/kg steviol equivalents. As well the bitterness intensity increased at levels greater than 200 mg/kg steviol equivalents. The Applicant concluded from these and some other taste studies that a maximum level of 200 mg/kg steviol equivalents is required for the two types of flavoured soft drinks.

The Applicant noted that although not all other beverages that they are seeking increased steviol glycoside limits have had taste testing performed on different formulations, they are all expected to require an increase in the limit to 200 mg/kg steviol equivalents. Such products include brewed soft drinks, formulated beverages and flavoured soy beverages, since the Applicant claims such products generally require 10% sucrose equivalents or 10 Brix (in the case of flavoured soy beverages), to be acceptable for consumers.

However the Applicant further notes that not all versions of beverage categories will require the maximum usage levels as there is a consumer category for lighter styles of drinks where the sweetness level will be lower. The examples the Applicant referred to was to current sugar based beverages ice tea and sports beverages which are generally produced and marketed as up to 40% less sugar than regular soft drinks.

For both ice cream and various soft drinks the conclusions were that the taste testers preferred the food or beverage that had been sweetened with more than currently permitted maximum levels of steviol glycosides in the Code.

2.7 Conclusion

The use of steviol glycosides as an intense sweetener and flavour enhancer in a range of foods is technologically justified. Steviol glycosides are high intensity sweeteners 200-300 times sweeter than sucrose, that also have a flavour enhancing effect when used in association with other flavours. They can be used in a wide range of foods and beverages that contain sugar, and can either be used in conjunction with sugar or intense sweeteners or as a total sugar or intense sweetener replacement.

The Applicant has provided trial results to support their claim that higher steviol glycoside maximum limits are required for ice cream and various flavoured drinks (specifically soft drinks, which has been used to justify amended limits for other drinks) to produce consumer acceptably sweetened products. The current maximum limits in the Code for these products were determined by taste test analysis to be too low.

3. Hazard Assessment

3.1 Background

3.1.1 Chemistry

Details of the physicochemical properties of steviol glycosides, including product specifications, are included in the Food Technology Assessment (Section 2).

3.1.2 Previous FSANZ Assessment

FSANZ conducted a hazard assessment for steviol glycosides as part of the assessment of Application A540 - Steviol Glycosides as Intense Sweeteners (FSANZ 2008). FSANZ concluded that the toxicological database for steviol glycosides provided an adequate basis for establishing a full ADI. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose was considered to be adequate so that the additional 2-fold safety factor used by JECFA in 2005 for uncertainty surrounding its effect in normotensive or diabetic individuals was not required.

An ADI of 0-4 mg/kg bodyweight (bw), derived by applying a 100-fold safety factor to the no-observed-adverse-effect level (NOAEL) of 970 mg/kg bw/day of stevioside (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat carcinogenicity study, was established by FSANZ. This ADI covers steviol glycoside mixtures with different ratios of stevioside/rebaudioside.

3.1.3 Assessments by Other Agencies

Joint FAO/WHO Expert Committee on Food Additives (JECFA)

JECFA assessed steviol glycosides at its 51st, 63rd, 68th and 69th meetings.

At its 51st meeting, JECFA evaluated toxicological data on stevioside and steviol and concluded that further information was needed (WHO 1999).

At its 63rd meeting (WHO 2005, 2006), JECFA determined that the commercial material should be known as “steviol glycosides” and established tentative specifications for material containing not less than 95% of the total of four specified glycosylated derivatives of steviol (i.e. stevioside, rebaudioside A, rebaudioside C and dulcoside A). Additionally, the sum of stevioside and rebaudioside A content was specified at not less than 70% of the four steviol glycosides. JECFA reviewed additional biochemical and toxicological data on the major steviol glycosides and on the aglycone steviol. It was noted that steviol glycosides are poorly absorbed and are metabolised by the intestinal microflora by successive hydrolytic removal of glucose units to steviol which is well absorbed. Therefore, the toxicity of the glycosides was related to the steviol content. A temporary ADI of 0-2 mg/kg bw for steviol glycosides expressed as steviol was established on the basis of the NOAEL of 2.5% stevioside in the diet, equal to 970 mg/kg bw per day, or 383 mg/kg bw per day expressed as steviol, in a 2-year carcinogenicity study in rats and with a safety factor of 200. The safety factor of 200 incorporated an additional 2-fold factor because of uncertainty surrounding the pharmacological effects of steviol glycosides in humans.

JECFA requested additional information on the effects of steviol glycosides on normotensive and hypotensive individuals and in patients with type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes. This was because there was limited evidence available at the time to assess whether the reported pharmacological effects of steviol glycosides would also occur at dietary exposure levels.

At its 68th meeting (WHO 2007), JECFA considered the information that had become available since the 63rd meeting. This comprised two submissions, which included a summary of published toxicological studies and some unpublished data, additional information identified from the scientific literature and responses intended to resolve the outstanding issues relevant to the specifications. JECFA was also informed that results of an ongoing toxicity testing programme, including clinical studies, would be available by August 2007. JECFA considered that the newly available data did not raise additional concerns regarding the safety of steviol glycosides, but that the ongoing clinical studies would be essential for the evaluation. JECFA therefore extended the temporary ADI of 0-2 mg/kg bw, expressed as steviol, pending submission of the results of the ongoing studies. The existing tentative specifications were revised by requiring an assay of not less than 95% of the total of seven named steviol glycosides, by deleting the assay requirement for the sum of stevioside and rebaudioside A content to be not less than 70%. The tentative designation for the specifications was removed.

At its 69th meeting (WHO 2009), JECFA considered a submission that comprised a review of all the available information, including studies completed after the 68th meeting and some older studies not highlighted in the previous JECFA evaluations. The new studies included four toxicological studies with rebaudioside A in experimental animals and clinical trials on the effects of steviol glycosides on blood pressure in healthy volunteers with normal or low-normal blood pressure and on glucose homeostasis in men and women with type 2 diabetes mellitus. JECFA concluded that the new studies showed no adverse effects at levels up to 4 mg/kg bw per day, expressed as steviol, in individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure. The new data were considered sufficient evidence to remove the temporary ADI designation the Committee had set in 2005. A full ADI for steviol glycosides of 0-4 mg/kg bw, expressed as steviol, was established.

<u>European Food Safety Authority (EFSA)</u>

In 2010, EFSA assessed the available data on steviol glycosides and concluded that steviol glycosides complying with JECFA specifications are not carcinogenic, genotoxic or associated with any reproductive/developmental toxicity. EFSA established an ADI for steviol glycosides, expressed as steviol equivalents, of 0-4 mg/kg bw based on application of a 100-fold uncertainty factor to the NOAEL in the 2-year carcinogenicity study in rats (EFSA 2010).

3.1.4 Scope of the Hazard Assessment

The aims of the current assessment were to:

- Evaluate any newly submitted data relevant to the hazard assessment of steviol glycosides

- Search the published literature for any relevant studies on steviol glycosides which have not been previously considered by FSANZ; and

- Determine whether the new data indicate a need to change the ADI.

3.2 Evaluation of Submitted Data

No new unpublished studies were submitted by the Applicant. The Applicant submitted several published reviews and studies which were published subsequent to the assessment of Application A540 by FSANZ. These published studies are evaluated below (Sections 3.2.1 to 3.2.7).

A literature search conducted in November 2010 using PubMed did not result in the identification of any additional papers considered relevant for hazard assessment.

3.2.1 Absorption, metabolism and excretion

The absorption, metabolism and excretion of ^{14}C-rebaudioside A, ^{14}C-stevioside, and ^{14}C-steviol were examined in intact and bile duct-cannulated male and female Sprague-Dawley rats to determine whether toxicological studies conducted previously with stevioside would be applicable to rebaudioside A (Roberts & Renwick 2008).

The same single oral (gavage) dose levels were used in the absorption, metabolism and excretion phases of the study. The dose level of rebaudioside A (5 mg/kg bw) was based on a published estimate of the highest daily human intake of rebaudioside A (Renwick 2008). The dose levels of stevioside (4.2 mg/kg bw) and steviol (1.6 mg/kg bw) were equivalent on a molar basis to that of rebaudioside A. Radiolabelled compounds were synthesised with ^{14}C in the $=CH_2$ group of the steviol moiety.

The plasma kinetic data (Table 3.1) indicate that the peak concentration of total radioactivity (C_{max}) and area under the concentration versus time curve (AUC) for stevioside were slightly higher than those of rebaudioside A. For each compound, the data indicate a sex difference in C_{max} for total radioactivity. A sex difference for AUC was also observed for rebaudioside A and stevioside. However, sex differences were not observed in the excretion or metabolism data (below).

The observed peak plasma concentrations of total radioactivity in both males and females given stevioside or rebaudioside A occurred between 2 and 8 h after dosage. The peak plasma concentrations of steviol were detected in the first sample (taken at 0.25 h post-dose) and therefore the data may underestimate the true C_{max} value. The AUC values for females, but not males, were lower for steviol than for stevioside and rebaudioside A, possibly because a significant contribution to the AUC for steviol was not measured prior to the first sample. The half-lives for total radioactivity were between 5 and 16 h for all three compounds in both sexes.

Table 3.1 Pharmacokinetic parameters derived from mean total radioactivity concentrations in plasma following the administration of single oral doses of ^{14}C-rebaudioside A, ^{14}C-stevioside, and ^{14}C-steviol

	Administered substance					
	Rebaudioside A		Stevioside		Steviol	
Parameter	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
C_{max} (ng-equiv/g)	90	177	101	279	114	264
T_{max} (h)	2	8	4	8	0.25	0.25
$AUC_{0\text{-}72\ h}$ (ng-equiv•h/g)	645	3329	1617	4287	1251	1604
$AUC_{0\text{-}\infty}$ (ng-equiv•h/g)	630	3349	1607	4359	1926	1926
k (h^{-1})	0.146	0.072	0.080	0.046	0.044	0.043
$t_{1/2}$ (h)	5	10	9	15	16	16

C_{max} – maximum observed plasma concentration; T_{max} – time of maximum observed plasma concentration; $AUC_{0\text{-}72\ h}$ – Area under the concentration versus time curve calculated from 0 to 72 h after administration; $AUC_{0\text{-}\infty}$ – AUC extrapolated to infinity using the terminal slope; k - elimination rate constant; $t_{1/2}$ - elimination half-life.

In intact rats, radioactivity was predominantly excreted in the faeces which accounted for 97–98 % of the dose for rebaudioside A and stevioside, and about 90% for steviol. For all compounds, the majority of the faecal radioactivity was excreted in the first 24 h (64–89%) with a further 10–22% excreted in faeces between 24 and 48 h. Recovery of radioactivity from urine and cage wash fluid ranged from 1% to 3%. Radioactivity in the gastrointestinal tract 96 h after administration ranged from 0.02% to 1.2%. No radioactivity was detected in the carcasses of rats of either sex at 96 h after administration of ^{14}C-rebaudioside A, while 0.01–0.1% was recovered from carcasses of rats dosed with ^{14}C-stevioside and ^{14}C-steviol.

For intact rats administered ^{14}C-rebaudioside A, the two predominant radioactive components in the faeces were rebaudioside A (29% and 19% of the dose for males and females, respectively) and steviol (44% and 57% of the dose for males and females, respectively). Stevioside and steviol glucuronide(s) were also detected, but at levels of <5%. Steviol and steviol glucuronide(s) were the predominant radioactive components in the faeces of intact animals administered ^{14}C -stevioside, accounting for 56% and 14% of the dose, respectively, in males, and 72% and 10% of the dose, respectively, in females; stevioside accounted for 12% and 2% of the dose in males and females, respectively. Steviol was the predominant radioactive component of the faeces of rats dosed with ^{14}C -steviol, accounting for 69% and 74% of the dose in males and females, respectively. Rebaudioside A, stevioside, and steviol glucuronide(s) were not detected.

In bile-duct cannulated rats, the majority of the radioactivity was recovered in the bile. In both male and female rats, about 70–80% of the administered ^{14}C-rebaudioside A or ^{14}C-stevioside were recovered in bile within 24 h. Most of the biliary excretion of radioactivity occurred within the first 12 h after dosing with limited excretion in the first 3 h after dosing. In contrast, the biliary excretion of radioactivity after dosing of ^{14}C-steviol was much more rapid, with about 50% and 70% of the dose eliminated in the bile of male and female rats within the first 3 h. The total recovery in bile over 48 h was 97–98% of the administered dose of steviol. For both rebaudioside A and stevioside, the remaining radioactivity was recovered in the faeces (21–30%) and the urine and cage washings (1–2%). For steviol, only 1–2% of the administered dose was recovered in the faeces, while the urine and cage washings accounted for a further 1% of the dose. Very low levels of radioactivity were detected at 96 h in the gastrointestinal tract and liver; no radioactivity was detected in the carcasses of any dose group. The extent of absorption was estimated by summing the mean total amount of radioactivity in the bile, urine (including cage wash fluid), liver, and the remaining carcass.

Using this method, approximately 71% and 82% of the dose of ^{14}C-rebaudioside A was absorbed by males and females, respectively, 78% and 81% of the ^{14}C-stevioside dose was absorbed by males and females, respectively, and 97% and 99% of the ^{14}C-steviol dose was absorbed by males and females, respectively.

In cannulated rats administered ^{14}C-rebaudioside A, the predominant radioactive component in faeces was steviol, accounting for 25% and 16% of the dose for males and females, respectively. Rebaudioside A, stevioside, and steviol glucuronide(s) were also detected but at levels of <2%. Steviol was also the predominant radioactive component in the faeces of cannulated rats dosed with ^{14}C-stevioside, accounting for 18% and 16% of the dose for males and females, respectively. The faeces from cannulated rats given ^{14}C-steviol were not analysed due to the low levels of radioactivity present. Steviol glucuronide conjugates were the predominant radioactive components in the bile of rats dosed with ^{14}C-rebaudioside A, ^{14}C-stevioside, or ^{14}C-steviol. The parent glycoside compounds and steviol were also detected in bile at levels <1% in all groups. Unidentified compounds in bile represented about 9%, 3% and 5% for rebaudioside A, stevioside and steviol, respectively. No significant sex differences were apparent in the routes or extents of elimination, or in the pattern of metabolites excreted.

3.2.2 Repeat-dose toxicity

In a dose range finding study, groups of Han Wistar rats (10/sex/group) received 0, 25000, 50000, 75000, or 100000 ppm rebaudioside A (purity 97%) in their diets for 4 weeks (Curry and Roberts 2008). The study was conducted at Huntingdon Life Sciences, Huntingdon, Cambridgeshire, England and was stated to be GLP and OECD guideline compliant.

There were no deaths and no signs of adverse reactions to rebaudioside A. Mean body weight gain for the entire experimental period was significantly lower in females receiving 100000 ppm rebaudioside A compared to the control females (48 g vs 65 g, $p < 0.01$). Over the entire experimental period, the only significant difference in mean daily food consumption was a small increase for high dose males compared to the controls (24 g vs 22 g, $p < 0.05$).

It was stated that a few statistically significant differences in haematology results were observed between control and treated rats, but that none were considered biologically relevant because they were small and not dose-dependent. It was also stated that clinical chemistry analyses revealed small but statistically significant increases in plasma creatinine in all groups of treated males and in the 75000 and 100000 ppm females. Urine specific gravity in the top two dietary concentrations in males and all treated groups of females was reported to be increased while urine volume was significantly reduced in the 75000 and 100000 ppm males. A significant decrease in total bile acid levels in males in the 75000 ppm and 100000 ppm groups was reported. All treated female rats were reported to have significantly lower bile acid levels compared to the control rats, but these decreases were not considered to be dose-dependent by the authors.

For males in the two highest dose-groups, heart weights (when adjusted for bodyweight) were significantly lower compared to control males. High-dose males had significantly lower testes weights compared to the controls. Females in the three highest dose-groups had significantly lower relative adrenal gland weights compared to controls, but these decreases did not follow a dose-response pattern. It was stated that there were no macroscopic findings considered to be related to treatment at necropsy and that microscopic examination of the testes and epididymides did not reveal any treatment-related findings (i.e., no histopathological correlates for the organ weight findings). Other organs were not examined microscopically. Based on the results of this study, dietary concentrations of rebaudioside A for a 13-week study were established at 0, 12500, 25000, and 50000 ppm.

In the subsequent 13-week study, groups of Han Wistar rats (20/sex/group) received rebaudioside A in the diet at concentrations of 0, 12500, 25000, or 50000 ppm (Curry and Roberts 2008). This study was also conducted at Huntingdon Life Sciences, Huntingdon, Cambridgeshire, England and stated to be GLP and OECD guideline compliant.

Rats were 40–46 days old at the start of treatment. Achieved mean intakes for weeks 1 and 13 of the study are shown in Table 3.2. Mean intakes over the entire study period were reported for the 50000 ppm animals only as 4161 and 4645 mg/kg bw/day in males and females, respectively.

Table 3.2 Achieved mean intakes of rebaudioside A (mg/kg bw/day)

	Males			Females		
	12500 ppm	*25000 ppm*	*50000 ppm*	*12500 ppm*	*25000 ppm*	*50000 ppm*
Week 1	1506	3040	5828	1410	2841	5512
Week 13	698	1473	3147	980	1914	3704

There were no treatment-related deaths and the appearance, behaviour, and sensory reactivity (approach response, auditory startle reflex, tail pinch response, and touch response) of the rats were reported to be unaffected by treatment. Ophthalmological analyses revealed no differences between high-dose rats and controls. Reported sporadic differences in activity scores in females and forelimb and hindlimb grip strength in males were considered to be unrelated to treatment. Apparent deficits in each test were observed in only one sex, and at only one evaluation period for the females, and were not corroborated by the results in other functional activity tests. Moreover, histopathological findings in muscle and nerve tissues were reported to be negative.

During the first 4 days of treatment, body weight gains were significantly lower in males and females receiving 25000 or 50000 ppm. Following the first 4 days of treatment to study termination, body weight gains were significantly lower in all male groups compared to the controls. Overall body weight gains were significantly lower in treated males. In females treated at 25000 and 50000 ppm the overall body weight gains were slightly, but significantly, lower than the controls (Table 3.3).

Table 3.3 Body weight gain (g) in the 13-week study

	Males				Females			
	0 ppm	*12500 ppm*	*25000 ppm*	*50000 ppm*	*0 ppm*	*12500 ppm*	*25000 ppm*	*50000 ppm*
Days 1-4	18	17	15**	11**	8	10	7*	3**
Days 4-92	236	217*	218*	194**	102	99	95	95
Days 1-92	255	234*	233*	205**	111	109	101*	98**

Compared to the control *p < 0.05; **p < 0.01.

Decrease body weight gain was accompanied by lower food consumption. When corrected for lower caloric density of the diets containing rebaudioside A, overall food consumption was 95%, 98%, and 95% of the controls in the low- through high-dose males, respectively, and 105%, 98%, and 95% of the controls in low- through high-dose females, respectively. In comparison to the controls, the greatest decreases in food consumption occurred in the first 2 weeks of the study, with decreases of 9% to 15% in high-dose males and 5% to 19% in high-dose females. Food conversion efficiency was significantly decreased at various times in treated males early in the study (days 1–14). Sporadic decreases in food conversion efficiency in males were also noted later in the study.

In females, food conversion efficiency was significantly lower during days 1 to 3 in the high-dose group and a significantly higher in all dose groups during days 8 to 10. Over the course of the entire study, statistically significant decreases in food conversion efficiency values were limited to high-dose males. As described for other high intensity sweeteners, it is likely that the observed effects on food consumption and body weight gain are attributable to taste aversion and the lower caloric density of the diets containing very high concentrations of the test article.

A few statistically significant differences in haematology results were observed between control and treated rats, but all were considered biologically irrelevant because they were small, did not occur in a dose-related pattern and/or occurred in only one sex.

As in the 4-week study, there was a tendency in both males and females for the plasma urea and creatinine to be higher in treated animals especially in the higher dose groups. These increases were relatively small with mean values within the laboratory's reference ranges. There was also a tendency in both males and females for urine specific gravity to be higher and urine volume lower in treated animals especially in the higher dose groups. These effects were not considered to adverse, a conclusion supported by the lack of renal toxicity as indicated by the macroscopic and microscopic evaluation of the kidneys.

On days 10, 46 and 89 all the treated-males, and on day 46 all treated females, had significantly lower levels of total bile acids. The large amounts of rebaudioside A metabolised by the liver may have altered normal bile acid homeostasis. Liver enzyme activities and hepatic histopathology were within normal limits and similar to control results and it is therefore concluded that the effect on bile acids is not toxicologically significant. Plasma triglycerides and cholesterol results were slightly but statistically significantly lower in some groups of treated male and female rats on days 10, 46 and 89. These reductions were also likely to be attributable to the altered bile acid homeostasis. Other statistically significant differences in clinical chemistry parameters were observed, but they were seen in only one sex, did not follow a dose-dependent pattern, and/or were within the laboratory's normal limits for rats.

The absolute epididymal weights of the high-dose males were significantly lower compared to the controls. Absolute weights of the ovaries and the adjusted weights of the heart and kidneys of the high-dose females were significantly lower relative to the controls. Macroscopic examination revealed 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. These findings were therefore not considered to be treatment-related. All histopathology findings were considered to be within the normal limits for rats of this age and unrelated to treatment. As a result, the sporadic organ weight findings in the high-dose males and females were not considered to be toxicologically significant.

It is concluded that the NOAEL was the high dietary concentration of 50000 ppm which corresponded to mean rebaudioside A doses of approximately 4161 and 4645 mg/kg bw/day in male and female rats, respectively (1370 mg/kg bw/day in males and 1530 mg/kg bw/day in females, expressed as steviol).

3.2.3 Genotoxicity

Three *in vitro* and two *in vivo* genotoxicity studies were conducted on rebaudioside A (Williams and Burdock 2009). These studies are summarised in Table 3.1. All studies were conducted at BSL Bioscience, Scientific Laboratories, Behringstrasse 6, 82152 Planegg, Germany. There was no statement regarding whether these studies were conducted according to GLP. It was stated that the studies were conducted according to OECD guidelines. The *in vitro* studies were conducted in the presence and absence of an

exogenous source of metabolic activation (S9 liver preparations from Aroclor 1254-induced rats). The test article in each study was rebaudioside A (purity 95.6%, Batch 0703134). Positive and negative (vehicle) controls were tested in each study and gave expected results, however the identity of the vehicle was not stated for the Ames test and the mammalian forward mutation assay. In the mammalian chromosome aberration assay, the cell system described in the Methods section of the paper (cultured human lymphocytes) differed from that in the Results section (Chinese hamster V79 lung fibroblasts). In addition, cytotoxicity data were not presented for this assay.

The inconsistencies in data presentation noted in the published report present some difficulties for data evaluation. Unfortunately none of the underlying raw experimental data were available to determine whether the missing information represents a genuine deficiency. However, comparing the results presented by Williams and Burdock (2008) with previously reported genotoxicity results in FSANZ, JECFA and EFSA assessments shows that there appears to be good agreement with the weight of evidence indicating that steviol glycosides are unlikely to be genotoxic.

Table 3.2: Summary of genotoxicity studies described in Williams and Burdock (2009)

Test	Test system	Concentrations / doses tested	Result
Bacterial reverse mutation (Ames test)	*S. typhimurium* strains TA98, TA100, TA1535 & TA1537. *E. coli* strain WP2uvrA. Plate incorporation and pre-incubation methods (±S9).	32-5000 µg/plate	Negative No cytotoxicity
Mammalian chromosome aberration	Human lymphocytes or Chinese Hamster V79 lung fibroblasts[2]. Incubation times of 4 h and 20 h (±S9).	1000-5000 µg/mL (Vehicle: 0.9% sodium chloride)	Negative Cytotoxicity data not reported
Mammalian forward mutation	Mouse lymphoma L5178Y cells. Incubation times of 4 h and 24 h (±S9).	10-5000 µg/mL (4 h, ±S9) 20-5000 µg/mL (24 h, -S9) 100-5000 µg/mL (24 h, +S9)	Negative No cytotoxicity
Mouse micronucleus	Mice (NMRI), IP injection. Blood collected 44 and 68 h post-injection.	Doses not stated for preliminary toxicity test. Main test: 0, 150, 375 & 750 mg/kg bw (44 h sampling time, 5/sex/group) 0 & 750 mg/kg bw (68 h sampling time, 5/sex/group)	Negative Toxicity at the highest dose[1]
Unscheduled DNA synthesis	Wistar rats, oral gavage. Hepatocytes collected 2 h and 16 h post-administration.	0 & 2000 mg/kg bw (4 males/group)	Negative No signs of toxicity

[1] All animals in the highest dose group exhibited signs of toxicity in the form of reduction of spontaneous activity, rough fur, prone position and cramps.
[2] The cells described in the Methods section of the paper (cultured human lymphocytes) differed from those in the Results section (Chinese hamster V79 lung fibroblasts).

3.2.4 Reproductive toxicity

A preliminary single-generation study and a subsequent two-generation study with rebaudioside A in rats were conducted by Huntingdon Life Sciences, Ltd., Cambridgeshire, England according to GLP and US FDA guidelines (Curry et al. 2008).

In the preliminary study, female Han Wistar rats and their litters received rebaudioside A (purity 97%) at concentrations of 0, 25000, 37500, and 50000 ppm in the diet. Parental (F_0) females (6/group) were treated from days 14–21 of lactation. Male and female offspring (F_1) animals were treated from day 14 of age and 10/sex/group from each treatment group (maximum of 2/sex/litter) were selected for continuation of treatment after weaning (day 21) until day 35 of age. Daily and weekly clinical observations, physical examinations, body weights and food consumption measurements were conducted. All rats were evaluated for gross lesions during necropsy and testes from high-concentration group males were examined microscopically for abnormalities. Statistical analyses were not conducted in this preliminary study.

There was no effect of treatment on the general condition of the F_0 animals or on body weight, body weight gain, food consumption, or on the incidence of macroscopic abnormalities noted at necropsy. Microscopic examination of the testes from the high dietary-concentration group animals indicated no effects on testicular morphology or spermatogenesis.

In F_1 animals, there was a reduction in body weight gain in the two highest concentration groups in males and females during the first 21 days post-partum. After weaning, in the selected F_1 rats, reductions in body weight gain in both sexes continued until 35 days of age in the 37500 and 50000 ppm groups. Reduction in body weight gain was most notable during days 21–24 of age. Food consumption during this time interval in the 37500 and 50000 ppm groups was stated to be lower than the controls. No effects of treatment on body weight gain or on food consumption post-weaning were noted in the 25000 ppm F_1 animals. Macroscopic examination of the selected F_1 juveniles revealed enlarged parotid salivary glands in 10/10 males and 8/10 females in the 50000 ppm groups and in one male treated at 37500 ppm.

It was concluded that the 25000 ppm dietary concentration would be suitable as the highest dietary concentration for a two-generation reproductive toxicity study.

In the subsequent two-generation study, Han Wistar rats received rebaudioside A (purity 97%) at concentrations of 0, 7500, 12500, and 25000 ppm in the diet. F_0 rats (30/sex/group, initial age 6 weeks) received test diet for 10 weeks prior to mating and throughout mating, gestation and lactation until termination. Rats from the F_1 generation were allocated to their specific treatment group when they were approximately 25 days of age. A minimum of one male and one female were randomly selected from as many litters as possible within each group until 25/sex/group were obtained.

For the F_0 generation, the average achieved rebaudioside A doses were 586, 975, and 2048 mg/kg bw/day for the males receiving the 7500, 12500, and 25000 ppm diets, respectively. The average achieved rebaudioside A doses were 669, 1115, and 2273 mg/kg bw/day for females in the 7500, 12500, and 25000 ppm groups, respectively, during the pre-pairing phase of the study. During the gestation and lactation phases of the study, ranges of 648–713, 1119–1169, and 2263–2381, and; 715–1379, 1204–2388, and 2602–5019 mg/kg bw/day were reported for females in the 7500, 12500, and 25000 ppm groups, respectively.

For F_1 adults, the average achieved rebaudioside A doses were 734, 1254, and 2567 mg/kg bw/day for the F_1 males receiving the 7500, 12500, and 25000 ppm diets, respectively. In the F_1 females, during the pre-pairing phase, the average achieved rebaudioside A doses were 798, 1364, and 2768 mg/kg bw/day for the 7500, 12500, and 25000 ppm groups, respectively. During the gestation and lactation phases of the study, achieved dose ranges of 562–625, 911–1058, and 2036–2212 and 976–1406, 1752–2394, and 3289–4893 mg/kg bw/day were calculated for females in the 7500, 12500, and 25000 ppm groups, respectively.

Expressed as steviol, the mean intake in animals fed the 25000 ppm diet was equivalent to 674 mg/kg bw/day in males and 748 mg/kg bw/day in females.

In the F_1 and F_2 generations, pre-weaning righting, auditory and visual reflexes were measured. The F_1 and F_2 offspring culled on day 4 of age and considered to be externally normal were discarded without examination. F_0 and F_1 adult animals were subjected to necropsies involving full macroscopic examinations and weighing of the adrenals, brain, kidneys, liver, pituitary, spleen, and ovaries and uterus with cervix and oviducts or epididymides, ventral prostate, testes, and seminal vesicles were conducted. In females, the number of implantation sites in each uterine horn was counted. Sperm samples were taken as soon as possible after death for analysis. Sperm count was evaluated for each group while sperm morphology was assessed in the high-concentration and control groups.

Microscopic examinations were performed on preserved tissues from F_0 and F_1 adult animals in the control and high dietary-concentration groups euthanized upon completion of the treatment period and for animals euthanized or dying during the study. Additional tissues reported at macroscopic examination as being grossly abnormal from F_0 and F_1 adult animals and F_2 offspring examined at scheduled termination on day 30 were examined microscopically.

No treatment related deaths occurred. Food conversion efficiency, oestrous cycle, mating performance, fertility, gestation length, gestation index, pregnancy outcome, general condition of offspring, litter size, survival, sex ratio, surface- and air-righting reflexes, auditory startle responses, pupil reflex and reproductive capability were all unaffected by treatment in the F_0 and F_1 generations.

Slight differences in food consumption and body weight gain were noted at various time points in the F_0 and F_1 generations, but they did not show a dose–response relationship and are therefore not considered to be treatment related.

Gross pathology and histopathology showed no treatment-related adverse effects in the F_0, F_1 or F_2 generation.

The NOAEL for reproductive performance and survival, growth and general condition of the offspring is therefore considered to be 25000 ppm in the diet. Expressed as steviol, the mean intake in animals fed the 25000 ppm diet was equivalent to 674 mg/kg bw/day in males and 748 mg/kg bw/day in females.

3.2.5 Human pharmacokinetics

A randomised, double-blind, two-way cross-over study assessed the effects of single doses of stevioside (purity 96.6%) and rebaudioside A (purity 98.7%) in healthy adult male subjects (n = 8) (Wheeler et al. 2008).

Subjects ingested single doses of 5 mg/kg bw rebaudioside A or 4.2 mg/kg bw stevioside as aqueous formulations, providing exposure of approximately 1.6 mg/kg bw of steviol equivalents. It was not stated whether subjects ingested the formulations in a single draft or over a short period of time.

Plasma samples were collected for analysis of steviol and steviol glucuronide prior to dosing, and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 36 and 72 h after dosing. Urinary output was collected during the following periods: -12 to 0 h pre-dose, and at 0–3, 3–6, 6–12, 12–24, 24–48 and 48–72 h post-dose. Faecal collections were made from 12 h pre-dose through 72 h post-dose.

Steviol glucuronide appeared in the plasma of all subjects after administration of rebaudioside A or stevioside, with median T_{max} values of 12 and 8 h, respectively. Steviol glucuronide was eliminated from plasma with $t_{1/2}$ values of approximately 14 h for both compounds. Ingestion of rebaudioside A resulted in a significantly lower steviol glucuronide mean C_{max} value (1472 ng/mL) than for stevioside (1886 ng/mL). The AUC_{0-t} value for steviol glucuronide after ingestion of rebaudioside A (30790 ng·h/mL) was approximately 10% lower than for stevioside (34090 ng·h/mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72 h collection period, accounting for 59% and 62% of the rebaudioside A and stevioside doses, respectively. Faeces accounted for approximately 5% of the dose in both cases. No steviol glucuronide was detected in faeces. Rebaudioside A and stevioside underwent similar metabolic and elimination pathways with steviol glucuronide excreted primarily in the urine and steviol in the faeces. No safety concerns were noted as determined by reporting of adverse events, clinical chemistry, haematology, urinalysis or vital signs.

3.2.6 Haemodynamic effects in humans

A randomised, double-blind placebo-controlled clinical trial evaluated the haemodynamic effects of 4 weeks consumption of 1000 mg/day rebaudioside A (n = 50) vs. placebo (n = 50) in individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Maki et al. 2008a). Subjects were predominantly female (76%, rebaudioside A and 82%, placebo) with a mean age of 41 years. The mean rebaudioside A dose was equivalent to 4.6 mg/kg bw/day expressed as steviol. The purity of rebaudioside A, which was administered as four 250 mg/capsules/day, was 97%. Two capsules were taken with the first meal of the day and two with the evening meal.

It was stated that the study was designed to provide 80% power to detect a 4.5 mmHg difference in SBP response for rebaudioside A vs. placebo. However, due to lower than anticipated subject variability, the study had >90% power which increased the sensitivity to detect minor changes in blood pressure.

Values for resting, seated SBP did not significantly differ between the rebaudioside A and placebo groups at baseline or during the treatment period. Values for resting, seated DBP and mean arterial blood pressure (MAP) were also not significantly different between rebaudioside A and placebo groups at baseline or in the changes from baseline to treatment.

Twenty-four hour ambulatory blood pressure monitoring of SBP and DBP revealed no significant differences between rebaudioside A and placebo treatment groups at week 0 or in the changes from week 0 to week 4 for morning, day time, night time, and overall continuous 24-h readings.

At week 0, there were no significant differences between groups in pre-meal values or the changes from pre-meal to post-meal values for supine SBP, DBP, MAP and HR. This was also true for the pre-meal measurements of these parameters at week 4. The placebo group

showed slight reductions in both supine SBP and DBP (-0.8 and -1.9 mmHg, respectively) compared to the pre-meal value at week 4.
In contrast, the rebaudioside A group showed slight increases for pre- to post-meal changes in DBP (0.6 mmHg, p = 0.045) and MAP (0.9 mmHg, p = 0.043). Supine heart rate at week 4 did not differ significantly between treatment groups. At week 0, there were no significant differences between rebaudioside A and placebo groups in pre-meal or the changes from pre-meal to post-meal values for standing SBP, DBP and MAP. Pre-meal standing HR was significantly higher at week 0 in the placebo group compared with the rebaudioside A group (78.9 vs. 74.7 bpm; p = 0.045), but the pre-meal to post-meal changes were not significantly different between groups. Pre-meal levels for standing DBP (74.7 vs. 72.4 mmHg; p = 0.016), MAP (87.9 vs. 85.9 mmHg; p = 0.036) and HR (79.9 vs. 74.6 bpm; p = 0.020) were significantly higher in the placebo group at week 4. Similar to the pattern observed for supine pressures, the rebaudioside A group showed relative increases from pre-meal in DBP (1.4 mmHg) and MAP (1.3 mmHg) that were significantly different from small declines in the placebo group (-1.3 mmHg, p <0.001 and -0.7 mmHg, p = 0.020, respectively). No significant differences were observed for standing SBP or HR responses at week 4. Standing SBP and DBP at week 4 were also similar for rebaudioside A and placebo in the first 2 h after dosing.

Rebaudioside A was well tolerated. There was no statistically significant difference in the number of adverse events reported in the rebaudioside A group compared to the placebo group. None of the adverse events were considered by the investigators to be related to the study test articles. No signs or symptoms of hypotension (e.g., light-headedness or dizziness) were reported in association with rebaudioside A. Clinical chemistry, haematology, and urinalysis indicated no clinically meaningful or statistically significant differences between the rebaudioside A and placebo groups.

3.2.7 Effects on type II diabetics

A randomised, double-blind, placebo-controlled clinical trial evaluated the effects of 16 weeks of consumption of 1000 mg/day rebaudioside A (n = 60) compared to placebo (n = 62) in men and women (33–75 years of age) with type 2 diabetes (Maki et al. 2008b). The mean rebaudioside A dose equivalent to 3.4 mg/kg bw/day expressed as steviol. The purity of rebaudioside A, which was administered as four 250 mg/capsules/day, was 97%. Two capsules were taken with the first meal of the day and two with the evening meal.

To be eligible, men and women (18–74 years of age) were required to have type 2 diabetes mellitus that was diagnosed at least one year prior to screening; $HbA_{1C} \leq 9.0\%$ at screening; and to have been treated for at least 12 weeks with stable dose(s) of one to three oral hypoglycaemic agents, basal insulin (intermediate or long-acting injections that provide a steady, low level of insulin throughout the day and night), or a combination of basal insulin plus one to three oral hypoglycaemic agents. Subjects were required to have a body mass index of 25–45 kg/m^2, be willing to maintain their habitual diets and physical activity patterns, and have no plans to change their smoking habits during the study period.

Mean changes in glycosylated haemoglobin levels did not differ significantly between the rebaudioside A and placebo groups. Changes from baseline for rebaudioside A and placebo, respectively, in fasting glucose (7.5 mg/dL and 11.2 mg/dL), insulin (1.0 IU/mL and 3.3 IU/mL), and C-peptide (0.13 ng/mL and 0.42 ng/mL) did not differ significantly (p > 0.05 for all). Measurements of blood pressure, body weight, and fasting lipids indicated no statistically significant differences by treatment. Clinical chemistry and haematology results were unremarkable. Rebaudioside A was well-tolerated, and records of hypoglycaemic episodes showed no excess vs. placebo. These results suggest that a daily intake of 1000 mg of rebaudioside A for 16 weeks does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

3.3 Discussion

No new unpublished studies have been provided as part of the current Application. The Applicant submitted several published reviews and studies which have been considered by JECFA but which were not yet published at the time of the assessment of Application A540 by FSANZ. FSANZ had considered these studies in unpublished form immediately following the completion of Application A540.

A review of published data on the metabolism of stevioside and rebaudioside A by human and animal intestinal microbiota showed close similarities across species and provides further confirmation that the rat is an appropriate species for safety studies on steviol glycosides (Renwick and Tarka 2008). A review of published genotoxicity studies of steviol and steviol glycosides concluded that these substances do not pose a risk of genotoxicity following oral consumption by humans (Brusick 2008).

Studies comparing the toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol in rats indicated that rebaudioside A and stevioside are handled in an almost identical manner. These studies support the use of toxicological studies conducted with stevioside for the safety assessment of rebaudioside A and vice versa (Roberts and Renwick 2008).
Two publications describing toxicity studies in rats administered rebaudioside A via the diet reported no adverse effects at any of the dietary concentrations tested. In a 13-week repeat dose toxicity study, the no-observed-adverse-effect level (NOAEL) corresponded to steviol doses of 1370 mg/kg bw/day for males and 1530 mg/kg bw/day for females (Curry and Roberts 2008) while a two-generation reproductive toxicity study in rats resulted in a NOAEL of 674 mg/kg bw/day for males and 748 mg/kg bw/day for females, expressed as steviol (Curry et al. 2008). The NOAEL values obtained in the repeat dose toxicity and reproductive toxicity studies on rebaudioside A are greater than the NOAEL of 383 mg/kg bw/day (expressed as steviol equivalents) from a 2-year rat study on stevioside that was used to derive the steviol glycosides ADI of 0-4 mg/kg bw, expressed as steviol equivalents (FSANZ 2008).

A published paper described three *in vitro* and two *in vivo* genotoxicity studies on rebaudioside A. No mutagenic or clastogenic activity was evident in these assays. As discussed in previous assessments by FSANZ, JECFA and EFSA, the weight of evidence from an extensive database indicates that steviol glycosides are unlikely to be genotoxic.

A randomised, double-blind, cross-over study assessed the pharmacokinetics of steviol and steviol glucuronide following single oral doses of rebaudioside A and stevioside in healthy adult males (Wheeler et al. 2008). Rebaudioside A and stevioside underwent similar metabolic and elimination pathways with steviol glucuronide excreted primarily in the urine and steviol in the faeces.

Two other human studies published in 2008 reported no adverse findings attributable to rebaudioside A. These studies were randomised, double-blind, placebo-controlled trials. Four weeks consumption of 1000 mg/day rebaudioside A did not significantly affect blood pressure (seated and 24 h ambulatory) in individuals with normal and low-normal systolic blood pressure and diastolic blood pressure (Maki et al. 2008a). The mean rebaudioside A dose was equivalent to 4.6 mg/kg bw/day, expressed as steviol. When blood pressure was measured during meal tests (supine pre-meal and standing post-meal), statistically significant ($p < 0.05$) differences between the rebaudioside A and placebo groups in diastolic blood pressure and mean arterial pressure were observed. However, the differences were small (<3 mmHg) and it is possible that these statistically significant differences arise by chance due to the large number of statistical comparisons undertaken in the study.
In a trial on individuals with type 2 diabetes, 16 weeks consumption of rebaudioside A at

1000 mg/day (3.4 mg/kg bw/day steviol equivalents) did not significantly affect any of the study endpoints (e.g. bodyweight, glycosylated haemoglobin, fasting glucose, insulin, blood pressure, fasting lipids) (Maki et al. 2008b). A lack of adverse findings for rebaudioside A in this study and in the above blood pressure study is consistent with the results of human studies conducted with other preparations of steviol glycosides. Those studies were evaluated as part of the original hazard assessment conducted by FSANZ which concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 4 mg/kg bw/day, expressed as steviol equivalents (FSANZ 2008).

3.4 Conclusion

The additional published toxicokinetics, metabolism, toxicity, and human data on steviol glycosides adds to the extensive database available for the hazard assessment of steviol glycosides. There were no findings in these publications which would indicate a need to change the ADI of 0-4 mg/kg bw, expressed as steviol equivalents, which was established by FSANZ in 2008.

4. Dietary Exposure Assessment

4.1 Approach to estimating dietary exposure to steviol glycosides

Dietary exposure assessments (DEAs) require data on concentrations of the chemical of interest in food and food consumption data. The approach for this DEA was to use chemical concentration data as listed in Schedule 1 of Standard 1.3.1 and the amendments proposed by the Applicant, which included higher permissions for some foods with existing permissions. This is then combined with food consumption data available from the most recent Australian and New Zealand national nutrition surveys. The dietary exposure assessment was conducted using FSANZ's customised dietary modelling computer program, DIAMOND.

A summary of the FSANZ approach to conducting dietary exposure assessments is at Appendix 1. A detailed discussion of the FSANZ methodology and approach to conducting dietary exposure assessments is set out in the *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

4.1.1 Proposed foods and concentration data used

Steviol glycosides are currently permitted to be used in numerous food categories in Schedule 1 to Standard 1.3.1 – Food Additives. They can be used in a range of foods and beverages that contain sugar, and can either be used in conjunction with, or as a replacement for, sugar or other intense sweeteners. The Applicant has requested an increase in the maximum permitted levels (MPLs) for steviol glycosides for some foods with existing permissions in Australia and New Zealand as shown in Table 4.1.

For the purposes of estimating dietary exposure to steviol glycosides, the food categories with existing permissions for steviol glycosides were assigned to DIAMOND food additive classification codes. These DIAMOND codes are based on the food additive classification system for Australia and New Zealand used in Schedule 1 of Standard 1.3.1 – Food Additives.

The proposed increased MPLs were included in the DEA along with the other existing MPLs for steviol glycosides as listed in Schedule 1 of Standard 1.3.1. In the absence of more specific data on actual use levels or actual concentrations of steviol glycosides in foods, MPLs were used. This is likely to lead to an overestimate in dietary exposure because in reality the maximum level may not be used, or they may not be used in all food categories. The concentrations of steviol glycosides for each food group used in the dietary exposure assessment are set out in Table 4.1.

TABLE 4.1: SUMMARY OF PERMITTED AND PROPOSED FOOD CATEGORIES, CORRESPONDING DIAMOND FOOD CLASSIFICATION AND STEVIOL GLYCOSIDES CONCENTRATION USED FOR DIETARY EXPOSURE ASSESSMENT.

DIAMOND Food Code	Food Name	Steviol glycosides[#] concentration (mg/kg)*
1.1.2	Liquid milk products and flavoured liquid milk	115
1.2.2	Fermented milk products and rennetted milk products	176
3	Ice cream & edible ices	**200**[~]
3.1	Ice confection sold in liquid form	115
	Ice cream & ice confection reduced & low fat	208
4.3.2	Fruits & vegetables in vinegar/oil/brine/alcohol	160
4.3.4.2	Low joule chutneys, jams & spreads	450
4.3.6	Fruit & vegetable preparations including pulp	208
5.1	Chocolate & cocoa products	550
5.2	Sugar confectionery	1100
6.3	Processed cereal & meal products	250
7.1.4	Fancy breads	160
7.2	Biscuits, crackers, cakes, pastries & scones	160
11.4	Tabletop sweeteners	400000^
13.3	Formula meal replacements & formulated supplementary foods	175
13.4	Formulated supplementary sports foods	175
14.1.2.1	Fruit & vegetable juices	50
14.1.2.2.2	Low joule fruit & vegetable juice products	125
14.1.3	Water based flavoured drinks	**200**[~]
14.1.4	Formulated beverages	**200**[~]
14.1.5	Coffee (or substitute), tea, herbal infusion & similar products	100
14.1.7.1	Soy beverage, unflavoured	**100**[~]
14.1.7.2	Soy beverage, flavoured	**200**[~]
20.2.1.1	Desserts, dairy [except ice cream]	150
20.2.1.1.1.2	Custard mix, custard powder & blancmange mix/powder	80
20.2.1.2	Desserts, no-dairy	150
20.2.1.2.3.1	Jelly	260
20.2.4.1	Snack foods, dairy or fat based	150
20.2.6.1	Sauces & syrups, sweet	320
20.2.6.2	Gravy, sauces & condiments	320
20.2.6.3.1	Dips, dairy or fat based	150
20.2.6.3.3	Spreads, dairy or fat based	320
20.2.7	Mayonnaise & salad dressings	320

[#] Expressed as steviol equivalents.

* Steviol glycosides concentration data as listed in Schedule 1 of Standard 1.3.1 and the amendments proposed by the Applicant.

[~] Changes proposed in this Application - A1037. Existing permissions for these foods are as follows: ice cream and edible ices 64 mg/kg; water based flavoured drinks 160 mg/kg; formulated beverages 160 mg/kg, soy beverage, unflavoured 65 mg/kg; soy beverage, flavoured 175 mg/kg.

^ Concentration value used as a proxy for GMP (Good Manufacturing Practice) permission.

4.1.2 Scenario for Dietary exposure assessment

The dietary exposure assessment for steviol glycosides was undertaken based on two main assumptions:

- Firstly, that for foods groups where there was a permission or a request to add a higher concentration of steviol glycosides, all foods in that group were assumed to contain steviol glycosides at maximum levels specified, and
- Secondly, that steviol glycosides at its maximum use level would only attain a 30% uptake of the market for all intense sweeteners available. Due to the limitations of the national nutrition survey data and DIAMOND, modelling for 30% market uptake was achieved by using 30% of the MPL as a proxy (achieved by multiplying the concentration assigned to the food group (mg/kg of steviol glycosides) by 0.3).

An exception to these assumptions was made for the food group 'coffee (or substitute), tea, herbal infusion and similar products', where instant coffee, ground coffee from beans and loose leaf tea and tea bags were excluded from the DEA. Assuming that these products (which are highly consumed by adult population groups) contain steviol glycosides is unrealistic and likely to skew the results of the assessment. The use of tabletop sweeteners in tea and coffee, where reported in the national nutrition surveys, is accounted for in category 11.4 – tabletop sweeteners. Similarly, while MPLs for steviol glycosides apply to all liquid milk products and flavoured liquid milk, this assessment excluded non-flavoured milk products, assuming high intensity sweeteners are not added to these products. These assumptions were included in the DEA, because including beverages on a broader level may affect the more realistic calculation of the major contributors to steviol glycosides.

Assuming that all foods contain the maximum permitted concentration of steviol glycosides, where a permission exists, grossly overestimates dietary exposure due to the broad range of foods with permissions and the assumption that no other intense sweeteners are used. While a 30% uptake of the market for steviol glycosides was considered as appropriate for the purpose of this assessment, it may be argued that this scenario results in a very protective overestimation of dietary exposure. This assumption discounts the use of any other sweeteners currently available in the market. In reality, there may be a 30% uptake of all sweeteners in the market (where permitted) of which steviol glycosides makes up a proportion of this 30%.

This scenario is consistent with FSANZ's previous DEA for A540 steviol glycosides which was based on JECFA estimates at its 63rd meeting that the dietary exposures to steviol glycosides would likely be 20-30% of total sugar replacement in foods (WHO 2005, 2006). However, it should be noted that the JECFA 20-30% assumption is based on total sugar replacement, while the FSANZ scenario assumes this is a 30% market uptake. The subsequent JECFA dietary exposure estimate (WHO 2009) considered the use of steviol glycosides in similar food categories to its original assessment, however most of them were at a much higher use level than those proposed in Table 4.1 for Australia and New Zealand.

4.1.3 Food consumption data used

Food additive permissions contained in the Code apply to food produced or sold in both Australia and New Zealand. Therefore this dietary exposure assessment has been conducted for both countries.

Food consumption data used for these assessments include:

- 2007 Australian National Children's Nutrition and Physical Activity Survey (also known as 'Kids Eat Kids Play') (2007 AusNNS), two non-consecutive 24 hour food recall surveys covering 4,487 Australian children aged 2-16 years.
- 1995 Australian National Nutrition Survey (1995 AusNNS), one 24 hour food recall survey covering 13,858 Australians aged 2 years and above.
- 2002 New Zealand National Nutrition Survey (2002 NZNNS), one 24 hour food recall survey covering 3,275 New Zealanders aged 5-14 years.
- 1997 New Zealand National Nutrition Survey (1997 NZNNS), one 24 hour food recall survey covering 4,636 New Zealanders aged 15 years and above.

The design of these surveys varies somewhat and key attributes of each are set out in Appendix 1. Also note that NNS data have limitations in predicting patterns of consumption. A detailed discussion of these limitations is included in Section 6 of the *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

A general limitation of national nutrition surveys which is relevant to this dietary exposure assessment is that it is not possible to predict consumer behaviour in relation to new products in the market. Specifically, it is not possible to predict the uptake of steviol glycosides by manufacturers and the consequent consumption of steviol glycoside sweetened products by consumers. This limitation may be compounded where there is an element of 'brand loyalty' in consumers' food selection, for example, always choosing the same brand of artificially sweetened soft drink. In recognition of this limitation, conservative 'worst case' scenarios and assumptions are also evaluated as part of the DEA (see Section 4.1.5, below).

4.1.4 Population group assessed

The hazard assessment (Section 3) did not identify any population sub-groups for which there were specific safety considerations in relation to steviol glycosides. Therefore, the population groups selected for the DEA were matched with the most recent food consumption data available. The sub groups included in this assessment were:

- Australian populations aged 2-6 years, 7-16 years, and 17 years and above
- New Zealand populations aged 5-14 years and 15 years and above.

Children aged 2-6 years are included as they have the highest food intake on a per kilogram body weight basis, due to their lower body weights and proportionally higher energy needs as they are growing and developing (FSANZ 2009, Section 4.3).

4.1.5 Assumptions in the Dietary Exposure Assessment

Assumptions made in the dietary exposure assessment are:

- Where permission for steviol glycosides was given to a food classification code, every food in that group contained steviol glycosides, except for instant coffee, ground coffee, tea from loose leaf or tea bags, and non-flavoured milk which were assumed not to contain steviol glycosides
- Unless otherwise specified, 30% of foods with a permission to add steviol glycosides actually contain them at their respective maximum permitted levels as specified in Table 4.1, above (30% market uptake). This was modelled by assuming that all foods with permission to contain steviol glycosides contain them at 30% of the maximum permitted level

- There are no other intense sweeteners in the market
- Where a food was not included in the exposure assessment, it was assumed to contain a zero concentration of steviol glycosides
- Where a food has a specified steviol glycosides concentration, this concentration is carried over to mixed foods where the food has been used as an ingredient
- There are no reductions in steviol glycosides concentrations from food preparation or due to cooking
- There are no other contributions to steviol glycosides dietary exposure, for example through the use of medicines.

These assumptions, particularly the first two listed, are likely to lead to a considerable over-estimate for steviol glycosides dietary exposure, as together they assume that every food in every specified food category contains steviol glycosides at 30% of the MPL as a proxy for market share. For example, there is a permission for fruit and vegetable juices to contain steviol glycosides to a MPL of 50 mg/kg. Products in this category include fresh, sweetened, fortified and concentrated, pure and blended fruit and vegetable juices. Only the sweetened versions of these products are likely to contain steviol glycosides. However, for the purposes of the dietary exposure assessment, every juice in this category is assumed to contain steviol glycosides.

4.2 Estimated dietary exposure to steviol glycosides for Australia and New Zealand

DEA results for steviol glycosides were calculated for 'consumers' only, that is, those people in each NNS who reported consuming foods permitted to containing the additive. Population statistics (mean and 90th percentile estimated exposure, on a milligrams per day basis) for each population group assessed were derived from each individual's exposures. Exposures were derived on a per kilogram body weight basis, using each individual's body weight, and reported as a proportion of the ADI. Major dietary contributors to the total intake of steviol glycosides were also calculated for each population group assessed.

4.2.1 Dietary exposure estimates for each population group assessed

The proportion of consumers of steviol glycosides to all survey respondents was almost 100% for all population groups assessed. This is because of the large range of food products that have existing permissions in the Code to add steviol glycosides.

Using the 30% market uptake scenario, based on the MPLs specified in Table 4.1, the estimated exposures were highest for Australian children aged 2-6 years at 1.5 mg/kg bw/day and 2.5 mg/kg bw/day for mean and 90th percentile, respectively. They were followed by New Zealand children aged 5-14 years at approximately 1 mg/kg bw/day and 2 mg/kg bw/day for mean and 90th percentile estimated exposure respectively.

Compared with the reference health standard of 4 mg/kg bw/day, for a 30% market uptake scenario, estimated mean and 90th percentile dietary exposures were less than 60% of the ADI for all population groups assessed. Estimated mean and 90th percentile exposures for steviol glycosides as a percentage of the ADI were higher for children compared to adults, which is expected as a result of their lower body weight ratio compared to food consumption. These results are summarised in Figure 4.1 below, with detailed results set out in Tables A2.1 and A2.2 of Appendix 2.

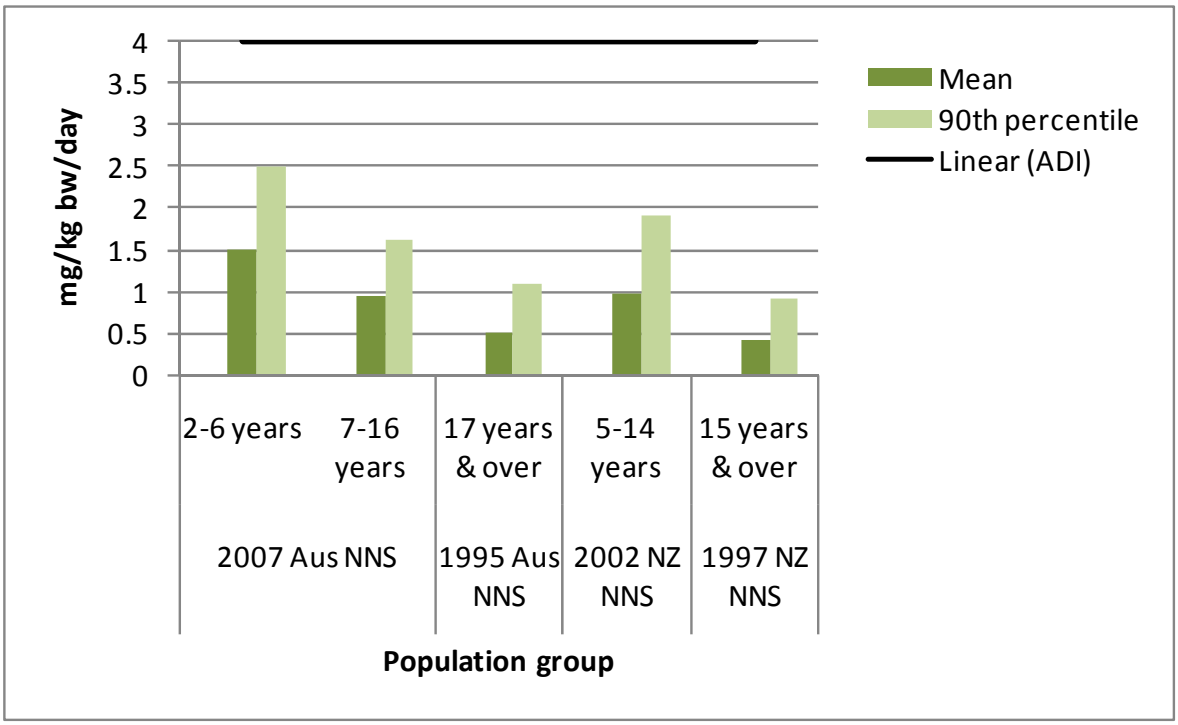


FIGURE 4.1: MEAN AND 90TH PERCENTILE ESTIMATED DIETARY EXPOSURE TO STEVIOL GLYCOSIDES FOR ALL POPULATION GROUPS ASSESSED COMPARED TO THE ACCEPTABLE DAILY INTAKE (4 MG/KG BW/DAY) FOR THE 30% MARKET UPTAKE SCENARIO

4.2.2 Major foods contributing to steviol glycosides exposure

Major foods contributing to steviol glycosides dietary exposure were calculated from consumers' total dietary exposure from foods included in the DEA. Given the inclusion of broad food groups in the assessment, these data should be interpreted with caution. For example, for the purposes of the DEA, in line with current permissions for steviol glycosides in the Code, all flavoured milk products and water based flavoured drinks (soft drinks, cordials, formulated beverages) were included in the assessment, where in reality, not all products in these groups will contain steviol glycosides. Most beverages included in this assessment were predicted to be major contributors to steviol glycosides exposures, most likely due to the large volume of these products consumed. Flavoured milk products are a major contributor to steviol glycosides exposure for children, but not for adults, because children may consume more milk products compared to adults. Similarly, including all water based flavoured drinks in the assessment make this food group the major contributor of steviol glycosides in adults. Water based flavoured drinks were also a major contributor for children second to flavoured milk products. As the assessments are done separately for adults and children, the major contributors fluctuate in line with the general consumption pattern of the population group assessed.

For the purposes of calculating the major food groups predicted to contribute to steviol glycosides exposure, similar food groups set out in Table 4.1 were combined. For example all food codes relating to ice cream were combined under the group 'ice cream & edible ices'.

Water based flavoured drinks (soft drinks, cordials, formulated beverages) were the major contributor to total steviol glycosides exposure for all the population groups assessed except for the Australian children aged 2-6 years ranging from 36% (Australian children aged7-16 years) to 41% (New Zealand children aged 5-14 years). The greatest contributors to total

steviol glycosides exposure for Australian children aged 2-6 years were flavoured milk products including yoghurt (21%) followed by water based flavoured drinks (19%). In addition to water based flavoured drinks, tabletop sweeteners were also major contributors for the older age groups; Australians aged 17 years and above (20%) and the New Zealand population aged 15 years and above (22%). Other food groups including flavoured milk products, confectionary, sauces & toppings and fruit & vegetable juice products were also a major contributor for different population groups assessed reflecting their consumption pattern.

The major contributors (≥5%) are shown in Figure 4.2 for Australians aged 2-6 years, Figure 4.3 for Australians aged 7-16 years, Figure 4.4 for Australians aged 17 years and above, Figure 4.5 for New Zealanders aged 5-14 years and Figure 4.6 for New Zealanders aged 15 years and above. A detailed list of all the food groups and their contributions to steviol glycosides dietary exposure can be found in Table A2.3 of Appendix 2.

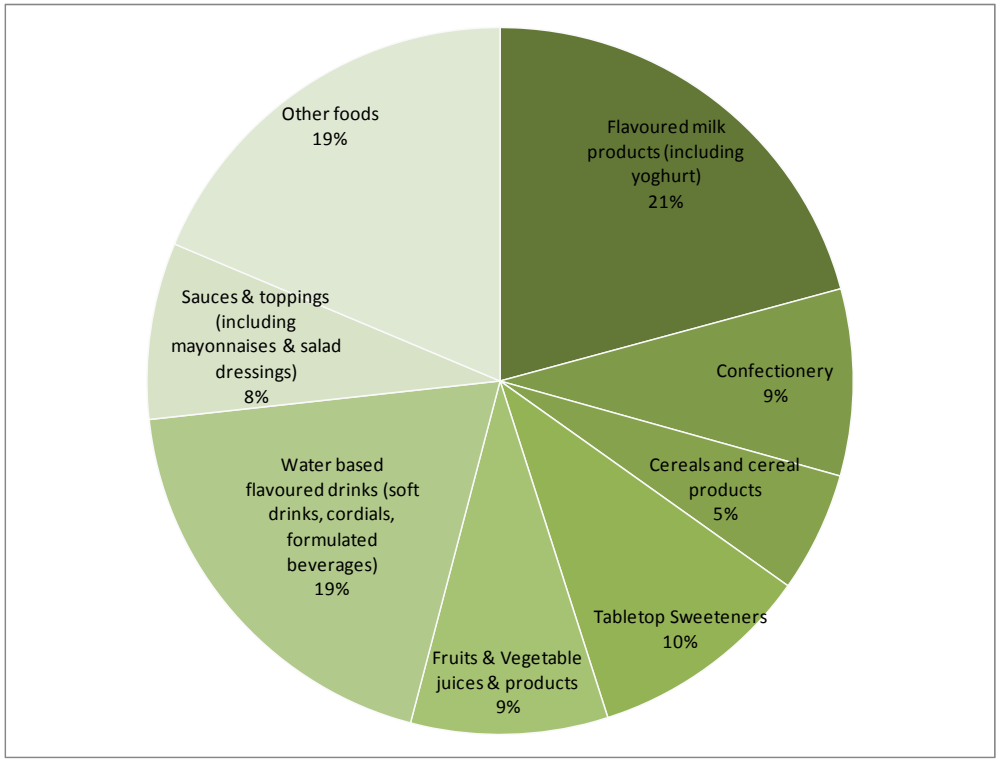


FIGURE 4.2: MAJOR CONTRIBUTORS TO ESTIMATED STEVIOL GLYCOSIDES DIETARY EXPOSURE FOR AUSTRALIAN CHILDREN AGED 2-6 YEARS

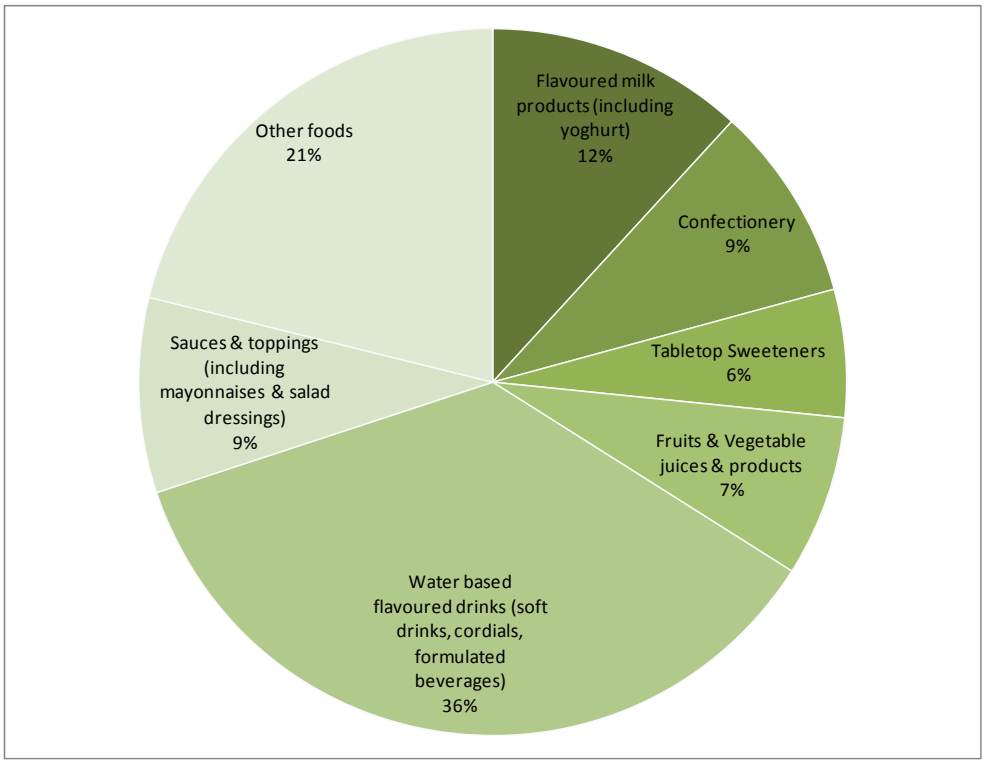


FIGURE 4.3: MAJOR CONTRIBUTORS TO ESTIMATED STEVIOL GLYCOSIDES DIETARY EXPOSURE FOR AUSTRALIAN CHILDREN AGED 7-16 YEARS

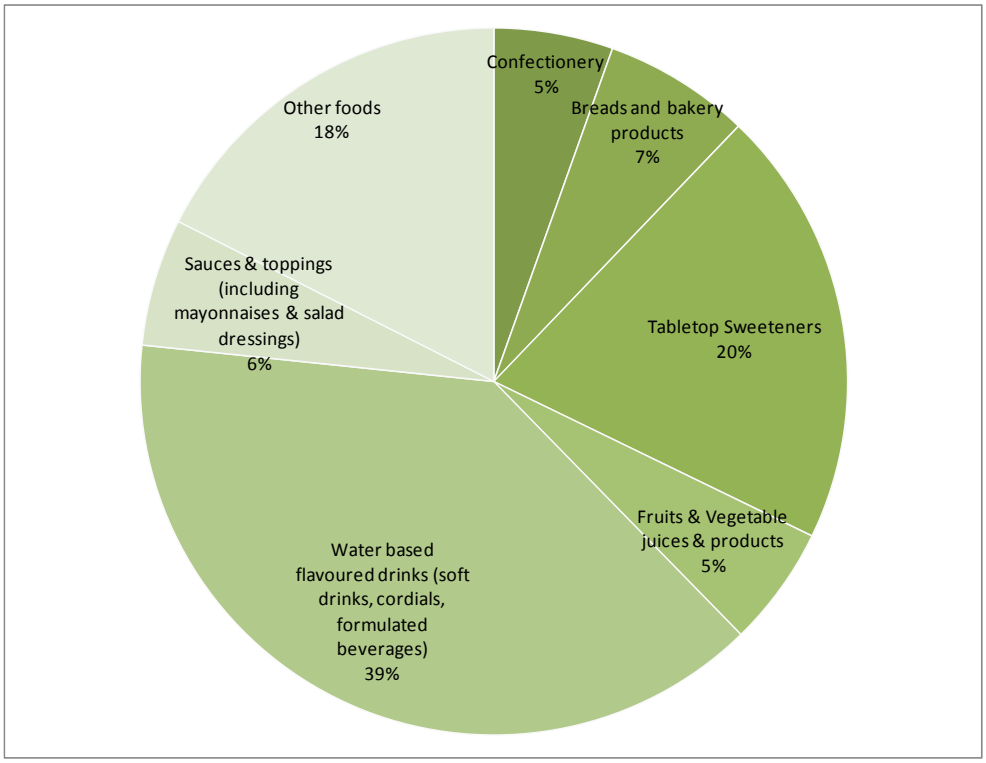


FIGURE 4.4: MAJOR CONTRIBUTORS TO ESTIMATED STEVIOL GLYCOSIDES DIETARY EXPOSURE FOR THE AUSTRALIAN POPULATION AGED 17 YEARS AND OVER

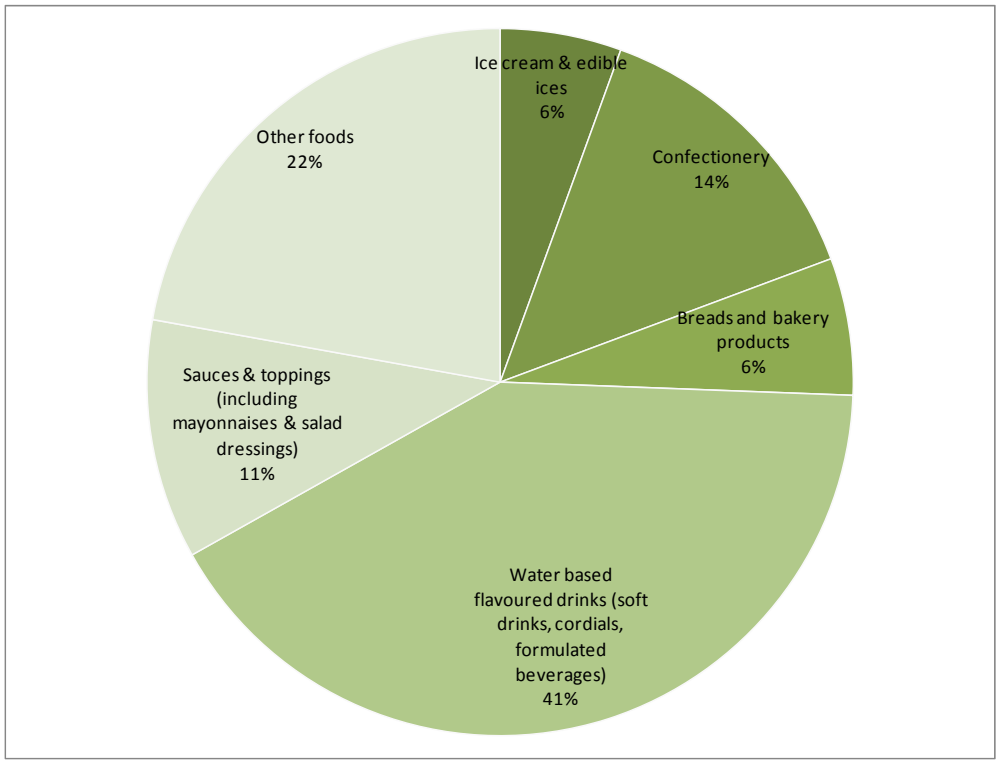


FIGURE 4.5: MAJOR CONTRIBUTORS TO ESTIMATED STEVIOL GLYCOSIDES DIETARY EXPOSURE FOR THE NEW ZEALAND POPULATION AGED 5-14 YEARS

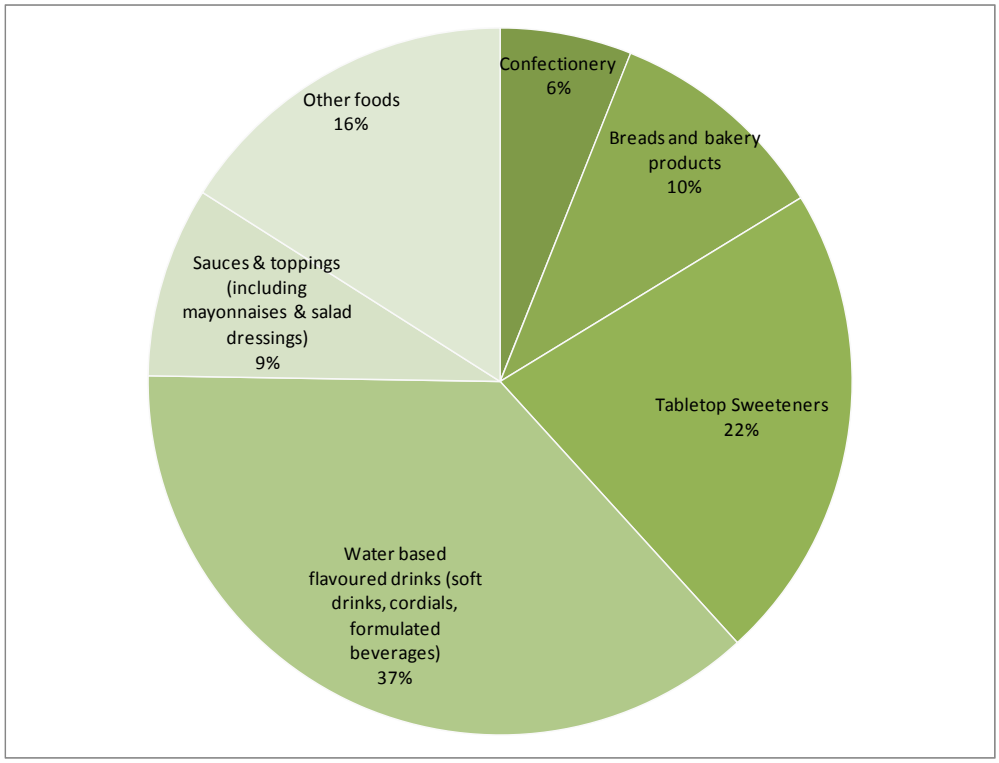


FIGURE 4.6: MAJOR CONTRIBUTORS TO ESTIMATED STEVIOL GLYCOSIDES DIETARY EXPOSURE FOR THE NEW ZEALAND POPULATION AGED 15 YEARS AND OVER

4.3 'Consumer behaviour' scenarios

Given the limitations of the data available to conduct the dietary exposure assessment, it is not possible to predict consumers' preferences and behaviours in relation to food selection. For example, would consumers of intense sweetened products replace all other sweeteners in their diets, across all food groups, substituting steviol glycosides sweetened products? The '30% market uptake' scenario used in this assessment takes consumer behaviour into account to a certain extent by broadly representing consumer selection of steviol glycoside sweetened foods 30% of the time. However, a 30% market uptake assumption may not be justified as a best estimate, particularly for 'brand loyal' consumers who may always choose the same product within a food category which may contain steviol glycosides up to the MPL. This assessment indicated that water based flavoured drinks (soft drinks, cordials, formulated beverages) were the major contributor to steviol glycosides dietary exposure for all population groups assessed except Australian children aged 2-6 years, for which flavoured milk products (including yoghurt) were the major contributor. In addition, recent market share data for Australia (Retail World's Australasian Grocery Guide, 2009) indicates that the proportion of people consuming water based flavoured drinks containing intense sweeteners is increasing compared with those consuming sugar sweetened drinks, up to approximately 35% in 2009. Therefore, both water based flavoured drinks (soft drinks, cordials, formulated beverages) and flavoured milk products were further investigated to predict potential exposures for brand loyal consumers.

In order to provide an indication of the potential estimated dietary exposure for 'brand loyal' consumers of water based flavoured drinks (soft drinks, cordials, formulated beverages) a further scenario was considered; a 30% market uptake was used for all food categories except for category 14.1.3 - Water based flavoured drinks, which was assumed to contain 100% of the MPL of steviol glycosides, as proposed by the applicant.

Estimated mean and 90th percentile dietary exposures to steviol glycosides using this 'consumer behaviour' scenario were approximately 55% and 110% of the ADI, respectively, for Australian children aged 2-6 years and 45% and 100% of the ADI, respectively, for New Zealand children aged 5-14 years. For all other population groups assessed estimated mean and 90th percentile exposures were less than or equal to 85% of the ADI. Detailed results are provided in Table A2.4 of Appendix 2.

Similarly, flavoured milk products (including yoghurt), as the highest contributor to steviol glycosides exposure for Australian children aged 2-6 years, were also investigated. The assessment was undertaken by assuming a 30% market uptake scenario for all other food categories and 100% uptake for the flavoured milk products. Using this scenario, estimated mean and 90th percentile dietary exposures for Australians aged 2-6 years was 55% and 100% of the ADI respectively. Estimated mean and 90th percentile dietary exposures for Australian children aged 7-16 and New Zealand children aged 5-14 were all less than 55% of the ADI. Given that flavoured milk products were not major contributors for any of the adult population groups (based on the 30% market share scenario) these groups were not further analysed. Detailed results are provided in Table A2.4 of Appendix 2.

As with the 30% market uptake scenario, each 'consumer behaviour' scenario is based on very protective assumptions that are likely to lead to a considerable overestimate of dietary exposure. In addition to the underlying assumption that 30% of all other foods, where permitted, contain steviol glycosides at the maximum permitted level for that food[1], each scenario assumed all water based flavoured beverages, or flavoured milk product (including yoghurt) contained the MPL of the sweetener and was always selected by the consumer.

[1] Modelled by assuming that all other foods, where permitted, contain steviol glycosides at 30% of their respective maximum permitted levels.

These ‘consumer behaviour’ models also discount the use of any other sweetener in the market.

4.4 Summary of JECFA estimated dietary exposure to steviol glycosides

Dietary exposure assessments for steviol glycosides were undertaken by JECFA at its 63rd and 69th meetings. The 69th JECFA dietary exposure estimate was a further elaboration from that done for its 63rd meeting and considered three estimate types for the assessment. This JECFA assessment considered similar food categories included in the FSANZ DEA for steviol glycosides, most of them at a much higher use level than that proposed in Table 4.1 for Australia and New Zealand

The 69th JECFA exposure estimates for steviol glycosides ranged between 1-5 mg/kg bw/day for the 13 national diet clusters considered. For these estimates, it was assumed that steviol glycosides would replace all sweeteners used in or as food, at a ratio of 200:1 based on equivalent sweetness. However, it was concluded that this assumption was highly conservative and likely exposures would be 20-30% of these values, that is, no more than 2 mg/kg/bw/day (WHO 2009).

4.5 Conclusion

The DEA for steviol glycosides, based on a 30% market uptake scenario for broad food groups at maximum levels specified, indicated that estimated dietary exposures to steviol glycosides were less than 60% of the ADI for both mean and 90th percentile exposures for all population groups assessed.

Overall the DEA indicated that water based flavoured drinks (soft drinks, cordials, formulated beverages) were the major contributor for all the population groups assessed, except for Australian children aged 2-6 years, ranging from 36% (Australian children aged 7-16 years) to 41% (New Zealand children aged 5-14 years) of total steviol glycosides exposure. The greatest contributors to total steviol glycosides exposure for Australian children aged 2-6 years were flavoured milk products (21%) followed by water based flavoured drinks (19%). In addition to water based flavoured drinks, tabletop sweeteners were also major contributors for Australians aged 17 years and above (20%) and the New Zealand population aged 15 years and above (22%).

Using a scenario to represent ‘brand loyal’ consumers of water based flavoured drinks, 90th percentile estimated dietary exposures were 110% of the ADI for Australian children aged 2-6 years and 100% of the ADI for New Zealand children aged 5-14 years.

A further scenario considered ‘brand loyal’ consumers of flavoured milk products (including yoghurt), the highest contributor to steviol glycosides exposure for Australian children aged 2-6 years. This scenario predicted that estimated mean and 90th percentile dietary exposures for Australian children aged 2-6 years were approximately 55% and 100% of the ADI, respectively.

However, it should be noted that the 30% market uptake scenario and subsequent ‘brand loyal’ consumer scenarios are based on broadly protective assumptions that are likely to lead to a considerable overestimation of dietary exposure.

JECFA exposure estimates for steviol glycosides ranged between 1-5 mg/kg bw/day and the likely exposures were predicted to be 20-30% of this, that is, no more than 2 mg/kg bw/day (WHO 2009). Even though the steviol glycosides use levels and the overall DEA approach

based on the consumption data available varied between both FSANZ and JECFA exposure assessments, the overall results were comparable.

5. Risk Characterisation

The updated hazard assessment resulted in no findings which would indicate a need to change the ADI of 0-4 mg/kg bw, expressed as steviol equivalents, which was established by FSANZ in 2008.

Assuming a 30% market uptake of steviol glycosides, the dietary exposure assessment indicated that for all groups of Australian and New Zealand consumers assessed (including children), estimated mean and 90th percentile dietary exposures were less than 60% of the ADI for all population groups assessed.

Using a scenario to represent 'brand loyal' consumers of water based flavoured drinks, 90th percentile estimated dietary exposures were 110% of the ADI for Australian children aged 2-6 years and 100% of the ADI for New Zealand children aged 5-14 years. A further scenario considered 'brand loyal' consumers of flavoured milk products (including yoghurt), the highest contributor to steviol glycosides exposure for Australian children aged 2-6 years. This scenario predicted that estimated mean and 90th percentile dietary exposures for Australian children aged 2-6 years were approximately 55% and 100% of the ADI, respectively.

However, it should be noted that the 30% market uptake scenario and subsequent 'brand loyal' consumer scenarios are based on broadly protective assumptions that are likely to lead to a considerable overestimation of dietary exposure. Dietary exposure was estimated to slightly exceed the ADI in one population group, however due to the conservative assumptions in the dietary exposure calculations, it is concluded that there no public health and safety concerns associated with the proposed increases in the maximum permitted levels in ice cream and certain beverages.

6. Conclusions

The proposed increases in the maximum permitted levels of steviol glycosides in ice cream and selected beverages are technologically justified and supported by taste trials as providing a more acceptable taste profile to consumers.

Toxicological and other relevant data published subsequent to the original FSANZ assessment raise no concerns regarding the safety of steviol glycosides and do not indicate a need to change the existing ADI of 0-4 mg/kg bw/day, expressed as steviol equivalents.

Dietary exposure assessment, based on a 30% market uptake scenario for broad food groups at maximum levels specified, indicated that estimated dietary exposures to steviol glycosides were less than 60% of the ADI for both mean and 90th percentile exposures for all population groups assessed, including children.

Using a scenario to represent 'brand loyal' consumers of water based flavoured drinks, 90th percentile estimated dietary exposures were 110% of the ADI for Australian children aged 2-6 years and 100% of the ADI for New Zealand children aged 5-14 years. A further scenario considered 'brand loyal' consumers of flavoured milk products (including yoghurt) which are the highest contributor to steviol glycosides exposure for Australian children aged 2-6 years. This scenario predicted that estimated mean and 90th percentile dietary exposures for Australian children aged 2-6 years were approximately 55% and 100% of the ADI, respectively. However, the 30% market uptake scenario and subsequent 'brand loyal' consumer scenarios are based on broadly protective assumptions that are likely to lead to a considerable overestimation of dietary exposure. On this basis, there are no public health and safety concerns associated with the proposed increases in the maximum permitted levels in ice cream and certain beverages.

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Appendices

Appendix 1: Dietary Exposure Assessments at FSANZ

Appendix 2: Dietary Exposure Assessments of Steviol Glycosides

Appendix 1: Dietary Exposure Assessments at FSANZ

A dietary exposure assessment is the process of estimating how much of a food chemical a population, or population sub group, consumes. Dietary exposure to (or intake of) food chemicals is estimated by combining food consumption data with food chemical concentration data. The process of doing this is called 'dietary modelling'.

Dietary exposure = food chemical concentration x food consumption

FSANZ's approach to dietary modelling is based on internationally accepted procedures for estimating dietary exposure to food chemicals. Different dietary modelling approaches may be used depending on the assessment, the type of food chemical, the data available and the risk assessment questions to be answered. In the majority of assessments FSANZ uses the food consumption data from each person in the national nutrition surveys to estimate their individual dietary exposure. Population summary statistics such as the mean exposure or a high percentile exposure are derived from each individual person's exposure.

An overview of how dietary exposure assessments are conducted and their place in the FSANZ Risk Analysis Process is provided on the FSANZ website at:
http://www.foodstandards.gov.au/scienceandeducation/scienceinfsanz/dietaryexposureassessmentsatfsanz/dietaryexposureandin4438.cfm

FSANZ has developed a custom built computer program 'DIAMOND' to calculate dietary exposures. More information on DIAMOND is available on the FSANZ website at:
http://www.foodstandards.gov.au/scienceandeducation/scienceinfsanz/dietaryexposureassessmentsatfsanz/fsanzdietaryexposure4439.cfm

Further detailed information on the principles and practices of conducting dietary exposure assessments at FSANZ is provided in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ, 2009), available at:
http://www.foodstandards.gov.au/_srcfiles/Principles%20&%20practices%20exposure%20assessment%202009.pdf

A1.1 Food consumption data used

The most recent food consumption data available were used to estimate exposures to steviol glycosides for the Australian and New Zealand populations. The national nutrition survey (NNS) data used for these assessments were:

- The 2007 Australian National Children's Nutrition and Physical Activity Survey (also known as '*Kids Eat Kids Play*') (2007 AusNNS)
- The 1995 Australian National Nutrition Survey (1995 AusNNS)
- The 1997 New Zealand National Nutrition Survey (1997 NZNNS)
- The 2002 New Zealand National Nutrition Survey (2002 NZNNS)

The design of each of these surveys varies somewhat and key attributes of each are set out below.

A1.1.1 2007 Australian Children's Nutrition & Physical Activity Survey (2007 AusNNS)

The 2007 AusNNS collected data on nutrition and physical activity for 4,487 children aged 2-16 years across Australia. The survey was conducted over a seven month time period, from February to August 2007.

In contrast to other national nutrition surveys used to date by FSANZ (the 1995 Australian and 1997 New Zealand surveys), in the 2007 AusNNS each respondent completed two 24-hour recalls on non-consecutive days. The availability of two days of food consumption data provides a more realistic estimate of long term consumption of infrequently consumed foods, because it takes account of those who may eat a food on one day of the survey but not on the other. Using one 24-hour recall may capture an unusual eating occasion for an individual that does not describe how they normally eat.

In this assessment, exposure to steviol glycosides was estimated from each consumer's average exposures from foods containing steviol glycosides across Day 1 and Day 2. The results of the 2007 AusNNS were weighted to represent the overall population of Australian children because stratified sampling with non-proportional samples was used.

A1.1.2 1995 Australian National Nutrition Survey (1995 AusNNS)

The 1995 AusNNS provides comprehensive information on dietary patterns of a sample of 13,858 Australians aged from 2 years and above (McLennan & Podger 1998). It is the most recent NNS for Australians aged 17 years and above. The survey used a 24-hour recall method for all respondents, with 10% of respondents also completing a second 24-hour recall on a second, non-consecutive day. The data were collected over a 13 month period. Food frequency data are available for a subset of the national sample (respondents aged 12 years and above) as are responses to a series of short dietary questions about food habits. These data are used unweighted in DIAMOND.

A1.1.3 1997 New Zealand National Nutrition Survey (1997 NZNNS)

The 1997 NZNNS provides comprehensive information on the dietary patterns of a sample of 4,636 respondents aged from 15 years and above. The survey was conducted on a stratified sample over a 12 month period. The survey used a 24-hour recall methodology with 15% of respondents also completing a second 24-hour recall with an additional food frequency questionnaire and questions on food consumption patterns. These data are used unweighted in DIAMOND.

A1.1.4 - 2002 New Zealand National Nutrition Survey (2002 NZNNS)

The 2002 New Zealand Children's National Nutrition Survey provides comprehensive information on the dietary patterns of a nationally representative sample of 3,275 New Zealand children aged 5-14 years, including sufficient numbers of children in the Mäori and Pacific groups to enable ethnic-specific analyses. The survey was conducted using a 24-hour recall methodology and collected data on dietary supplements as well as foods and beverages. A repeat 24-hour diet recall was obtained from a subsample, which enabled the statistical adjustment of the data to present the 'usual' intake distribution for nutrients by subgroup.

Further information on how the Australian National Nutrition Surveys are used to conduct dietary exposure assessments is available on the FSANZ website at:
http://www.foodstandards.gov.au/scienceandeducation/scienceinfsanz/dietaryexposureassessmentsatfsanz/foodconsumptiondatau4440.cfm

Further information on the New Zealand National Nutrition Surveys is available from the New Zealand Ministry of Health website at:
http://www.moh.govt.nz/moh.nsf/indexmh/dataandstatistics-subjects-nutrition

A1.2 Limitations of dietary exposure assessments

Dietary exposure assessments based on the most recently available food consumption data provides the best estimate of actual consumption of a food and the resulting estimated dietary exposure for the Australian New Zealand populations. However, it should be noted that NNS data do have limitations. Further details of the limitations relating to dietary exposure assessments undertaken by FSANZ are set out in the FSANZ document, *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

Appendix 2: Dietary Exposure Assessments of Steviol Glycosides

TABLE A2.1: ESTIMATED DIETARY EXPOSURES TO STEVIOL GLYCOSIDES BASED ON THE 30% MARKET UPTAKE SCENARIO

Survey	Age group	Consumers as a % of Respondents	Dietary Exposure for 30% market uptake (mg/day)	
			Mean	90th percentile
2007 Aus NNS	2-6 years	100%	27.6	45.0
	7-16 years	100%	40.5	69.7
1995 Aus NNS	17 years and above	100%	36.4	79.0
2002 NZNNS	5-14 years	100%	34.8	68.1
1997 NZNNS	15 years and above	99%	31.5	65.9

TABLE A2.2: ESTIMATED DIETARY EXPOSURES TO STEVIOL GLYCOSIDES BASED ON THE 30% MARKET UPTAKE SCENARIO COMPARED TO THE ADI

Survey	Age group	30% market uptake			
		mg/kg bw/day		%ADI (ADI = 4 mg/kg bw/day)	
		Mean	90th percentile	Mean	90th percentile
2007 Aus NNS	2-6 years	1.5	2.5	35	60
	7-16 years	0.9	1.6	25	40
1995 Aus NNS	17 years and above	0.5	1.1	10	25
2002 NZNNS	5-14 years	1.0	1.9	25	50
1997 NZNNS	15 years and above	0.4	0.9	10	20

TABLE A2.3: MAJOR CONTRIBUTORS TO ESTIMATED STEVIOL GLYCOSIDES EXPOSURE BASED ON THE 30% MARKET UPTAKE SCENARIO

	% contribution to steviol glycosides exposure				
	Australia			New Zealand	
Food group	**2-6 years[1]**	**7-16 years[1]**	**17 years & above[2]**	**5-14 years [3]**	**15 years & above[4]**
Flavoured milk products	**21**	**12**	<5	<5	<5
Ice cream & edible ices	<5	<5	<5	6	<5
Fruit and vegetable products	<5	<5	<5	<5	<5
Confectionery	9	9	5	**14**	6
Cereals and cereal products	5	<5	<5	<5	<5
Breads and bakery products	<5	<5	7	6	10
Tabletop Sweeteners	10	6	**20**	<5	**22**
Special purpose foods	<5	<5	<5	<5	<5
Fruits & Vegetable juices & products	9	7	5	<5	<5
Water based flavoured drinks (soft drinks, cordials, formulated beverages)	**19**	**36**	**39**	**41**	**37**
Coffee & tea products, herbal infusion & similar products	<5	<5	<5	<5	<5
Soy beverage	<5	<5	<5	<5	<5
Dairy and fat based deserts, dips and snacks except ice cream	<5	<5	<5	<5	<5
Sauces & toppings (including mayonnaises & salad dressings)	8	9	6	11	9
Other foods	<5	<5	<5	<5	<5

1. 2007 Aus NNS
2. 1995 Aus NNS
3. 2002NZNNS
4. 1997 NZNNS

TABLE 2.4: ESTIMATED DIETARY EXPOSURES TO STEVIOL GLYCOSIDES USING 'CONSUMER BEHAVIOUR' SCENARIOS COMPARED TO THE ADI

Survey	**Age group**	**%ADI (ADI = 4 mg/kg bw/day)**			
		30% market uptake plus 100% market uptake for water based flavoured drinks only		**30% market uptake plus 100% market uptake for flavoured milk products only**	
		Mean	**90th percentile**	**Mean**	**90th percentile**
2007 Aus NNS	2-6 years	55	110	55	100
	7-16 years	40	85	30	55
1995 Aus NNS	17 years and above	25	60	NA	NA
2002 NZNNS	5-14 years	45	100	25	55
1997 NZNNS	15 years and above	20	50	NA	NA

NA = Not assessed for this scenario.