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Te Mana Kounga Kai – Ahitereiria me Aotearoa

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FINAL ASSESSMENT REPORT

APPLICATION A540

STEVIOL GLYCOSIDES AS INTENSE SWEETENERS

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to http://www.foodstandards.gov.au/standardsdevelopment/

Executive Summary

FSANZ received an Application (A540) on 31 May 2004 from the Plant Sciences Group, Central Queensland University and Australian Stevia Mills Pty Ltd to amend Standard 1.3.1 – Food Additives of the *Australia New Zealand Food Standards Code* (the Code) to approve the use of steviol glycosides[1] (extracts of the herb *Stevia rebaudiana*) as an intense sweetener for a wide variety of foods. Approval is therefore specifically being sought to include steviol glycosides in Schedule 1 or 2 of Standard 1.3.1. There are currently no permissions for steviol glycosides in the Code. This Application is a Group 3 (cost-recovered) Application.

Steviol glycosides are high intensity sweeteners, extracted from *S. rebaudiana*. They are 250-300 times sweeter than sucrose and have been approved for use for several years in a number of countries as sweeteners for a range of food products. In particular, Japan has used stevia as its main non-sucrose sweetener source for more than 30 years. Other countries which allow the use of steviol glycosides include China, Russia, Korea, Brazil, Paraguay, Argentina, Indonesia and Israel.

FSANZ released the Draft Assessment Report for public comment in May 2007. However, subsequent to the release of the Draft Assessment Report, FSANZ became aware of an additional study investigating the potential pharmacological effects of steviol glycosides in humans. Only an abstract of the study was available and the full results were not published. As this data could have had an impact on the establishment of an Acceptable Daily Intake (ADI), FSANZ deferred the Final Assessment of steviol glycosides until this additional data was available for evaluation. The results of this study recently became available and FSANZ has included it in the Final Assessment Report.

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

A dietary exposure assessment estimated that, for the majority of consumers, the ADI is not exceeded when steviol glycosides were added to the range of foods requested in the Application. Two scenarios were modelled: a full sugar replace scenario; and a 30% market share uptake scenario. Based on the full sugar replacement scenario, the estimated exposure for high consumers (children aged 2-6 years) at the 90th percentile was at the ADI. However, this estimate is based on very conservative assumptions.

[1] The most common names used for steviol glycosides are stevia, stevioside and sometimes stevia extract and stevia sugar

When a dietary exposure estimate was undertaken with concentrations of steviol glycosides that reflect a more realistic level of use (a 30% market share uptake scenario), it was estimated that dietary exposure for high consumers (children aged 2-6 years) at the 90th percentile was only 55% of the ADI.

Due to the conservative assumptions in the dietary exposure calculations, FSANZ concludes that there are no public health and safety concerns for steviol glycosides when used as a food additive at the maximum levels proposed by the Applicant.

Purpose

The Applicant seeks approval for the use of steviol glycosides (extracts of the herb *S. rebaudiana*) as an intense sweetener for a wide variety of foods. Approval was therefore specifically being sought to include steviol glycosides in Schedule 1 or 2 of Standard 1.3.1.

Decision

Approve the draft variation. This means that FSANZ has decided to vary Standards 1.2.4 and 1.3.1 to permit the use of steviol glycosides in specified food at specified levels.

Reasons for Decision

This draft variation is proposed for the following reasons.

- The proposed draft variation to the Code is consistent with the section 18 objectives of the FSANZ Act. In particular, at the levels of use requested by the Applicant, it does not raise any public health and safety concerns. The risk assessment of steviol glycosides is based on the best available scientific evidence and the draft variation helps promote an efficient and internationally competitive food industry.

- Use of steviol glycosides is technologically justified since it has desirable qualities that are of interest to the food manufacturing industry.

- The regulation impact assessment concluded that the benefits of permitting use of steviol glycosides outweigh any costs associated with its use.

To achieve what the Application seeks, there are no alternatives that are more cost-effective than a variation to Standard 1.3.1 and Standard 1.2.4.

Consultation

FSANZ has now completed the assessment of Application A540 and held two rounds of public consultation. The draft variations to the Code have been approved by the FSANZ Board and the decision has been notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council). If the Ministerial Council does not request FSANZ to review the draft variations to the Code, an amendment to the Code will be published in the *Commonwealth Gazette* and the *New Zealand Gazette* and adopted by reference and without amendment under Australian State and Territory food law.

CONTENTS

INTRODUCTION....2

1. BACKGROUND....2
   1.1 Nature of Application....3
   1.2 Historical Background....4
2. THE PROBLEM....4
3. OBJECTIVES....5

RISK ASSESSMENT....5

4. KEY ASSESSMENT QUESTIONS....5
5. RISK ASSESSMENT SUMMARY....5
   5.1 Hazard assessment....5
   5.2 Dietary exposure assessment....6
   5.3 Risk characterisation....8
   5.4 Food Technology Assessment....9

RISK MANAGEMENT....9

6. RISK MANAGEMENT OF ISSUES RAISED IN SUBMISSIONS....9
   6.1 Establishment of the ADI....9
   6.2 Specifications....9
   6.3 Steviol equivalents....10
   6.4 Methods of analysis....11
7. REGULATORY OPTIONS....11
   7.1 Option 1 – reject the Application, thus not approving the use of steviol glycosides as an intense sweetener and flavour enhancer....11
   7.2 Option 2 – approve the use of steviol glycosides as an intense sweetener and flavour enhancer in Schedule 1 of Standard 1.3.1....11
   7.3 Option 3 – approve the use of steviol glycosides as an intense sweetener and flavour enhancer in Schedule 2 of Standard 1.3.1....11
8. IMPACT ANALYSIS....12
   8.1 Affected Parties....12
   8.2 Benefit Cost Analysis....12
   8.3 Comparison of Options....13

COMMUNICATION AND CONSULTATION STRATEGY....14

9. COMMUNICATION....14
10. CONSULTATION....14
    10.1 Initial Assessment....14
    10.2 Draft Assessment....14
    10.3 World Trade Organization (WTO)....15

CONCLUSION....15

11. CONCLUSION AND DECISION....15
    11.1 Reasons for Decision....15
12. IMPLEMENTATION AND REVIEW....16

ATTACHMENT 1 - DRAFT VARIATIONS TO THE *AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE*....17
ATTACHMENT 2 - HAZARD ASSESSMENT....21
ATTACHMENT 3 - DIETARY EXPOSURE ASSESSMENT....62
ATTACHMENT 4 - SUMMARY OF SUBMISSIONS TO DRAFT ASSESSMENT REPORT....90

INTRODUCTION

1. Background

Stevia rebaudiana is an herb belonging to the chrysanthemum family which grows wild as a small shrub in parts of Paraguay and Brazil. The leaves have traditionally been used as a sweetener. The leaves of *S. rebaudiana* contain 10 different steviol glycosides. Steviol glycosides are considered high intensity sweeteners (250-300 times that of sucrose) and have been used for several years in a number of countries as a sweetener for a range of food products.

Stevioside and rebaudioside A are the major components of stevia. The Applicant claims that the ratio of these two components 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.

The Applicant indicates that steviol glycosides are a natural extract of the leaves of *S. rebaudiana* with reduced or no calories. The common names used for the purified extract of the stevia plant have included stevia, stevioside, steviol and various other names. The extract is a mixture of one or more glycosides of steviol, the most predominant being stevioside and rebaudioside A. The Joint Expert Committee on Food Additives (JECFA) recently (2004) concluded that the most appropriate name to be used for this extract was 'steviol glycosides' Steviol glycosides has the food additive number INS 960.

In addition, the extract was previously expressed as a weight (usually mg) of steviol glycosides, although it was actually a mixture of very similar glycosides. As the molecular weights of the various glycosides are different, JECFA has determined that the concentrations/amounts of steviol glycosides should be expressed as steviol content, which is equivalent to approximately 40% of the stevioside and 33% of rebaudioside A content.

JECFA evaluated steviol glycosides in 2004 at its 63rd meeting, setting a temporary ADI of 2 mg/kg bw/day (expressed as steviol). The temporary ADI included a 100-fold safety factor applied to the No Observed Effect Level (970 mg/kg bw/day of stevioside (approximately 400 mg/kg bw/day steviol equivalents)) and an additional two-fold safety factor for uncertainty related to potential pharmacological effects of steviol glycosides in humans. JECFA specified the need for additional studies involving normotensive and hypotensive individuals and insulin-dependent and insulin-independent diabetics.

At the 68th meeting, JECFA evaluated new studies on normotensive and hypotensive individuals and diabetics. JECFA considered that the newly available data did not raise additional concerns regarding the safety of steviol glycosides. However, JECFA maintained the temporary ADI until the 2008 meeting, pending the submission of the results of an ongoing study, in diabetics.

The additional information requested by JECFA was evaluated at the 69th meeting in June 2008. JECFA's recommendation is that the temporary ADI be raised to 4 mg/kg bw/day and the tentative assignation be removed[2].

[2] http://www.fao.org/ag/agn/agns/files/jecfa69_final.pdf

Steviol glycosides are approved for use in a number of countries. In particular, Japan has used stevia as its main non-sucrose sweetener source for more than 30 years. Other countries which allow the use of steviol glycosides include China, Russia, Korea, Brazil, Paraguay, Argentina, Indonesia and Israel.

Steviol glycosides do not have Generally Recognised As Safe (GRAS) status in the United States of America (US) and are therefore not currently permitted to be added to foods in the US; although they are sold as a dietary supplement. The European Union (EU) considers steviol glycosides to be a food additive and has not approved them for use in food. The Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) has been requested to provide an opinion on steviol glycosides as a food additive by the European Commission.

The same data constraints that have delayed the FSANZ assessment and JECFA evaluation of steviol glycosides have contributed to a lack of specific approvals of steviol glycosides as a food additive in the US and EU, and the delay in an opinion of the AFC.

S. rebaudiana is approved for use as an active and/or excipient ingredient in Listed medicines in Australia. Stevioside is permitted in Listed medicines only in conjunction with the use of *S. rebaudiana* (it is not approved as an ingredient in its own right). There have been no known adverse effects for stevioside reported to the Therapeutic Goods Administration to date.

1.1 Nature of Application

FSANZ received an Application on 31 May 2004 (revised version provided on 31 January 2006) from the Plant Sciences Group, Central Queensland University and Australian Stevia Mills Pty Ltd to amend Standard 1.3.1 – Food Additives of the *Australia New Zealand Food Standards Code* (the Code) to approve the use of steviol glycosides (extracts of the herb *S. rebaudiana*) as intense sweeteners/flavour enhancers for a wide variety of foods.

The Applicant requests that steviol glycosides be used as a food additive (sweetener and/or flavour enhancer) in the following food categories:

- milk products – flavoured;
- yoghurts etc – flavoured;
- ice confection (including liquid, reduced and low fat);
- ice creams (including reduced and low fat);
- canned fruit, jams and preparations;
- soy products;
- low joule chocolate and confectionary products (including chewing gum);
- processed breakfast cereals;
- biscuits, sweet (excluding chocolate coated);
- cakes, slices and muffins;
- pastries, sweet;
- tabletop sweeteners (including tablets and liquid);
- fruit and vegetable juice drinks (including low joule);
- soft drinks and cordials (including low joule);
- coffees, teas & infusions;

- desserts (including dairy);
- cereal products (including muesli and breakfast bars);
- gravy & sauces – sweetened only; and
- toppings, mayonnaises & salad dressings.

Work on this Group 3 (cost-recovered) Application commenced on 30 September 2005.

1.2 Historical Background

1.2.1 Application A397

FSANZ received an Application (A397) on 26 August 1999 to amend the former Australian *Food Standards Code* to use Stevia herbal extract as an additive in general consumer foods such as bakery products and dairy products. The Application was at Full Assessment and FSANZ requested additional information to clarify the specific intent of the Application and in relation to public health and safety concerns. The Applicant could not supply this information and advised FSANZ on 28 June 2000 that they were withdrawing the Application.

1.2.2 Application A457

FSANZ received an Application (A457) on 31 October 2001 for approval of Sunlabel Stevia Manni-Stevia as a novel food. FSANZ requested further information on public health and safety issues and clarification on a range of other issues, in particular, clarification of whether the Applicant required permissions under Standard 1.5.1 or Standard 1.3.1. The Applicant responded, but provided no data and requested further time to undertake studies in order to address the public health and safety concerns. However, despite repeated requests from FSANZ, no data were provided. As the Applicant failed to provide a reasonable excuse for failure to comply with the repeated requests for data from FSANZ, the Application was taken to be withdrawn on 23 December 2002.

2. The Problem

Standard 1.3.1 prohibits the addition of food additives to food unless the additive is expressly permitted in that standard. Before FSANZ considers new permissions, it requires that food additives undergo a pre-market risk assessment through an application to FSANZ.

Steviol glycosides are being requested to be approved as a new intense sweetener (food additive) in Australia and New Zealand. There is currently no permission within Standard 1.3.1 for using steviol glycosides as a food additive; therefore a pre-market risk assessment, including a dietary modelling assessment, is required.

Steviol glycosides are proposed for use primarily as a non-calorie sweetener and/or flavour enhancer in a wide range of products that contain sugar and/or are permitted to contain approved intense sweeteners. As steviol glycosides are 250 to 300 times sweeter than sucrose, they can be used at rates as little as 0.004 times the rate of sugar used. They can be used in conjunction with sugar or other sweeteners and will replace some of the sweeteners currently approved for use in foods. The main purpose of using steviol glycosides in foods is to enhance the taste and sweetness without needing to use high calorie sweeteners (sucrose, glucose, fructose, honey etc) or synthetic chemical sweeteners.

3. Objectives

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 18 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

RISK ASSESSMENT

4. Key Assessment Questions

The key assessment questions considered at Draft and Final Assessment were:

- Considering the information provided by the Applicant and other available information, would the approval of steviol glycosides as a food additive pose any risk to public health and safety?
- What would be the potential dietary intake of steviol glycosides for mean and high consumers if it were approved as a food additive?
- What are the food technology implications of this Application?

5. Risk Assessment Summary

5.1 Hazard assessment

'Steviol glycosides' is a collective term for steviol derivatives which are glycosylated with different side chains. There are around ten steviol glycosides present in *S. rebaudiana* extracts. Of these, stevioside and rebaudioside A are present in the highest concentrations. In all toxicological studies the administered extract (dose), expressed as mg/kg bw of stevioside, was used to assess the toxicity of steviol glycosides.

In particular, FSANZ has reviewed supplementary metabolism, pharmacological, mechanistic and toxicity studies to determine whether sufficient uncertainty remains to apply an additional two-fold safety factor to the acceptable daily intake (ADI) for steviol glycosides.

Stevioside is completely metabolised to steviol by the microbial flora of the caecum, with excretion occurring via the faeces in animals or by the urine in humans. Stevioside has very low acute toxicity and there is no evidence of carcinogenicity, developmental, reproductive or genotoxicity effects. Several studies in animals and humans indicate that steviol glycosides have antihypertensive and antiglycaemic effects. While the exact mechanism of action has not been fully elucidated, the absence of urinary metabolites apart from conjugated steviol suggests that unconjugated steviol is pharmacologically active in humans.

Steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day. The adequacy of the existing database and a new study in humans provides a basis for revising the uncertainty factor used by JECFA to derive the temporary ADI for steviol glycosides in 2005 (2 mg/kg bw/day, expressed as steviol equivalents). In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore, FSANZ has established a full ADI of 4 mg/kg bw (expressed as steviol equivalents), derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study. This ADI covers steviol glycoside mixtures with different ratios of stevioside/rebaudioside (the full Hazard Assessment report is at **Attachment 2**).

5.2 Dietary exposure assessment

A dietary exposure assessment was undertaken by FSANZ to estimate dietary exposure to steviol glycosides. Food consumption data from the 1995 Australian and 1997 New Zealand National Nutrition Surveys were used for the exposure assessments. The population groups assessed were the Australian population (2 years and above), the New Zealand population (15 years and above) and children (2-6 years for Australia only).

Under the FSANZ Science Strategy 2006-2009, FSANZ agreed to review its dietary modelling procedures. As part of this review, an international peer review was sought. FSANZ has previously reported chronic dietary exposures for high consumers at the 95th percentile. The recommendation of the peer review by an international dietary exposure assessment expert from the US Food and Drug Administration was that FSANZ should consider aligning its reporting of food chemical dietary exposures with international best practice by reporting at the 90th percentile not the 95th percentile, if only one 24 hour recall record per person was used for the assessment[3]. This is because the 95th percentile results are likely to be an overestimate of dietary exposure on a daily basis over a life-time of exposure. Basing risk management decisions on the 95th percentile will potentially result in an overly conservative risk management approach.

[3] Lambe, J., Kearney, J., Leclercrq, C., Berardi, D., Zunft,H., De Henauw, S., De Volder, M., Lamberg-Allardt, C., Karkkainen, M., Dunne, A. and Gibney, N. (2000) Enhancing the capacity of food consumption surveys of short duration to estimate long term consumer-only intakes by combination with a qualitative food frequency questionnaire. Food Additives and Contaminants, 17(3), pp. 177-187.

After Draft Assessment the Applicant requested some minor changes to the permissions included in the draft variation to Standard 1.3.1. The dietary exposure assessment reflects the amended levels of use requested by the Applicant. Amendments from the Draft Assessment include new requests for permission in peanut butter, fancy breads, formulated beverages and an increase in the level of use requested for bubble and chewing gum and hard boiled confectionery.

The Applicant provided FSANZ with information on proposed levels of use for steviol glycosides for specific food groups and the expected proportion of products in each food group in which sugar or intense sweeteners would be replaced by steviol glycosides over 20 years of availability to food suppliers. Based on this information, dietary exposure assessments were conducted for a *sugar replacement scenario* (Scenario 1).

At its 63rd meeting, JECFA estimated the intake of steviol glycosides would likely be 20-30% of total sugar replacement in foods (World Health Organization, 2004). Therefore, dietary exposure assessments were conducted for a *30% market share* scenario (Scenario 2) based on this assumption.

Estimated dietary exposures were compared with the ADI of 4 mg/kg bw (expressed as steviol equivalents).

For both scenarios, the major contributors to steviol glycosides dietary exposure for Australians aged 2 years and above and for New Zealanders aged 15 years and above were predicted to be formulated beverages, tabletop sweeteners and carbonated soft drinks. For Australian children aged 2-6 years, the major contributors were fruit and vegetable juices and products, formulated beverages, carbonated soft drinks, breakfast cereals, mueslis and muesli bars, and flavoured milks and yoghurts for both scenarios. Beverages were predicted to be major contributors to steviol glycosides exposures because they are consumed in large volumes.

For both the *sugar replacement* model (Scenario 1) and the *30% market share* model (Scenario 2), estimated mean and 90th percentile exposures for all population groups assessed were at or below the ADI. For the *sugar replacement* scenario, Australian children aged 2-6 years had estimated 90th percentile dietary exposures to steviol glycosides at 100% ADI.

Summary results are in the two tables below: For full dietary modelling methodology and results, see Attachment 3.

Estimated dietary exposure to steviol glycosides (Scenario 1)

Population group	**Mean consumers mg/day (% ADI)**	**90th percentile consumers mg/day (% ADI)**
2 years and above (Australia)	57 (25%)	107 (50%)
2-6 years (Australia)	40 (55%)	74 (100%)
15 years and above (New Zealand)	40 (15%)	74 (25%)

Estimated dietary exposure to steviol glycosides (Scenario 2)

Population group	Mean consumers mg/kg bw/day (% ADI)	90th percentile consumers mg/kg bw/day (% ADI)
2 years and above (Australia)	37 (15%)	63 (30%)
2-6 years (Australia)	24 (35%)	41 (55%)
15 years and above (New Zealand)	29 (10%)	49 (20%)

5.3 Risk characterisation

Based on the hazard assessment, steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day. The adequacy of the existing database and a new study in humans provides a basis for revising the uncertainty factor used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore FSANZ has established a full ADI of 4 mg/kg bw (expressed as steviol equivalents), derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a two-year rat study. This ADI covers steviol glycoside mixtures with different ratios of stevioside/rebaudioside.

Estimated mean and 90th percentile exposures for all population groups assessed were below the ADI except for the 90th percentile exposure of Australian children aged 2-6 years in Scenario 1 (100% of the ADI). However, Scenario 2 (which would reflect a more realistic exposure estimate) indicates that the highest estimated consumption for this population group was at 55% of the ADI. The highest exposure is still likely to be an overestimate due to the following reasons:

- steviol glycosides would have to be added to all the foods proposed, which is extremely unlikely;
- all the foods would have to contain steviol glycosides at the maximum concentrations proposed by the Applicant; and
- the data used for modelling is a 24-h record which overestimates food consumption for consumers (the use of multiple day records tends to significantly reduce predicted high consumer exposure).

Mean exposure, which is a more realistic representation of exposure over a longer period of time for both scenario 1 and 2, was below the ADI for all population groups. Furthermore, as the ADI is based on a NOEL from an animal study using a 100-fold safety factor, there is an inbuilt safety margin if exposure reaches, or is marginally above the ADI.

Overall, although some groups were shown to reach the ADI (in Scenario 1), due to the conservative assumptions in the dietary exposure calculations, FSANZ concludes that there are no public health and safety concerns for steviol glycosides when used as a food additive at the maximum levels proposed by the Applicant.

5.4 Food Technology Assessment

The Food Technology Assessment conducted at Draft Assessment concluded that 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 250-300 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.

RISK MANAGEMENT

6. Risk Management of Issues Raised in Submissions

FSANZ has considered the management of any risks identified through the risk assessment and submissions received during the public consultation period.

6.1 Establishment of the ADI

At Draft Assessment, FSANZ recommended that steviol glycosides be approved for use as a Schedule 1 food additive in a range of foods and beverages and that the temporary ADI (2 mg/kg bw) set by JECFA in 2005 should be raised to 4 mg/kg bw. Following this, FSANZ became aware of an additional study investigating the potential pharmacological effects of steviol glycosides in humans. These results, although still in abstract form, were recently made available and FSANZ has included this study in the risk assessment of this Final Assessment Report.

At Draft Assessment, a number of submitters requested that FSANZ defer further consideration of steviol glycosides until after JECFA had finalised its evaluation. One submitter questioned whether FSANZ was the appropriate agency to set an ADI for Australia and New Zealand. FSANZ is a pivotal body charged with establishing reference health standards for food in Australia and New Zealand. In setting reference health standards, FSANZ takes full account of any FAO/WHO Assessments that may be available, but is still charged with undertaking an independent scientific assessment. Furthermore, through its participation in JECFA, FSANZ contributes to the setting of reference health standards used internationally by other regulatory agencies.

In June 2008, JECFA reconsidered its temporary ADI of 2 mg/kg bw (set at its 2005 meeting). At this meeting, data were evaluated that had not been available in 2005. On the basis of the new data, JECFA established a new ADI for steviol glycosides of 4 mg/kg bw. The new JECFA ADI is based on the same studies used by FSANZ in this risk assessment.

6.2 Specifications

Standard 1.3.4 – Identity and Purity adopts specifications for food additives (and other substances in foods) by reference to specific sources, including JECFA specifications. Standard 1.3.4 also contains distinct specifications for some ingredients and substances where there is not a suitable specification included in the sources referenced in the Standard.

At Draft Assessment, FSANZ did not propose to include a separate specification for steviol glycosides, which would result in other recognised specifications for steviol glycosides being included in the Standard by reference to the applicable sources listed in clauses 2 and 3 of Standard 1.3.4.

The current JECFA specification for steviol glycosides defines steviol glycosides:

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

The Applicant indicated that some Australian producers would extract steviol glycosides with water and not wish to use methanol in the manufacturing process. The Applicant expressed concern that the reference in the JECFA specification to the use of methanol in washing and recrystallising may result in the use of other solvents, particularly water, not being compliant with the JECFA specification.

The Purpose in Standard 1.3.4 makes it clear that the Standard is intended to regulate the identity and purity of substances, not methods of manufacture. FSANZ considers that as the purpose of Standard 1.3.4 is concerned with identity and purity, it does not matter by which method the required identity and purity specifications are met. Therefore, FSANZ has not considered it necessary to include a method of extraction for steviol glycosides.

The Applicant also requested that the level of purity of steviol glycosides in the JECFA specification be reduced from 95% to 90% total glycosides. The Applicant suggested that 95% purity will exclude a large portion of the current world supply of steviol glycosides and that 95% purity is achieved only by reprocessed glycosides powder, often associated with the separation of Rebaudioside A or with enzymatic modification.

However, as the pivotal study in setting the ADI for steviol glycosides used a 95% pure product, FSANZ considers this purity is appropriate. Therefore, FSANZ has not included a distinct specification for steviol glycosides in Standard 1.3.4. Manufacturers of steviol glycosides will have to meet the identity and purity parameters set out in the specification included in any of the sources referenced in clauses 2 and 3 of the Standard.

6.3 Steviol equivalents

Some submissions requested additional clarity in the terminology used to refer to quantities of steviol glycosides, particularly in relation to the ADI and the draft variations to the Code. The ADI and the permissions in the draft variations to the Code refer to steviol glycosides expressed as steviol equivalents. FSANZ has clarified this in the draft variations to the Code and when referring to the ADI for steviol glycosides in this Report.

6.4 Methods of analysis

FSANZ does not ordinarily prescribe methods as this can inhibit method development and require the prescribed method to be used even though better, cheaper or more sophisticated methods may be developed in the future. Prescribing a method may also prevent the use of equivalent methods for monitoring purposes and restricts the flexibility of industry and compliance agencies. In the absence of a prescribed method, analysts would still need to develop and use methods that are 'fit for purpose' and suitably validated.

FSANZ acknowledges that there are implementation issues associated with adding levels for a new intense sweetener in Standard 1.3.1. However, the role of FSANZ does not extend to developing or validating methods or determining specific arrangements for compliance monitoring. These aspects will need to be implemented by compliance agencies either individually or collectively.

7. Regulatory Options

Food additives used in Australia and New Zealand are required to be listed in Standard 1.3.1. As steviol glycosides are considered food additives and require pre-market approval under Standard 1.3.1, it is not appropriate to consider non-regulatory options to address this Application.

Three regulatory options are identified for this Application:

7.1 Option 1 – reject the Application, thus not approving the use of steviol glycosides as an intense sweetener and flavour enhancer

This option maintains the *status quo* by not permitting the use of steviol glycosides as a food additive in Standard 1.3.1.

7.2 Option 2 – approve the use of steviol glycosides as an intense sweetener and flavour enhancer in Schedule 1 of Standard 1.3.1

This option will result in an amendment to Schedule 1 of Standard 1.3.1 to permit the use of steviol glycosides as a food additive in a specified range of foods at restricted levels. This option will also result in a subsequent amendment to Standard 1.2.4 to include steviol glycosides in the list of food additives in Schedule 2.

7.3 Option 3 – approve the use of steviol glycosides as an intense sweetener and flavour enhancer in Schedule 2 of Standard 1.3.1

This option will result in an amendment to Schedule 2 of Standard 1.3.1 to permit the use of steviol glycosides as a food additive at levels according to Good Manufacturing Practice in processed foods specified in Schedule 1 of Standard 1.3.1. This Option would result in a wider range of foods being permitted to contain added steviol glycosides than for Option 2. This option will also result in a subsequent amendment to Standard 1.2.4 to include steviol glycosides in the list of food additives in Schedule 2.

8. Impact Analysis

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community, which includes consumers, the food industry, governments in both Australia and New Zealand and often public health professionals. The benefits and costs associated with any proposed amendment to the Code will be analysed in a Regulatory Impact Assessment.

8.1 Affected Parties

Parties possibly affected by the regulatory options outlined above include:

1. Consumers who may be affected by new products containing steviol glycosides.

2. Public health professionals because of the role of slow release carbohydrates in human nutrition.

3. Those sectors of the food industry wishing to market foods containing steviol glycosides, including potential importers, manufacturers of steviol glycosides and manufacturers of foods that may potentially contain steviol glycosides.

4. Government agencies enforcing the food regulations.

8.2 Benefit Cost Analysis

8.2.1 Option 1 – Reject the Application

Under Option 1, the affected parties and potential impacts are:

- Manufacturers of steviol glycosides, manufacturers wishing to produce foods containing steviol glycosides and importers of foods containing steviol glycosides, would be disadvantaged as they would be unable to innovate and take advantage of market opportunities for the development and sale of steviol glycosides-containing products.

- Consumers may be disadvantaged as they would be unable to take advantage of any potential steviol glycosides-containing foods, particularly in regard to food with lower energy values.

- Public health professionals may be disadvantaged as they would be unable to promote any potential health benefits of foods containing steviol glycosides.

- There is no perceived impact on government agencies, although lack of approval may be regarded as unnecessarily trade restrictive.

8.2.2 Option 2 – Permit the use of steviol glycosides as a food additive in Schedule 1 of Standard 1.3.1

Under Option 2, the affected parties and potential impacts are:

- Manufacturers of steviol glycosides, manufacturers wishing to produce foods containing steviol glycosides and importers of foods containing steviol glycosides, would benefit. There would be opportunities to innovate and take advantage of market opportunities, both domestically and overseas, for the development and sale of steviol glycosides-containing products.

- Including steviol glycosides in Schedule 1 of Standard 1.3.1 would benefit public health and safety. This option would impose limits on the use of the additive and would address the potential for high dietary exposure to exceed the ADI.

- Consumers may benefit from foods containing steviol glycosides as this would provide an alternative intense sweetener and reduce the risk of excess consumption of any one sweetener, including sugar, added to foods.

- Public health professionals may benefit as they would be able to promote any potential health benefits of foods containing steviol glycosides.

- Government agencies responsible for enforcement of the Code may have additional costs of enforcement of a new food additive with restricted permissions for use.

8.2.3 Option 3 – Permit the use of steviol glycosides as a food additive in Schedule 2 of Standard 1.3.1

Under Option 3, the affected parties and potential impacts are:

- Manufacturers of steviol glycosides, manufacturers wishing to produce foods containing steviol glycosides and importers of foods containing steviol glycosides, would benefit. There would be greater opportunities (than under Option 2) to innovate and take advantage of market opportunities, both domestically and overseas, for the development and sale of steviol glycosides-containing products due to a wider range of foods being permitted to contain steviol glycosides.

- Consumers may benefit from an additional range of foods containing steviol glycosides (than under Option 2) as this would provide an alternative intense sweetener and reduce the risk of excess consumption of any one sweetener, including sugar, added to foods.

- Public health professionals may benefit as they would be able to promote any potential health benefits of foods containing steviol glycosides.

- There is no perceived impact on government agencies.

8.3 Comparison of Options

Option 1 appears to provide no benefits to industry, consumers, public health professionals or government. Option 1 denies industry access to a new food additive which has been assessed as safe. It also denies consumers access to foods containing steviol glycosides, and any associated benefits.

Option 2 and 3 do not appear to impose any significant costs on industry, consumers, public health professionals or government.

Option 2 and 3 provide benefits to industry in terms of product innovation and development and potential sales of foods containing steviol glycosides, while consumers may benefit from possible improved flavour/taste profiles and the potential of reduced levels of other intense sweeteners and sugars in foods.

Option 3 would provide industry with a greater potential for innovation due to a wider range of foods being permitted to contain added steviol glycosides than would be permitted under Option 2. However, dietary modelling indicated that the 90th percentile of consumers in the 2-6 year old population group in Australia has the potential to exceed the ADI for steviol glycosides. Although, in reality this is not considered to be a likely scenario, consideration should be given to this potential dietary exposure.

An assessment of the costs and benefits of the three Options indicates that there would be a net benefit in permitting the use of steviol glycosides (Option 2 or 3). Option 2 provides a greater level of protection for high consumers of steviol glycosides in certain population sub groups that could theoretically exceed the ADI.

Therefore, Option 2 is the preferred option.

COMMUNICATION AND CONSULTATION STRATEGY

9. Communication

FSANZ has reviewed the nature of the feedback received from submitters at Initial and Draft Assessment and does not intend to undertake specific communication and consultation work in addition to the two statutory public consultation periods.

Once the draft variations to the Code are approved by the FSANZ Board, that decision will be notified to the Ministerial Council. The Applicant and stakeholders, including the public, will be notified of any changes to the Code in the national press and on the website.

10. Consultation

10.1 Initial Assessment

The Initial Assessment was advertised for public comment between 7 December 2005 and 1 February 2006.

Sixteen submissions were received during this period. Fourteen submissions supported the progression of the Application to Draft Assessment with industry submissions strongly supporting the approval of steviol glycosides as an intense sweetener. Two submissions suggested deferring the Draft Assessment until after JECFA had evaluated the additional studies requested at its 63rd meeting.

10.2 Draft Assessment

The Draft Assessment was advertised for public comment between 23 May 2007 and 4 July 2007.

Nineteen submissions were received during this period. 15 submissions supported the approval of steviol glycosides as an intense sweetener. Three of these submissions noted that support was based on favourable outcomes from JECFA and AFC evaluations of steviol glycosides. Two submissions did not support the progression of the Application until after JECFA had finalised its evaluation of steviol glycosides. Two submissions did not specifically comment on the options and provided reports on steviol glycosides, published in other sources.

A full summary of submissions received at Draft Assessment is at Attachment 4.

10.3 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

There are no relevant international standards for steviol glycosides. Amending the Code to allow the use of steviol glycosides as a food additive is unlikely to have a significant effect on international trade, as the proposed variations to the Code constitutes minor technical changes. As such, they are not expected to significantly impact on trade issues for either technical or sanitary or phytosanitary reasons. However, there may be unforeseen trade implications in regard to other approved intense sweeteners, due to the liberalising effect of approving the use of steviol glycosides in a range of foods. Therefore, at Draft Assessment FSANZ notified the WTO under the Sanitary and Phytosanitary Agreement to enable other WTO member countries to comment on the proposed changes to the standards in case approval of steviol glycosides has a significant impact on them. FSANZ received no comments in response to the WTO notification.

CONCLUSION

11. Conclusion and Decision

Approval of steviol glycosides as a food additive is proposed. Permission is proposed to be provided by amending Schedule 1 of Standard 1.3.1 – Food Additives.

Decision

Approve the draft variation. This means that FSANZ has decided to vary Standards 1.2.4 and 1.3.1 to permit the use of steviol glycosides in specified food at specified levels.

11.1 Reasons for Decision

This draft variation is proposed for the following reasons.

- The proposed draft variation to the Code is consistent with the section 10 objectives of the FSANZ Act. In particular, at the levels of use requested by the Applicant, it does not raise any public health and safety concerns. The risk assessment of steviol glycosides is based on the best available scientific evidence and the draft variation helps promote an efficient and internationally competitive food industry.

- Use of steviol glycosides is technologically justified since it has desirable qualities that are of interest to the food manufacturing industry.

- The regulation impact assessment concluded that the benefits of permitting use of steviol glycosides outweigh any costs associated with its use.

To achieve what the Application seeks, there are no alternatives that are more cost-effective than a variation to Standard 1.3.1 and Standard 1.2.4.

12. Implementation and Review

Approval of the Application will result in the proposed variations to Standard 1.3.1 and Standard 1.2.4 commencing on gazettal.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code*
2. Hazard Assessment Report
3. Dietary Exposure Assessment Report
4. Summary of submissions to Draft Assessment

Attachment 1

Draft variations to the *Australia New Zealand Food Standards Code*

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunsetting.

To commence: On gazettal

[1] ***Standard 1.2.4*** *of the Australia New Zealand Food Standards Code is varied by –*

[1.1] *inserting in* Part 1 *of* Schedule 2 –

Steviol glycosides	960

[1.2] *inserting in* Part 2 *of* Schedule 2 –

Steviol glycosides	960

[2] ***Standard 1.3.1*** *of the Australia New Zealand Food Standards Code is varied by –*

[2.1] *inserting in* Schedule 1, *under item* 1.1.2 Liquid milk products and flavoured liquid milk* –

960	Steviol glycosides (calculated as steviol equivalents)	115	mg/kg

[2.2] *inserting in* Schedule 1, *under item* 1.2.2 Fermented milk products and rennetted milk products* –

960	Steviol glycosides (calculated as steviol equivalents)	176	mg/kg

[2.3] *inserting in* Schedule 1, *under item* 3 ICE CREAM AND EDIBLE ICES* –

960	Steviol glycosides (calculated as steviol equivalents)	64	mg/kg

[2.4] *inserting in* Schedule 1, *under item* 3, *sub-item* Ice confection sold in liquid form –

960	Steviol glycosides (calculated as steviol equivalents)	115	mg/kg

[2.5] *inserting in* Schedule 1, *under item* 3 ICE CREAM AND EDIBLE ICES* –

Reduced and low fat ice cream and edible ices

960	Steviol glycosides (calculated as steviol equivalents)	208	mg/kg

[2.6] *inserting in* Schedule 1, *under item* 4.3.2 Fruits and vegetables in vinegar, oil, brine or alcohol* –

960	Steviol glycosides (calculated as steviol equivalents)	160	mg/kg

[2.7] *inserting in* Schedule 1, *under item* 4.3.4, *sub-item* low joule chutneys, low joule jams and low joule spreads –

960	Steviol glycosides (calculated as steviol equivalents)	450	mg/kg

[2.8] *inserting in* Schedule 1, *under item* 4.3.6 Fruit and vegetable preparations including pulp* –

960	Steviol glycosides (calculated as steviol equivalents)	208	mg/kg

[2.9] *inserting in* Schedule 1, *under item* 5.1 Chocolate and cocoa products –

960	Steviol glycosides (calculated as steviol equivalents)	550	mg/kg

[2.10] *inserting in* Schedule 1, *under item* 5.2 Sugar confectionery* –

960	Steviol glycosides (calculated as steviol equivalents)	1100	mg/kg

[2.11] *inserting in* Schedule 1, *under item* 5.2, *sub-item* low joule chewing gum –

960	Steviol glycosides (calculated as steviol equivalents)	1100	mg/kg

[2.12] *inserting in* Schedule 1, *under item* 6.3 Processed cereal and meal products* –

960	Steviol glycosides (calculated as steviol equivalents)	250	mg/kg

[2.13] *inserting in* Schedule 1, *under item* 7.1 Breads and related products* –

fancy breads

960	Steviol glycosides (calculated as steviol equivalents)	160	mg/kg

[2.14] *inserting in* Schedule 1, *under item* 7.2 Biscuits, cakes and pastries* –

960	Steviol glycosides (calculated as steviol equivalents)	160	mg/kg

[2.15] *inserting in* Schedule 1, *under item* 11.4 Tabletop Sweeteners* –

960	Steviol glycosides (calculated as steviol equivalents)	GMP	

[2.16] *inserting in* Schedule 1, *under item* 11.4.1 Tabletop Sweeteners-liquid preparation* –

960	Steviol glycosides (calculated as steviol equivalents)	GMP		

[2.17] *inserting in* Schedule 1, *under item* 11.4.2 Tabletop Sweeteners-tablets or powder or granules packed in portion sized packages* –

960	Steviol glycosides (calculated as steviol equivalents)	GMP		

[2.18] *inserting in* Schedule 1, *under item 13.3 Formula meal replacements and formulated supplementary foods** –

960	Steviol glycosides (calculated as steviol equivalents)	175	mg/kg	

[2.19] *inserting in* Schedule 1, *under item* 13.4 Formulated supplementary sports foods* –

960	Steviol glycosides (calculated as steviol equivalents)	175	mg/kg	

[2.20] *inserting in* Schedule 1, *under item* 14.1.2.1 Fruit and vegetable juices –

960	Steviol glycosides (calculated as steviol equivalents)	50	mg/kg	

[2.21] *inserting in* Schedule 1, *under item* 14.1.2.2 Fruit and vegetable juice products* –

soy bean beverage (plain or flavoured)

960	Steviol glycosides (calculated as steviol equivalents)	65	mg/kg	Plain soy bean beverage only
960	Steviol glycosides (calculated as steviol equivalents)	175	mg/kg	Flavoured soy bean beverage only

[2.22] *inserting in* Schedule 1, *under item* 14.1.2.2, *sub-item* low joule fruit and vegetable juice products –

960	Steviol glycosides (calculated as steviol equivalents)	125	mg/kg	

[2.23] *inserting in* Schedule 1, *under item* 14.1.3 Water based flavoured drinks* –

960	Steviol glycosides (calculated as steviol equivalents)	160	mg/kg	

[2.24] *inserting in* Schedule 1, *under item* 14.1.3.1 Brewed soft drink* –

960	Steviol glycosides (calculated as steviol equivalents)	160	mg/kg	Clause 4 limits do not apply

[2.25] *inserting in* Schedule 1, *under item* 14.1.1 Formulated beverages* –

960	Steviol glycosides (calculated as steviol equivalents)	160	mg/kg	

[2.26] *inserting in* Schedule 1, *under item* 14.1.5 Coffee, coffee substitutes, tea, herbal infusions and similar products –

960	Steviol glycosides (calculated as steviol equivalents)	100	mg/kg	

[2.27] *inserting in* Schedule 1, *under item* 20.2, *sub-item* custard mix, custard powder and blanc mange powder –

960	Steviol glycosides (calculated as steviol equivalents)	80	mg/kg	

[2.28] *inserting in* Schedule 1, *under item* 20.2, *sub-item jelly* –

960	Steviol glycosides (calculated as steviol equivalents)	260	mg/kg	

[2.29] *inserting in* Schedule 1, *under item* 20.2, *sub-item* dairy and fat based desserts, dips and snacks –

960	Steviol glycosides (calculated as steviol equivalents)	150	mg/kg	dairy and fat based dessert products only

[2.30] *inserting in* Schedule 1, *under item* 20.2, *sub-item* sauces and toppings (including mayonnaises and salad dressings) –

960	Steviol glycosides (calculated as steviol equivalents)	320	mg/kg	

Attachment 2

Hazard Assessment

Executive summary

Steviol glycosides[4] are a mixture of high intensity sweeteners which are readily extracted from the leaves of the plant *Stevia rebaudiana*. The steviol glycosides in highest concentration in extracts, namely stevioside and rebaudioside A, are two of around ten other steviol glycosides present. In all toxicological studies the administered extract (dose), expressed as mg/kg bw of stevioside, was used to assess the toxicity of steviol glycosides. In particular, supplementary metabolism, pharmacological, mechanistic and toxicity studies have been reviewed to determine whether sufficient uncertainty remains to apply an additional two-fold safety factor to the acceptable daily intake (ADI) for steviol glycosides.

Stevioside is completely metabolised to steviol by the microbial flora of the caecum, with excretion occurring via the faeces in animals or by the urine in humans. Stevioside has very low acute toxicity and there is no evidence of carcinogenicity, developmental, reproductive or genotoxicity effects. Several studies in animals and humans indicate that steviol glycosides have antihypertensive and antiglycaemic effects. While the exact mechanism of action has not been fully elucidated, the absence of urinary metabolites apart from conjugated steviol suggests that unconjugated steviol is pharmacologically active in humans.

Steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day. The adequacy of the existing database and a new study in humans provides a basis for revising the uncertainty factor used by JECFA to derive the temporary ADI for steviol glycosides in 2005 (2 mg/kg bw/day steviol). In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore a full ADI of 4 mg/kg bw/day, derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study, has been established. This ADI covers steviol glycoside mixtures with different ratios of stevioside/rebaudioside.

Background

S. rebaudiana is an herb belonging to the chrysanthemum family which grows as a small shrub in parts of Paraguay and Brazil. The leaves of *S. rebaudiana* contain 10 different sweetening substances which are referred to as steviol glycosides and have traditionally been used as intense sweeteners. In leaf extracts 2 major (stevioside, rebaudioside A) and two minor (rebaudioside C and dulcoside) sweetening components can be isolated in a mixture to sweeten foods. Six other minor steviol analogues have been identified in extracts; namely, rebaudioside B, D, E, F, steviolbioside and rubudioside. The steviol glycoside content varies between 4 and 20 % of the dry leaf weight depending on variety and growth conditions, but is approximately 10 % in most crops grown in the field.

[4] Steviol glycosides is a collective term for steviol derivatives which are glycosylated with different side chains

Steviol glycosides are extracted from the leaves of *S. rebaudiana* Bertoni with hot water, followed by solvent purification of the water-soluble extract (Nabors, 2001; Hanson & Oliveira, 1993; Starratt et al 2002; Geuns 2004). Stevioside is a white crystalline powder with a molecular weight of 809 and has limited solubility in water (1.25g/L or 0.125%) (Nabors 2001). However, its solubility can be increased to 30% if at least 12% rebaudioside A is present in the steviol glycoside mixture (Goto & Clemente 1998).

The approximate concentrations of the four main steviol glycosides when extracted from S. *rebaudiana* leaves are listed in Table 1 (Nabors 2001). There is limited information on the concentrations of other constituents namely, rebaudioside B, D, E, F, steviolbioside and rubusoside when extracted from S. *rebaudiana* suggesting that these glycosides may occur in trace amounts or are unstable. It has been suggested that steviolbioside and rebaudioside B are generated as artefacts of the extraction process (Geuns 2004).

Table 1: Concentrations of steviol glycosides extracted from *S. rebaudiana*

Analogue	Approximate concentration (w/v)	Sweetness relative to sugar (times as sweet)
Stevioside	5-10	300
Rebaudioside A	2-4	250-450
Rebaudioside B	No data	300-350
Rebaudioside C	1-2	50-120
Rebaudioside D	No data	250-400
Rebaudioside E	No data	150-300
Rebaudioside F	No data	No data
Dulcoside A	0.4-0.7	50-120
Steviolbioside	No data	100-125
Rubusoside	No data	No data

Structure of stevioside and its analogues

Stevioside is a glycoside of the diterpene derivative steviol (ent-13-hydroxykaur-16-en-19-oic acid). Various analogues are produced by substitution on the R1 and R2 steviol skeleton (Figure 1) with either hydrogen, glucopyranosoyl (Glc) (glucose) or rhamnopyranosyl (Rha) (Rhamnose) groups (Table 2). In rebaudioside D and E, R1 is composed of 2 *b*-Glc-*b*-Glc(2®1). In rebaudioside A, B, C, D, E and F, the R2 group has an additional sugar moiety added on carbon 3 of the first b-Glc. In rebaudioside F one *b*-Glc is substituted for by -*b*-Xyl (Geuns 2004).

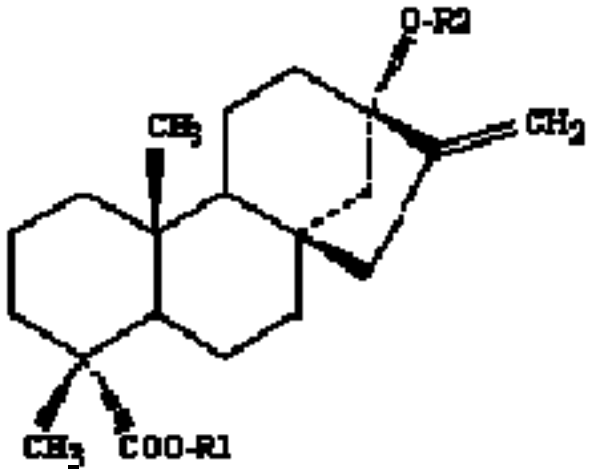

Figure 1: Diagram of steviol skeleton

Table 2: Comparison of R1 and R2 grouped on stevioside analogues

Compound name	R1	R2
steviol	H	H
steviolbioside	H	*b*-Glc-*b*-Glc(2-1)
stevioside	*b* -Glc	*b*-Glc-*b*-Glc(2-1)
rubsoside	*b* -Glc	*b* -Glc
rebaudioside A	*b*-Glc	*b*-Glc-*b*-Glc(2-1) | *b*-Glc(3-1)
rebaudioside B	H	*b*-Glc-*b*-Glc(2-1) | *b*-Glc(3-1)
rebaudioside C (dulcoside B)	*b*-Glc	*b*-Glc-*a*-Rha(2-1) | *b*-Glc(3-1)
rebaudioside D	*b*-Glc-*b*-Glc(2-1)	*b*-Glc-*b*-Glc(2-1) | *b*-Glc(3-1)
rebaudioside E	*b*-Glc-*b*-Glc(2-1)	*b*-Glc-*b*-Glc(2-1)
rebaudioside F	*b*-Glc	*b*-Glc-*b*-Xyl(2-1) | *b*-Glc(3-1)
dulcoside A	*b*-Glc	*b*-Glc-*a*-Rha(2-1)

Previous considerations of stevioside by the Joint Expert Committee on Food Additives

Fifty-first meeting of JECFA (WHO 1999)

The Joint (FAO/WHO) Expert Committee on Food Additives (JECFA) first assessed the toxicity of stevioside at its 51st meeting (WHO 1999). The following is an overview of this toxicological assessment:

- Following oral administration in rats, stevioside was hydrolysed by microflora in the colon to steviol with limited absorption of stevioside occurring in the upper small intestine. Steviol was completely absorbed from the gastro-intestinal tract, excreted in the bile and underwent enterohepatic circulation with an elimination half-life of 24h. Steviol is the only faecal metabolite of stevioside that has been identified and faecal excretion is the major route of elimination.

- Stevioside and/or steviol affected a variety of biochemical parameters in models *in vitro*, indicating possible mechanisms of antihypertensive and antiglycaemic effects that involve modulation of various ion channels.

- Stevioside has very low acute toxicity via the oral route, with LD_{50} ranges from >2-15g/kg bw in either rats, mice or hamsters. Dietary administration of stevioside to rats up to concentrations of 2.5% for two years had no significant toxicological effects (equivalent to 970 and 1100 mg/kg bw/day in males and females, respectively). At a higher dose of 5% (equivalent to 2000 and 2400 mg/kg bw/day in males and females, respectively) reduced bodyweight gain and survival were observed. There was no evidence of carcinogenic potential in long-term studies in rats.

- Oral administration of stevioside at doses up to 2500 mg/kg bw/day in hamsters and 3000 mg/kg bw/day in rats showed no reproductive effects. No teratogenic or developmental effects were observed in rats at doses up to 1000 mg/kg bw/day by gavage.

- Genotoxicity tests on stevioside were found to be negative; however, following metabolic activation steviol showed positive responses in *in vitro* forward mutation assays in bacteria, gene mutation and chromosomal aberration assays in Chinese Hamster lung fibroblasts. An *in vivo* mouse micronucleus assay was negative.

Owing to an incomplete toxicological database JECFA was unable to recommend an ADI. Additional information was sought on the metabolism of stevioside in humans, and further genotoxicity studies on the potential mutagenicity of steviol *in vivo*. In addition, JECFA requested that precise specifications should be developed that was representative of the material of commerce (namely, stevioside) that would be used in food.

Sixty-third meeting of JECFA (WHO 2005)

JECFA reconsidered stevioside at its 63rd meeting (WHO 2005) and noted that most of the data requested at the 51st meeting was available. Additional *in vivo* studies on DNA damage and micronucleus formation in rats, mice and hamsters indicated no evidence of genotoxicity of steviol up to 8000 mg/kg bodyweight.

JECFA concluded that stevioside and rebaudioside A are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*.

JECFA reviewed a number of *in vivo* studies on the effects of stevioside on blood glucose and blood pressure in animals and humans (Summarised in Part 1). JECFA noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to approximately 12.5–25 mg/kg body weight per day[5] (11 to 22 mg/kg bw/day for a 70 kg adult). The evidence available was inadequate to assess whether these pharmacological effects would also occur at lower concentrations of dietary exposure, which could lead to adverse effects in some individuals (e.g. those with hypotension or diabetes). JECFA therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans.

[5] Based on 60 kg adult weights

A temporary ADI of 2 mg/kg bw, expressed as steviol, was established for steviol glycosides on the basis of the No Observed Effect level (NOEL) for stevioside of 970 mg/kg body weight per day (or 383 mg/kg body weight per day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. At higher doses reduced bodyweight gain and survival were observed. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. JECFA noted that this temporary ADI only applies to products complying with the specifications.

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

At this same meeting JECFA established tentative specifications for stevioside and its other glycosides (rebaudioside A, C and dulcoside A). However, in order to remove the tentative designation from the specifications, further information was requested by the Committee to be submitted by 2007 on the following:

- analytical data on distribution and concentrations of all component steviol glycosides, including those that are not identified in the current specifications;
- method of analysis for the determination of all component steviol glycosides, including those that are not identified in the current specifications;
- the nature and concentration of the non-steviol glycosides fractions;
- the quantities of residual solvents from purifications steps of the manufacturing process; and
- the hydrolytic stability of the steviol glycosides in acidic foods and beverages.

Sixty eighth meeting of JECFA (WHO 2007)

No additional safety concerns were raised based on the newly available data. The Committee extended the temporary ADI of 0–2 mg/kg bw for steviol glycosides (expressed as steviol) pending submission of the results of ongoing toxicological and clinical studies, in particular studies addressing pharmacological effects, by the end of 2008. The specifications were revised and the tentative assignation was removed. The method of assay includes a minimum requirement of 95% of the total of 7 steviol glycosides. The Committee concluded that steviol glycosides are stable for use in foods (including acidic beverages) under normal conditions of use. All other outstanding issues regarding the method of manufacture were resolved.

JECFA will be considering additional safety data at its 69th meeting in June 2008.

Aims of the current assessment

FSANZ has not previously assessed the safety of stevioside. Therefore, the aims of the current assessment were to:

- review supplementary data on the absorption, distribution, metabolism and excretion (ADME), pharmacology and mechanism of action of stevioside in laboratory animals and humans to determine its safety as a food additive; and

- determine whether the new data affects the temporary ADI for stevioside.

Summary of supplementary data

The database for stevioside is adequate and consists of studies previously evaluated by JECFA in addition to supplementary data on its ADME, pharmacology, mechanism of action and toxicology. The data assessed were provided by the Applicant (unpublished studies) and from published papers. The full assessments of the supplementary data are at Parts 2-5.

Absorption, Distribution, Metabolism and Excretion

A number of *in vitro* and *in vivo* studies indicated that stevioside is not absorbed across the digestive tract in rats or humans and is not metabolised by any of the normal digestive enzymes (Wingard et al 1980; Nakayama et al 1986; Hutapea et al 1997; Koyama et al 2003 a & b; Geuns & Pietta 2004). In rats, stevioside is metabolised to steviol via an intermediate compound, steviolbioside, with enterohepatic recycling producing unidentified conjugated metabolites in the bile. Excretion is via the faeces, with 68% of the initial dose of stevioside excreted in the faeces by day 5 post-dose, 2% in the urine and the remainder in expired air (Nakayama et al 1986).

Steviol is produced via the bacterial conversion of stevioside in the caecum of rats and humans (Wingard et al 1980; Hutapea et al 1997; Koyama et al 2003b). In humans, steviol is transported to the liver where a steviol-glucuronide conjugate is formed with excretion via the kidneys and urine. Steviolbioside may also be an intermediate compound in humans. The principle excretion route for the steviol glucuronide conjugate is the urine (68%) and a small amount of free steviol is excreted via the faecal route (8%) with a total recovery of steviol (free and conjugated) of 76% (Geuns et al 2006 a & b).

Rat and human liver microsomes metabolised steviol to a number of monohydroxy and dihydroxy derivatives, which required an NADPH generating system; this suggested that cytochrome P450 may be involved in the oxidation of steviol and that phase 1 oxidation reactions may occur *in vitro* (Koyama et al 2003a). *In vivo* studies identified some steviol metabolites; namely, steviol-16,17α-epoxide in mice (Hutapea et al 1997) and isosteviol, 15-α hydroxysteviol and steviol-16,17, α-epoxide in the blood, faeces and urine of hamsters following a single oral dose of 1000 mg/kg bw (Hutapea et al 1999). However, there is no evidence of phase 1 metabolism *in vivo* in humans, as the recent studies in have not identified any steviol metabolites in blood, urine or faeces (Geuns & Pietta 2004; Simonetti et al 2004; Geuns et al 2006 a & b).

The production of steviol metabolites by human liver microsomes may be attributable to the use of dimethyl sulfoxide (DMSO) as a vehicle, which may have enhanced the solubility of steviol[6] and allowed phase 1 metabolism to occur (Koyama et al 2003a).

No stevioside or free steviol was detected in human plasma and a large variability in maximum concentrations (Cmax) of steviol glucuronide conjugates (0.7 to 21.3µg/mL) occurred from 0 to 7 h post-dose after a single 250 mg dose of stevioside, which followed two previous days of dosing at 750 mg stevioside/day (Geuns & Pietta 2004). *In vitro* studies with human faecal suspensions found that stevioside was degraded to the intermediate compound steviolbioside (peaking at 2-4h post incubation) followed by steviol (at 3-4h post incubation), with total conversion to steviol occurring within 10h (Gardana et al 2003).

[6] Steviol solubility <0.001%

Following a single oral dose of stevioside, steviol was also detected in the plasma of rats at 8 h, peaking at 24 h post dose (Wang et al 2004). A peak plasma steviol concentration of 18.31 μg/mL occurred 15 minutes post-dose following oral doses of steviol (Koyama et al 2003a).

Pharmacology studies

Effects of stevioside on blood glucose and insulin

A single intravenous (IV) dose of stevioside (200 mg/kg bw) to diabetic-induced rats increased the insulin response, decreased glucose and increased glucagon secretion (Jeppesen et al 2002. A single oral dose of stevioside at 500 mg/kg bw similarly reduced the product of the insulin and glucose incremental area-under-the-curve (AUC) (a measure inversely related to whole body insulin sensitivity) in obese insulin-resistant rats (Lailerd et al 2004). A reduction in the glucose and glucagon AUC and increased insulin secretion occurred in rats following repeated oral doses of 25 mg/kg bw/day for 6 weeks' duration (Jeppesen et al 2003).

Reductions in blood glucose and increased insulin release occurred following a single oral dose of stevioside of 14 mg/kg bw/day[7] in mildly obese patients with type 2 diabetes (Gregersen et al 2004). However, in normal healthy human subjects (Temme et al 2004; Barriocanal et al 2006 & 2008) or in type 1 or 2 diabetics (Barriocanal et al 2006 & 2008) administered oral doses of stevioside at 11 mg/kg bw/day or those with mild to moderate hypertension administered oral doses of stevioside at 11 mg/kg bw/day (Chan et al 2000) or 21 mg/kg bw/day (Hseih et al 2003) no effects on blood glucose and insulin concentrations were observed. This suggests that effects only occur when blood glucose concentrations are elevated, as in the diabetic state and that there is a relatively low risk of hypoglycaemia in normal subjects from consumption of dietary concentrations of stevioside.

Effects of stevioside on blood pressure (BP)

In rats, reduced BP occurred following repeated oral doses of stevioside of 25 mg/kg bw/day for 6 weeks (Jeppeson et al 2003) with a crude extract of stevia orally administered by gavage (2.67g dried leaves/day) for 30 days (Melis 1996) and with single oral and IV doses at 200 and 50 mg/kg bw respectively in dogs (Liu et al 2003).

Repeated oral doses of 11 to 21 mg/kg bw/day stevioside for a 1 or 2 year period, respectively, in mild to moderately hypertensive subjects significantly decreased BP compared to placebo controls (Chan et al 2000; Hseih et al 2003).

At doses up to 15 mg/kg bw/day crude stevioside preparations did not reduce BP in mildly hypertensive humans (Ferri et al 2006). At doses of stevioside of 11 mg/kg bw/day for a 3-month period in type 1 and 2 diabetics and non-diabetics with normal/low BP, no significant differences were observed in mean BP between control and treated subjects for all three groups (Barriocanal et al 2006 & 2008) or in normal subjects for a 3 day period (Guens et al 2007).

[7] Based on 70 kg adult weights

Mechanistic studies

Oral doses of stevioside lowered blood glucose concentrations in normal and diabetic induced rats in a dose-dependent manner with maximum reductions observed at 90 minutes post-dose at doses of 10 mg/kg bw/day with no further reductions noted when the period of blood sampling was extended to 15 days. Significant reductions in phosphoenol pyruvate carboxylase (PECK) mRNA and protein concentrations were observed, suggesting that a possible mechanism of action of stevioside may be to regulate blood glucose concentrations by decreasing PEPCK expression in the liver. The authors proposed that this may lead to a decrease in gluconeogenesis and a subsequent decrease in hyperglycaemia in diabetic-induced rats (Chen et al 2005).

Stevioside orally administered at a dose of 5.5 mg/kg bw/day for 15 days had no effect on fasting blood glucose concentrations; whereas, stevia powder at doses of 20 mg/kg bw/day decreased glucose concentrations in fasted rats. Reduction of hepatic gluconeogenesis via inhibition of the two key enzymes pyruvate carboxylase (PC) and PEPCK were proposed by the authors as a mechanism by which glucose may be reduced by oral doses of stevia powder in rats (Ferreira et al 2006).

Exposure of clonal α-TC1-6 cells to fatty acids resulted in glucagon hypersecretion and triglyceride accumulation. Stevioside was able to reduce the release of glucagon, possibly by enhanced expression of genes involved in fatty acid metabolism leading to increased mRNA expressions of carnitine palmitoyltransferase, PPARγ and stearoyl-CoA desaturase (Hong et al 2006).

Rebaudioside A was shown to dose dependently increase insulin secretion from mouse islets with the effects of rebaudioside A on insulin secretion glucose-dependent and only occurred with glucose concentrations > 6.6 mmol/L (Abudula et al 2004).

An increase in intracellular insulin concentration occurred in mouse pancreatic islets cells pre-treated with stevioside. In contrast, glibenclamide, a stimulator of insulin secretion that is used to treat diabetes, decreased the insulin concentration. Glibenclamide but not stevioside stimulated basal insulin secretion whereas glucose stimulated insulin secretion was increased by pre-treatment with stevioside (Chen et al 2006).

Proposed mechanisms by which BP reductions occur are via an increase in renal plasma and urine flow and sodium excretion leading to a vasodilating effect on the kidney resulting in blood pressure reductions (Melis et al 1996). Alternatively, a vasodilatation effect on vascular smooth muscle cells has been proposed, involving the inhibition of calcium into the blood vessels (Lee et al 2003).

More recently, a study by Wong et al (2006) suggested that isosteviol (a metabolite of steviol) inhibits angiotension-II-induced cell proliferation and endothelin-1-secretion via reductions in reactive oxygen species generation in the smooth muscle of rat aortas. However, the significance of this study for humans is unclear, as only limited or trace amounts of isosteviol would be expected to occur in stevioside mixtures used as a food additive suggesting that unless isosteviol was administered at very high doses it is unlikely to exert a pharmacological affect in humans at doses encountered in the diet.

In summary, studies in animal and humans suggested that the mechanism of action of stevioside on blood glucose concentrations may be mediated by increased insulin secretion, which occurs only when glucose concentrations are elevated. This effect may also be mediated by altering secretion of glucagon and possibly by key enzymes that control blood glucose concentrations in the liver. Although administration of stevioside to humans produced reductions of blood pressure in patients with already elevated blood pressure, the mechanism by which stevioside excreted this effect remains to be determined.

Toxicity studies

In the pivotal study of Toyoda et al (1997) used by JECFA as the basis of the temporary ADI, stevioside was admixed in the diet and fed *ad libitum* to rats for two years at dietary levels of 0, 2.5 or 5% (achieved doses of 0/0, 969/1120 and 1997/2387 mg/kg bw/day in males/females, respectively). Final survival was significantly lower in high-dose males relative to the controls. However, this result was attributed to the rapid onset of spontaneous large granular lymphocyte leukaemia resulting in more rats being sacrificed or dying during the last few weeks of the study. While a dose-related increase in the incidence of this leukaemia relative to the control occurred, it was not statistically significant or outside the normal range for age and sex-matched rats. On this basis the lower final survival in high-dose males was not considered treatment-related. An apparent treatment-related effect on bodyweight gain and terminal bodyweight was attributable to reduced caloric intake and was therefore not considered toxicologically-significant. There was no evidence of any carcinogenic potential. The NOEL was 1997 mg/kg bw/day in males and 2387 mg/kg bw/day in females, based on the absence of any toxicologically-significant effects at these doses. It should be noted that JECFA interpreted the decreased survival and bodyweight gain in high-dose males as treatment-related and toxicologically significant and therefore set the NOEL at 973 mg/kg bw/day.

Rebaudioside A was admixed in the diet and fed *ad libitum* to rats at nominal doses of 0, 500, 1000 or 2000 mg/kg bw/day for up to 93 days (actual doses of 0/0, 517/511, 1035/1019 and 2055/2050 mg/kg bw/day in males/females, respectively). High-dose males exhibited significantly reduced food conversion efficiency at weeks 1, 4 and 8, and terminal bodyweight, relative to the control, while food consumption was unaffected; this effect was therefore attributable to a reduced caloric intake due to the relatively high proportion of test material in the feed rather than to a direct toxicological effect of rebaudioside A. There were no other treatment-related effects. The NOEL was 2050 mg/kg bw/day (or 680 mg/kg bw/day steviol), based on the absence of any toxicologically-significant effects at this dose. (Nikiforov & Eapen 2008)

Discussion

The toxicological database for stevioside now covers an adequate range of endpoints. It consists of studies previously evaluated by JECFA in addition to supplementary data on its ADME, pharmacology, mechanism of action and toxicity.

The *in vitro* and *in vivo* ADME studies suggest that stevioside is not absorbed but is first metabolised to the metabolite steviol by microflora in the caecum (via successive hydrolysis of glucose units) and that the process is similar in animals and humans (Wingard et al 1980; Hutapea et al 1997; Koyama et al 2003b; Geuns et al 2006 & 2007).

A key difference between animals and humans is that in animals uncharacterised steviol conjugates are formed and excreted via the faeces (Nakayama et al 1986); whereas, in humans steviol is conjugated with glucuronide and excreted via the urine (Geuns et al 2006a&b).

There are a number of factors that are likely to influence the efficiency of conversion of stevioside to steviol thereby leading to interspecies and intraspecies differences in metabolism and the extent to which effects (e.g. pharmacological effects) are observed in animal and human studies:

- transit time through the gastrointestinal tract (faster times may lead to less ability for the microbial flora to convert stevioside to steviol);
- bile production (which varies between animals and individuals and will influence breakdown of steviol);
- the food matrix (which may also affect transit time, i.e. higher fat –containing foods will have slower transit times);
- differences in the gut microflora between animals and humans; and
- differences in gut microflora between individual humans due to the extensive variety of food a human is exposed to in their diets compared to a standard rat diet which would lead to less changes in microflora of the caecum.

In light of the above factors and that the human studies were conducted in Chinese (Chan et al, 2000; Hseih et al 2003), South American (Ferri et al 2006; Barriocanal et al 2006 & 2008) or European populations (Temme et al 2004; Gregersen et al 2004) it is plausible that there is likely to be a difference in the ability of subjects selected from different populations to convert stevioside to steviol, with some individuals being efficient converters and others less so.

This may account for the reductions in blood pressure observed at a dose of 750 mg/day (11 mg/kg bw/day) in Chinese subjects (Chan et al 2000) suggesting that they are more efficient converters; however, at the same dose no effects were observed in European (Temme et al 2004) or South American subjects (Barriocanal et al 2006 & 2008) suggesting they have poorer efficiency of conversion.

A parallel example exists in the literature in regard to the intense sweetener cyclamate. In humans non-absorbed cyclamate is converted to the metabolite cyclohexylamine by bacteria in the gastrointestinal tract. Once conversion has occurred, cyclohexylamine is rapidly and completely absorbed from the large intestine and primarily excreted unchanged in the urine. However, the extent of conversion varies substantially between individuals and within individuals over time, not all individuals are able to convert cyclamate and the proportion of converters may be slightly lower in European and North American populations and higher among Japanese populations (Bopp & Price 2001). For example, the high carbohydrate-based diets of the Chinese populations may alter the colonic microflora and lead to increased efficiency of conversion to steviol and hence greater ability to exert effects on blood pressures within a relatively short time period. Therefore, the observed blood pressure reductions at the lowest dose tested in humans (11 mg/kg bw/day) may therefore be specific and somewhat confined to the Chinese population and not be applicable to more general populations groups (e.g. European populations).

There have been no recent studies that have assessed the effects of direct exposure to steviol in the diet of either animals or humans. Whilst recent studies have detected steviol-glucuronide conjugates in the blood and urine (Geuns & Pietta 2004; Geuns et al 2006 & 2007) in humans and free steviol in the blood plasma of animals (Wang et al, 2004; Koyama et al 2003a) there appears to be an inability to detect the free form of steviol in human blood plasma. Possibly, blood samples should have been taken earlier in the dosing period (e.g. on day 1 or 2) in the human studies rather than on day 3 in order to detect free steviol (Geuns & Pietta, 2004; Geuns et al 2006 a & b).

Single or repeated doses of stevioside reduced blood glucose and increased insulin release in diabetic-induced rats. It has been proposed that these effects may be mediated by increased insulin secretion and insulin sensitivity[8] or glucagon reductions (Jeppeson et al 2002, 2003 & 2006; Lailerd et al 2004, Chen et al 2005, Hong et al 2006) or by decreased PEPCK[9] gene expression in the liver leading to a decrease in gluconeogenesis in diabetes-induced rats (Chen et al 2005) or more recently by regulation of acetyl-CoA carboxylase (Chen et al 1997). Studies in humans have suggested that oral administration of stevioside to normal healthy subjects at doses up to 750 mg/day (equivalent to 11 mg/kg bw/day steviol) does not affect glucose or insulin concentrations (Temme et al 2004; Barriocanal et al 2006 & 2008) or in subjects with mild to moderate hypertension, receiving repeated doses of stevioside from 11 to 21 mg/kg bw/day for either 1 or 2 years duration, respectively (Chan et al 2000; Hseih et al 2003).

Reductions in blood glucose and increased insulin released occurred post-prandial following single oral doses of stevioside of 14 mg/kg bw/day in mildly obese patients with type 2 diabetes (Gregersen et al 2004).

These studies in humans suggest that effects of stevioside or stevioside/rebaudioside mixtures (e.g. increased insulin secretion or reduced glucagon release) may only occur when blood glucose concentrations are elevated (as in the diabetic state) and at doses >11 mg/kg bw/day and that there is a relatively low risk of hypoglycaemia in normal healthy human subjects, particularly at concentrations that may be encountered in the diet. This is supported by recent *in vitro* studies in which high concentrations of glucose were required to stimulate the release of insulin following pre-incubation of Islet of Langerhan cells with stevioside (Jeppesen et al 2000; Chen et al 2006) or rebaudioside A (Abdula et al 2006) whereas, at normal glucose concentrations no or limited insulin secretion occurred. In summary, the weight-of-evidence indicates that stevioside would be unlikely to produce hypoglycaemia in humans at concentrations encountered in the diet.

In rats reductions were observed in BP following oral doses of stevioside of 25 mg/kg bw/day for 6 weeks (Jeppesen et al 2003) and with single oral and IV doses at 200 mg/kg bw and 50 mg/kg bw, respectively in dogs (Liu et al 2003). One proposed mechanism in rats is an increase in renal plasma and glomerular filtration rates and subsequent urinary output which lowers renal vasculature resistance leading to blood pressure reductions (Melis et al 1996).

[8] Insulin sensitivity refers to the effectiveness of insulin or the quantity of glucose that moves into cells as a result of the action of insulin

[9] PEPCK is the rate-limiting enzyme for gluconeogenesis

More recent studies have suggested that the vasodilatation effect is via the inhibition of calcium into vascular smooth muscle cells (Liu et al 2003; Lee et al, 2003) similar to the action of the human antihypertensive drugs nifedipine and verapamil[10] or hydralazine drugs (such as apresoline, vasodilan and loniten) which exert a peripheral vasodilating effect through direct relaxation of the smooth muscle tissue. A recent study suggested that isosteviol (a metabolite of stevioside) inhibits angiotension-II-induced cell proliferation and endothelin-1-secretion in rat aortic smooth muscle cells both of which have been implicated in the pathogenesis of chronic vascular disease (Wong et al 2006). Although studies have identified isosteviol as being a metabolite of stevioside in rats and hamsters (Hutapea et al, 1999; Nakayama et al 1986) recent studies in humans have not identified isosteviol as a metabolite of stevioside in humans following oral doses of stevioside.

Repeated oral doses of stevioside at doses of 11 or 21 mg/kg bw/day for a 1- or 2-year period respectively, in mild to moderately hypertensive subjects, significantly decreased BP compared to placebo controls (Chan et al 2000; Hseih et al 2003). At doses up to 15 mg/kg bw/day crude stevioside preparations did not reduce BP in mildly hypertensive humans up to a period of 11 weeks (Ferri et al 2006). Contributing factors to the lack of effect on blood pressure in this study may have been the limited number of subjects, purity of stevioside and/or rebaudioside A mixture, or that the frequency of administration was twice a day *versus* three times/day in the Chan et al (2000) and Hseih et al (2003) study.

In contrast, oral doses of stevioside at 11 mg/kg bw/day for 2-3 days duration did not reduce blood pressure in normal healthy subjects (Temme et al 2004) or subjects with Type 1 or 2 diabetes and normal/hypotensive subjects when administered for 3 months (Barriocanal et al 2006 & 2008). The reasons for the inconsistency in results at the same dose are unclear as effects on the smooth muscle of blood vessels would be expected to occur irrespective of the clinical state (hypertensive or hypotensive).

However, the recent results in the study by Barriocanal et al (2006 & 2008) suggests that there is no real evidence that stevioside administered to normotensive or hypotensive individuals would lower blood pressure to a degree that would be considered an adverse effect.

In addition, in the study by Chan et al (2000) and Hseih et al (2003) the subjects had higher initial baseline blood pressures as they were mild to moderately hypertensive to start with, compared to normal or normal/hypotensive subjects in the other studies (Temme et al 2004; Barriocanal et al 2006 & 2008) which may partly explain why reductions were observed at the same dose.

Although the database is extensive many of the key studies in humans which investigated the effects of stevioside on blood glucose and BP did not report the purity of stevioside or mixtures of stevioside/rebaudioside (Chan et al 2000, Hseih et al 2003, Ferri et al 2006, Barriocanal et al 2006 & 2008). This did not allow absolute assessment of whether the purity of stevioside would be representative of the expected specifications of stevioside that humans would be exposed to in the diet. In addition, as only one dose level is used in the pivotal human studies (Gregersen et al 2004, Temme et al 2004, Chan et al 2000, Hseih et al 2003) it precluded the establishment of a NOEL on which an ADI could be established.

[10] Nifedipine and Verapamil are used to treat high blood pressure by blocking calcium channels

A recently published 90-day dietary toxicity study conducted in Sprague-Dawley rats (Nikiforov & Eapen 2008) determined that rebaudioside A, which is also metabolised to steviol in the gastrointestinal tract [albeit more slowly than stevioside (WHO 2006)], did not cause any toxicologically-significant effects up to and including the highest dose of 2050/2055 mg/kg bw/day in males/females [equivalent to 680 mg/kg bw/day steviol using a conversion factor of 33% (relative molecular weight of rebaudioside A = 966 and steviol = 318)]. Similarly, when stevioside was administered to F344 rats for 2 years, no toxicologically-significant effects were evident up to and including the highest dose of 1997/2387 mg/kg bw/day in males/females [equivalent to 800 mg/kg bw/day steviol using a conversion factor of 40% (relative molecular weight of stevioside = 805 and steviol = 318)] (Toyoda et al 1997). It is noteworthy that in both of these studies, apparent treatment-related effects on bodyweight gain and/or final bodyweight were attributable to reduced caloric intake due to the relatively high proportion of test material in the feed. As these studies essentially used limit doses, it is likely that the true NOEL is greater than the above figures.

Conclusions

This review of supplementary data indicated that stevioside is metabolised completely to steviol in the gastrointestinal tract, which is absorbed into the blood stream and then exerts a pharmacological effect by lowering blood pressure and blood glucose. While the precise mechanism of pharmacological action remains to be defined, stevioside is unlikely to produce hypoglycaemia or hypotension in humans at concentrations encountered in the diet. Studies previously reviewed by JECFA confirm the low toxicity potential of stevioside. On this basis, there are unlikely to be any safety issues associated with the use of stevioside as a sweetener.

No suitable human study was identified that could serve as a basis of an ADI for stevioside. However, steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day.

The adequacy of the existing database and a new study in humans provides a basis for revising the uncertainty factor used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required.

As the ADME data indicated that stevioside is completely converted to steviol in animals and humans, the ADI is expressed in terms of steviol equivalents. This allows for any variability in the individual glycosides in mixtures of steviol glycoside extracts (e.g. different ratios of stevioside/rebaudioside) to be accounted for by the ADI being expressed in steviol equivalents. Therefore, based on this complete metabolism, a conversion factor of 40% from the steviol glycoside, stevioside (relative molecular mass: stevioside, 805; steviol, 318) to steviol is used to calculate the ADI.

Therefore a full ADI of 4 mg/kg bw/day, derived by applying a 100-fold safety factor to the JECFA NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study, has been established.

However, it should be noted that the true NOEL for steviol is likely to be higher than 383 mg/kg bw/day. As discussed in the current report, FSANZ does not interpret the lower survival and bodyweight gain in high-dose males in the 2-year rat study as toxicologically significant and therefore the NOEL would be 2000 mg/kg bw/day (equivalent to 800 mg/kg bw/day steviol), the highest dose tested. This NOEL is supported by the supplementary 90-day toxicity study in rats on rebaudioside A, where the NOEL was 2050 mg/kg bw/day (equivalent to 680 mg/kg bw/day steviol), the highest dose tested.

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PART 1: Summary of key *in vivo* studies reviewed by JECFA (WHO 1999 and 2005)

SUMMARY OF ANIMAL STUDIES

Authors	Dose	Animals	Effects on BP	Effects on glucose and insulin concentrations	Additional comments
Jeppesen et al (2002)	Single doses of 0 or 200 mg/kg bw stevioside intravenous in saline vehicle before administration of IV glucose at 2000 mg/kg bw Purity stevioside 96%	Groups of six Goto-Kakizaki (GK) rats or groups of 12-14 normal Wistar controls	Not studied	Stevioside increased insulin AUC 57% ($p<0.05$), decreased glucose AUC 32% ($p<0.05$) and glucagon AUC 34% ($p<0.05$) in GK rats at 120 minutes post-dose. In Wistar rats, insulin AUC was transiently increased 78% at 15 minutes ($p<0.001$) and returned to control concentrations after 90 minutes post-dose, but no significant effects were observed in blood glucose or glucagon concentrations.	
Lailerd N et al (2004)	Single doses of 0, 200 or 500 mg/kg bw stevioside by gavage before administration of glucose (1000 mg/kg bw) at 2h post stevioside dose. Purity stevioside not stated	Groups of 5 lean insulin-sensitive and obese insulin-resistant Zucker rats	Not studied	No effects on glucose or insulin were observed at doses of 200 mg/kg bw. At 500 mg/kg bw the product of the glucose and insulin incremental AUC was reduced 42% ($p<0.05$) in lean rats with no effect on glucose concentrations. In obese rats the AUC for insulin and glucose were reduced by 30 and 45% ($p<0.05$), respectively.	The product of the glucose and insulin incremental AUC is a measure that is inversely related to whole body insulin sensitivity.
Jeppesen et al (2003)	0 or 25 mg/kg bw/day stevioside in a water vehicle orally for 6 weeks followed by an arterial glucose tolerance test at 2000 mg/kg bw. Purity stevioside >99.6%)	Groups of Goto-Kakizaki (GK) rats (numbers not stated)	Significant decrease ($P<0.001$) in mean BP from 153±5 mm Hg/83±1 mm Hg to 135±2/74±1 in the treated group	Glucose AUC reduced 35% ($p<0.05$), insulin AUC increased 60% ($p<0.05$) and glucagon AUC decreased 42%.	

Authors	Dose	Animals	Effects on BP	Effects on glucose and insulin concentrations	Additional comments
Melis (1996)	0 or 2.67g stevia extract orally for 30 days following hypertension induced by a clip on left renal artery Purity stevioside not stated	Groups of 10 Wistar rats	In controls and hypertensive rats mean arterial pressure was significantly reduced from 155±3 mmHg to 108±4 mm Hg ($p<0.05$) following stevia administration.	Not studied	Increase in renal plasma and urine flow and sodium excretion in both normotensive and hypertensive rats following oral doses of stevia extract.
Liu et al (2003)	Single doses of 0 or 200 mg/kg bw stevioside powder administered nasogastrically or 50 mg/kg bw by IV in a saline vehicle to normal healthy dogs Purity stevioside not stated	8 healthy mongrel dogs and 8 dogs which were made hypertensive by ligation of left renal artery	Significant decrease ($p<0.01$) in mean BP from 148±33.3/104.3±19.2 mm Hg to 135.4±32.6/94.7±17 mm Hg after nasogastric doses of stevioside. Significant decrease ($p<0.01$) in mean BP from 165.8±16.3/108±21.5 mm Hg to 130.2±39.2/65.1±20 mm Hg 10 minutes after IV doses of stevioside.	Not studied	

SUMMARY OF HUMAN STUDIES

Authors	Dose	Subjects	Effects on BP	Effects on glucose and insulin concentrations	Additional comments
Gregersen et al (2004)	Single doses of 0 or 1000 mg/day stevioside capsules (14 mg/kg bw/day*) administered post-prandial Purity stevioside 91%/rebaud. A 4%	12 overweight or mildly obese patients with type 2 diabetes	No significant effects when measured from 15 to 240 minutes post-dose (compared to placebo-control)	Area under plasma glucose and glucagon curve (AUC) decreased 18% and 19% respectively ($p<0.02$). Non-significant increase in area under insulin response curve. The insulinogenic index (ratio of AUC insulin/AUC glucose) – a measure of insulin secretion, was increased by 40% ($p<0.001$).	Mean baseline Blood pressures: 146/86 mm
Temme et al 2004	750 mg/day stevioside capsules (11 mg/kg bw/day) in 3 (250 mg) divided doses for 2 days. No control groups Purity stevioside >97%	9 normal healthy subjects	No significant effect when measured at 30 or 60 minutes post-dose on the third day of treatment (compared to pre-treatment values)	No significant effect on blood glucose or insulin concentrations when measured at 30 or 60 minutes post-dose on the third day of treatment (compared to pre-treatment values)	No adverse effects observed. Stated in methods that stevioside was administered for 3 days; however, a dose of stevioside at 250 mg was administered on day 3 but it was not stated whether further doses were given Mean baseline Blood pressures: 116/74 mm

Authors	Dose	Subjects	Effects on BP	Effects on glucose and insulin concentrations	Additional comments
Chan et al (2000)	0 or 750 mg/day stevioside capsules (11 mg/kg bw/day) in 3 (250 mg) divided doses for 1 year. Purity stevioside not stated	106 patients with mild-moderate hypertension (60 treated and 46 placebo controls)	Significant ($p<0.05$) decrease in mean in blood pressure (166.5±7.4 mmHg / 102.1±4 mmHg to 152.6±6.8 / 90.3±3.6) in the treated group than in the placebo group (166.0±9.4 mmHg / 104.7±5.2 mmHg to 164.8±8.7 / 103.8±5.4)	No significant effect on blood glucose between controls and treated groups	Mean baseline Blood pressures: 166/104 mm
Hseih et al (2003)	0 or 1500 mg/day stevioside capsules (21 mg/kg bw/day) in 3 (500 mg) divided doses for 2 years. Purity stevioside not stated	168 patients with mild hypertension (82 treated and 86 placebo controls)	Significant decrease ($P<0.05$) in mean BP from 150±7.3 mm Hg/95±4.2 mm Hg to 140±6.8/89±3.2 in the treated group than in the placebo group (149.0±6.0 mmHg / 96±4.2 mmHg to 150±7.0 /95.8±4.8)	No significant effect on blood glucose between controls and treated groups	Baseline Blood pressures: 140-159 mm (systolic)/90-99 mm (diastolic)

***Based on 70 kg adult weight**

Part 2 – Evaluation of supplementary ADME studies

Animal studies

In vitro

Wingard RE, Brown JP, Enerlin FE et al (1980) Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. Experientia 36: 519-520

The intestinal degradation and absorption of stevioside and rebaudioside A was studied in whole cell suspensions of bacteria isolated from the rat caecum. Stevioside (2.5 mg/mL), rebaudioside (3 mg/ml) and steviol (0.2 mg/ml) (source and purities not stated) were incubated with whole cells for 2-6 days and the concentrations were measured by HPLC. Stevioside was metabolised into steviol within 2 days (100% recovery), whereas rebaudioside required 6 days (100% recovery). This study indicated that intestinal bacteria in the rat colon can metabolise stevioside and rebaudioside to steviol.

Hutapea AM, Toskulkao C, Buddhasukh D et al (1997) Digestion of stevioside, a natural sweetener, by various digestive enzymes. J. Clin. Biochem. Nutr. 23: 177-186

This study investigated whether stevioside (extracted and purified from dried S. *rebaudiana* leaves; purity not stated) in a water vehicle could be metabolised by digestive enzymes from animals (salivary and pancreatic α-amylases, saliva, pepsin, gastric secretions, pancreatin and intestinal brush border membrane enzymes) and by the intestinal microflora (caecal suspensions) of mice, rats and hamsters. The study also sought to determine the presence of key metabolites of stevioside (steviol, steviol-16, 17α-epoxide, 15α-hydroxysteviol, steviolbioside and isosteviol) using HPLC. Stevioside could not be hydrolysed by any of the digestive enzymes. Stevioside was degraded by the microflora from the caecum to steviol with a >90% recovery at the end of a 2-day incubation period in rats and hamsters and 64% in mice. At 4 days post-incubation 100% recovery was observed in rats, hamsters and 71% in mice. The only other metabolite identified was steviol-16, 17α-epoxide, with a recovery of 31% in mice and no detections in rats or hamsters.

Koyama E, Sakai N, Ohori Y et al (2003a) Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglyocne, steviol, in rats and humans. Food. Chem. Toxicol 41: 875-883

The absorption and hepatic metabolism in rats of stevia mixture (rebaudioside A 29%, rebaudioside C 25%, stevioside 17% and dulcoside A 10%; Japan Stevia Industrial Association, Tokyo) and steviol were investigated. Everted gastro-intestinal sacs were incubated with 0.5 mg/mL stevia mixture or 0.1 mg/mL steviol for 30 minutes. Salicylic acid was used as a positive control to confirm that the sacs were functional. Steviol was rapidly transported across the duodenum-jejunum and the ileum (76 and 95% of salicylic acid transport, respectively); however, stevia mixture was poorly absorbed with >93% remaining in the mucosal fluids.

This suggested that there is limited absorption of stevia mixture *in vitro* in the upper intestine (duodenum, jejunum and ileum), whereas, steviol was readily absorbed. Mass spectral analysis of rat liver microsomes following incubation with 1 mM steviol for 120 minutes detected monohydroxy and dihydroxy metabolites.

In Vivo

Wingard RE, Brown JP, Enerlin FE et al (1980) Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. Experientia 36: 519-520

Steviol synthesised by the performing laboratory from steviol acetate (1 ml, 1.7 µCi, 0.7 mg; purity not stated) radiolabelled with ^{14}C (position of radiolabel not reported) was administered intracaecally to 3 rats (strain, age & bodyweights not reported) in a 0.5% klucel suspension, the bile ducts ligated and urine, faeces and expired air was collected (period of collection not stated). Radiolabelled ^{14}C steviol (1 ml, 1.7-2.63µCi, 0.7 mg) was also administered intracaecally to 2 rats with the bile duct cannulated, 5 rats with the bile duct ligated and by gavage to 3 rats without the bile duct ligated. Bile, urine and faeces were collected for 72h post-dose. The principal excretion pathway in bile duct ligated rats was via the urine (94% of administered dose) and in the bile of the cannulated rats following intracaecal administration of steviol (105% recovery) and in faeces of non-canulated rats (96% recovery) following oral administration. Although there were limited numbers of rats used in this study, it suggested that there was absorption of steviol via the intracaecal and oral route of administration, enterohepatic circulation occurred in the rat and faecal excretion was a major route of elimination following oral administration of steviol.

Nakayama K, Kasahara D & Yamamoto F (1986) Absorption, distribution, metabolism and excretion of stevioside in rats. J. Food Hyg. Soc. Jpn. 27: 1-8

Stevioside (Technical grade; Tama Seikagaku Company) radiolabelled with ^{3}H (position of radiolabel not reported) was administered to groups of 3-7 male Wistar rats (weight between 180 to 300g, aged not stated) by single oral gavage at 125 mg/kw bw in a 2% gum arabic solution. Blood was collected from 0.5 to 120 h post-dose, urine and faecal samples at 24h intervals for 120h and expired air was collected (period not stated). In some animals (number not stated) the bile duct was cannulated and bile was collected at 24h intervals for 72h. Animals were sacrificed at 1, 4, 24 and 48h post dose and a range of organs and tissues were removed to determine the distribution of radioactivity.

Radioactivity reached a maximum level in the blood at 8h post dose (4.8 µg/mL) and then decreased slowly with an elimination half-life of 24h. At 4h the highest level of radioactivity was in the caecum (283 µg/g) with the fat containing concentrations of 18 µg/g. At 24h, the concentrations of radiolabel were as follows: blood (2.6 µg/g), liver (5.7µg/g), kidney (3.17 µg/g), adrenal gland (12µg/g), small intestine (8.8µg/g), caecum (40µg/g), large intestine (12µg/g) and fat (12µg/g). At 48 h, concentrations had decreased in the caecum (26 µg/g) but the fat contained 15 µg/g and the adrenal gland 21 µg/g.

Stevioside was mainly distributed to the GI tract. At 48h post-dose the concentrations in the caecum were approximately 2-5 times higher than in the blood and other tissues with the exception of the fat and adrenal glands.

The distribution of stevioside and its metabolites in the GI tract, faeces and bile were measured by thin-layer chromatography (TLC) and the results summarised in the following table.

Compound (% radioactivity)	Stomach	Small intestine	Caecum	Bile	Faeces
Stevioside	Major component (1h)	33% (1h) 7.6% (4h)	39.4% (4h) Not detected (24h)	Not measured	Not measured
Metabolites (steviolbioloside, steviol and isosteviol)	Not measured	67% (1h)	Not measured	Not measured	Not measured
Steviolbioside	Not measured	8% (4h)	16.7% (4h)		
Steviol	Not measured	7.5% (4h)	5.1% (4h) 15.6% (24h)	51% (24h) 63% (48h)	39% (72h)
Unidentified metabolites	Not measured	Not measured	68% (24h)	19% (48h)	Not measured

By day 5 post-dose, 68% of the administered dose had been excreted in the faeces, 2% in the urine and 24% in expired air. In bile duct cannulated rats initial excretion in the bile was low up to 24h but increased rapidly to 41% by day 3. This study suggested that stevioside is not absorbed in the GI tract of rats but is metabolised to steviolbioside, steviol and other unidentified metabolites. Although there was wide distribution of stevioside to key organs and tissues, the GI tract was the principle site of distribution in rats. The main route of excretion of steviol was in the faeces, with accompanying unidentified metabolites in the faeces. The results from the biliary excretion study suggested that enterohepatic circulation of steviol occurs in rats with the liver converting steviol to unidentified steviol conjugates, which were excreted into the GI tract through the bile. (Nakayama et al 1986).

Sung LH (2002) Report on the pharmacokinetic studies of T100 Sunstevia 95% stevioside in rats. Sunlabel Pty Ltd, 21 Marsiling Industrial Estate, Road 9, Singapore 739175. Unpublished report

T100 Sunstevia (Wako Pure Chemical Industry, Japan; containing 70% stevioside) in a water vehicle was administered by gavage to groups of 6-8 male Sprague-Dawley rats (weighing 200 to 250g, aged not stated) at doses of 500 or 2000 mg/kg bw. Blood was collected at 0, 5, 10, 30, 60, 120, 180, 300, 480 and 1440 minutes post-dose and urine and faeces after 48h post-dose.

The bile duct was cannulated and stevioside administered by gavage to groups of 3 rats at 500 or 2000 mg/kg bw and bile collected at 0-60, 60-120, 120-180, 180-240, 240-360, 360-420 and 420-480 minutes. Stevioside was detected by HPLC in the plasma at 5 minutes post-dose; however, there was a large variability in the Cmax of stevioside in plasma between 10 to 300 minutes post-dose. Stevioside was excreted in the faeces (5.7-16.9% for doses of 500 or 2000 mg/kg bw) and urine (1 to 6.7%, dose not stated in results) of the total administered doses; however, the authors noted that the urine may have been contaminated with faeces. Steviol was not detected in plasma or bile but was found in the faeces (1.05 to 5.36 mg at doses of 500 mg/kg bw and 2.34 to 11.68 mg at doses of 2000 mg/kg bw).

This study is in contrast to the previous animal studies demonstrating limited or no absorption of stevioside occurs. Due to the methodological problem outlined by the authors in this study no specific conclusions can be made.

Koyama E, Sakai N, Ohori Y et al (2003a) Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglyocne, steviol, in rats and humans. Food. Chem. Toxicol. 41: 875-883

The absorption of a stevia mixture (rebaudioside A 29%, rebaudioside C 25%, stevioside 17% and dulcoside A 10%; Japan Stevia Industrial Association, Tokyo) or steviol was investigated. Following single oral doses to four male Sprague-Dawley rats (8-9 weeks old, 312-390g) of steviol 45 mg/kg bw in a corn oil vehicle or stevia mixture (125 mg/kg bw) in a 2% w/v gum Arabic vehicle, the time-dependent portal plasma concentration profiles of steviol were observed. A peak plasma concentration for steviol of 18.31µg/mL was observed 15 minutes post-dose; however, the profile of the stevia mixture differed considerably, with an initial delay before steviol was detected at 2 h before a peak of 5µg/mL observed at 8h, possibly due to the time required to pass into the large intestine and breakdown to steviol and other metabolites.

Human studies

In vitro

Hutapea AM, Toskulkao C, Buddhasukh D et al (1997) Digestion of stevioside, a natural sweetener, by various digestive enzymes. J. Clin. Biochem. Nutr 23: 177-186

This study investigated whether stevioside (extracted from dried *S. rebaudiana* leaves, purity not stated) could be metabolised *in vitro* by intestinal microflora (caecal suspensions) from humans. The study also sought to determine the presence of key metabolites of stevioside (steviol, steviol-16, 17α-epoxide, 15α-hydroxysteviol, steviolbioside and isosteviol) using HPLC. Stevioside was degraded by the caecal suspensions to steviol with a >90% recovery at the end of a 2-day incubation period; at 4 days post-incubation 100% recovery was observed. The only metabolite identified was steviol-16, 17α-epoxide with a recovery of 14% at day 2 only and no detections at day 4 post-incubation.

Koyama E, Kitazawa Y, Ohori O et al (2003b) In vitro metabolism of the glycosidic sweeteners, stevia mixture and enzymatically modified stevia in human intestinal microflora. Food. Chem. Toxicol. 41: 359-374

The metabolism of stevia mixture, stevioside glycosides and enzymatically modified stevia incubated was investigated under anaerobic conditions for 0, 8 and 24h with human intestinal microflora from pooled faecal homogenates of 5 healthy Japanese males aged 29-34 years (bodyweight not stated). The test materials were of technical grade and obtained from Japan Stevia Industrial Association, Tokyo, Japan, and consisted of the following:

- stevia mixture (rebaudioside A and C, stevioside, dulcoside A);
- rebaudioside A, B and C, stevioside, steviol, rubusoside, dulcoside A;
- enzymatically modified stevia (α-glucosylrebaudioside A and C, α-glucosylstevioside, α-glucosyldulcoside);
- α-monoglucosylrebaudioside A and α-monoglucosylstevioside.

Stevia mixture, enzymatically modified stevia, stevioside and rebaudioside were completely metabolised `within 24h; whereas, no degradation of steviol was observed during the incubation period.

The results at substrate concentrations of 0.2 (low) or 10 mg/mL (high) in pooled human faecal homogenates for stevioside, rebaudioside A and steviol are summarised as follows:

Compound	**% of initial value at 8h (0.2 mg/mL)**	**% of initial value at 24h (0.2 mg/mL)**	**% of initial value at 8h (10 mg/mL)**	**% of initial value at 24h (10 mg/mL)**	**% conversion to steviol at 24h**
Stevioside	Below LOD	Below LOD	70	23	84 (low) 63 (high)
Rebaudioside A	65	Below LOD	90	44	109 (low) 22 (high)
Steviol	99.2*	99.2*	96.4*	96.4*	-

*Concentrations of 0.08 mg/mL (low) and 0.2 mg/mL (high) for steviol substrates.

The results suggest that stevioside and rebaudioside A are rapidly degraded by bacteria in human faeces to steviol and that there is no or limited degradation of steviol observed over a 24h period. There were no other peaks on chromatograms other than steviol when steviol, stevia mixture or enzymatically modified stevia was incubated for 24h, which suggested that the principle metabolite *in vitro* in humans is steviol.

From the analysis of the chromatograms the authors suggested that the principle metabolic pathways of stevioside and its analogues are as follows:

- Stevioside is hydrolysed via rubusoside to steviol;
- α-monoglucosylstevioside is metabolised similarly to that of stevioside after α-deglucosylation;
- Rebaudioside A is hydrolysed via stevioside to steviol (major pathway) and via rebaudioside B (minor pathway) to steviol; and
- the metabolism of α-monoglucosylrebaudioside A was similar to rebaudioside A after α-deglucosylation.

Gardana C, Simonetti P, Canzi E et al (2003) Metabolism of stevioside and rebaudioside A from stevia rebaudiana extracts by human microflora. J. Agric. Food. Chem. 51: 6618-6622

Stevioside (85% purity, source not stated) and rebaudioside A (90% purity, source not stated) were incubated with faecal suspensions from 6 men and 5 women aged 20-50 years (weight not stated) volunteers for 72h. Stevioside completely degraded to steviol in a 10h period. Steviolbioside concentration peaked after 2-4h of incubation, and then decreased to zero with steviol detected after 3-4h incubation. These results suggested that stevioside was initially hydrolysed to steviolbioside and then this intermediate was rapidly metabolised to steviol. After a period of 6-7h rebaudioside A was hydrolysed to steviolbioloside and then completely metabolised to steviol. Steviol remained unchanged during the 72h incubation and no other metabolites were observed.

In Vivo

Simonetti P, Gardana C, Bramati L & Pietta PG (2004) Bioavailability of stevioside from Stevia rebaudiana in humans: preliminary report. Proceedings of the first symposium on the safety of stevioside. Kuleuvan. Euprint Editions ISBN

Nine healthy male subjects (aged 25-50 years, weight not stated) received single oral doses (375 mg/day; mean dose of 5.16 mg/kg bw/day) of stevioside capsules (purity 85% w/w, source not stated). Plasma, urine and faecal samples were collected before administration of stevioside and at 60, 120, 240 or 300 minutes post-dose and analysed for stevioside or its metabolites (steviol, steviol-16, 17-α-epoxide and 15-α-hydroxysteviol) by LC-MS. Stevioside was detected in the plasma of 7/9 subjects, although there was a large inter-subject variation in the maximum plasma concentration (Cmax) of stevioside with 2 subjects with a Cmax of 0.1 µg/ml peaking between 60 to 120 minutes post-dose. A qualitative evaluation of the presence of stevioside and any metabolites in plasma and urine was made. Steviol, steviol-16, 17-α-epoxide and 15-α-hydroxysteviol were not detected in plasma or urine samples. Free steviol was found in the faeces of all subjects. The presence of steviol-glucuronate was found in plasma (5/9 subjects) and urine (9/9 subjects), however, the time of measurement of these samples was not stated in this study. This paper was a preliminary report, which suggested that the main metabolite in the urine is a steviol-glucuronide rather than other metabolites (steviol-16, 17-α-epoxide, 15-oxosteviol and 15-α-hydroxysteviol).

Geuns JMC & Pietta P (2004) Stevioside metabolism by human volunteers (unpublished report). Laboratory Functional Biology, Kuleuven, Kasteelpark Arenberg. Belgium

Italian study: Single oral doses (375 mg; mean dose of 5.16 mg/kg bw/day) of capsules of stevioside (source and purity not stated) were administered to 9 male subjects (Italian, aged 20 to 50 years with a normal body mass index) blood samples collected at 0, 1, 2, 3,4 and 5h post-dose and urine and faecal samples collected for 5 days.

Stevioside, steviol, steviolbioside, steviolglucuronide, steviol 16, 17α-epoxide, 15-hydroxy-steviol and 15-oxosteviol were analysed by LC-MS Total Ion Chromatography. Low concentrations of stevioside were detected in the plasma of seven subjects 1 to 3h post-dose with a maximum of 0.1µg/mL observed at 2h post-dose. Steviol glucuronide was detected in the plasma of 5 subjects; however, data was presented for only 1 subject (maximum level of 0.1µg/mL at 3h post-dose in this subject). No free steviol or other metabolites were detected in the plasma. Low concentrations of stevioside was detected in the urine of 2 subjects (data not shown) and steviol glucuronide was detected in the urine of all subjects reaching a maximum of 49.4±9.2 mg at day 5 post-dose (13% of administered dose). Free steviol or other metabolites were not detected in the urine. In the faeces only free steviol at a concentration of 56 mg. Although the data presented suggested that steviol glucuronide was the only metabolite detected in the plasma and urine in humans, there was a lack of detailed data available for an independent review.

Belgium Study: Stevioside capsules (source not stated, purity stevioside >97%, steviolbioside 2.7% and trace amounts of rebaudioside A) were administered to 5 male and 5 female Belgium subjects (aged 24±2 years, with normal body mass index) at doses of 750 mg/day for 3 days. On day 3 post-dose, blood was collected before breakfast and at 0, 0.5, 1, 3, 5 and 7h post-breakfast, and a 24h urine and faecal sample collected at day 3 and 4, respectively. An analysis of stevioside, steviol and any metabolites was undertaken by HPLC.

Bound steviol was eluted by hydrolysis with β-glucuronidase/sulfatase to determine whether steviol was bound as glucuronide or sulphate conjugates. A summary of the results is as follows:

Samples	**Stevioside (mg)**	**Free Steviol (mg)**	**Steviol glucuronide (mg)**
Blood plasma	Not detected	Not detected	Mean 33.93 (range 14.1 to 70.7)
Urine	Not detected	Not detected	Mean 101.8±21.3 (range 28 to 205)
Faeces	Not detected	Mean 22.8±3 (range 13 to 40)	Not detected

The studies performed in Italy and Belgium identified a steviol-glucuronide conjugate in plasma and urine.

These studies suggested that stevioside is not absorbed across the gastrointestinal tract in humans, no free steviol was detected in the blood or urine and that free steviol is detected in the faeces, with low recoveries. A large range of steviol glucuronide conjugates were observed in the blood plasma and urine from 0 to 7h post-dose, which may reflect normal variability in human subjects.

Geuns, J.M., Buyse, J., Vankeirsbilck, A., Temme, E.H., Compernolle, F., Toppet, S. (2006a). Identification of steviol glucuronide in human urine. J. Agric. Food. Chem. 5: 2794-2798

Geuns JMC, Buyse J, Vankeirsbilck A et al (2006b) Metabolism of stevioside in healthy subjects. Unpublished report. Laboratory Functional Biology, Kuleuven, Kasteelpark Arenberg. Belgium

Ten healthy male and female subjects (aged 21-29 years, with normal body mass index) received oral doses of stevioside capsules (source not stated, purity stevioside >97%; 2.8% steviolbioside and 0.2% rebaudioside) at 0 or 250 mg three times/day for 3 days. A 24h urine sample was collected at day 3 post-dose and analysed for metabolites of stevioside by MS, NMR, IR and UV. Blood was also taken on day 3 post-dose and the concentrations of alkaline phosphatase, alanine aminotransferase/glutamic pyruvate ratio, creatine kinase and lactate dehydrogenase measured. Bound steviol was eluted by hydrolysis with β-glucuronidase/sulfatase to determine whether steviol was bound as glucuronide or sulphate conjugates. No significant differences were observed in the blood chemistry parameters between controls and treated groups. No free steviol was detected in the urine and the only metabolite observed was steviol-glucuronide.

The Authors calculated that of a daily dose of 750 mg/day, 300 mg of free steviol is formed in the colon (assuming complete degradation of stevioside to steviol) with the percentage of free steviol, glucuronidated steviol in the blood and urine is as follows:

Dose of stevioside/day (mg)	Free steviol in colon (mg)	Free steviol in faeces(mg)	Steviol glucuronide in blood (mg)	Steviol glucuronide in urine (mg)	Total recovery of steviol (faecal and urinary routes)
750	300	23±2.7	101.8±16.4 (34%)	101.8±21.3 (34%)	76%

This study suggested that in humans, stevioside is completely metabolised to steviol via bacteria in the colon, transported to the liver where steviol glucuronide is formed. Although not measured, the glucuronide remaining in the blood would be expected to be excreted in the urine. The principle excretion route is the urine (68%) and small amount is excreted via the faecal route (8%) with a total recovery of steviol of 76% (Geuns et al 2006).

Guens JM, Buyse J, Vankeirsbilck A & Temme EH (2007) Metabolism of stevioside by healthy subjects. Exp. Biol. Med. 232: 164-167

Ten healthy subjects (5/sex; aged 21-29 years; mean bodyweight was 74 kg and 65 kg for men and women respectively; normal BMI of 23 kg/m^2) received oral doses of stevioside capsules (Stevita, Brazil; >97% purity; 2.8% steviolbioside & 0.3% rebaudioside) at 0 or 250 mg three times/day for 3 days. Doses, expressed as steviol, were calculated to be 294 mg/day or 4.5 mg/kg bw/day for females and 4 mg/kg bw/day for males. On day 3 post-dose, fasted blood was collected before breakfast and at 0, 0.5, 1, 3, 5 and 7 h post-breakfast, with a 24 h urine and faecal sample collected at day 3 and 4, respectively. Stevioside, steviol and any metabolites/conjugates were analysed in urine and faecal samples by HPLC. Blood pressure was measured prior to and at 30, 60, 90, 120 and 180 minutes after the first dose. The following clinical chemistry parameters were measured: blood glucose and insulin, alkaline phosphatase, alanine aminotransferase, glutamic pyruvate transaminase, creatinine kinase and lactate dehydrogenase. The following urinalysis parameters were analysed: creatinine, sodium, potassium, calcium and urine volume.

No significant differences were observed in mean blood glucose or insulin concentrations, BP, 24 h urine volume or any other clinical chemistry or urinary parameter between control and treated subjects. No stevioside or free steviol was detected in blood or urine, however, steviol glucuronide conjugates were detected at concentrations ranging from 0.7 to 21.3 μg/mL and up to 318 μg/24 h urine sample, respectively. Free steviol was detected in faecal samples at a concentration of 13-40 mg/24 h faecal sample, while no stevioside or steviol conjugates were detected.

Part 3 – Evaluation of supplementary pharmacological studies

Animal studies

Chen TH, Chen SC, Chan P et al (2005) Mechanism of the hypoglycaemic effect of stevioside a glycoside of Stevia rebaudiana. Planta Med. 71: 108-113

Oral doses of stevioside were administered to normal, streptozotocin[11] (STZ) induced diabetic rats (IDDM[12] model) and in a diabetic model induced by feeding rats with 60% fructose (NIDDM[13] model) for 2-weeks. Tolbutamide[14] (10 mg/kg; IP) was used to confirm whether or not rats had developed insulin resistance. Stevioside (extracted from dried *S. rebaudiana* leaves, purity 99%) in a physiological saline vehicle was administered by gavage to groups of 10 male Wistar rats (8 weeks old, weight 200 to 250 g) at doses of 0, 0.5, 1 or 5 mg/kg bw twice daily (total doses; 0, 1, 2 or 10 mg/kg bw/day). At 0, 60, 90 and 120 minutes post-dose, blood was collected for the measurement of glucose and insulin concentrations. A separate part of the study investigated oral administration of stevioside over a 15 day period in STZ and fructose-induced diabetic rats. A glucose tolerance test was performed following gavage administration of oral doses of stevioside at 0, 0.5, 1, or 5 mg/kg bw/day stevioside (10 rats/dose) followed by a 500 mg/kg bw intravenous injection of stevioside via the tail vein. Blood glucose measured at 30, 60, 90 or 120 minutes post-injection.

In STZ diabetic rats, following gavage doses of stevioside at 0, 0.5, 1 or 5 mg/kg twice daily for 15 days the rate-limiting enzyme for gluconeogenesis was examined. This was performed by reverse transcription combined with polymerase chain reaction (RT-PCR) and Northern/Western blotting to measure mRNA and protein concentrations of phosphoenol pyruvate carboxykinase (PEPCK), respectively.

Normal Wistar rats

A statistically significant dose-related maximum reduction in mean blood glucose compared of 18% ($p<0.05$), 28% ($p<0.01$) and 38% ($p<0.01$) at low, mid and high doses respectively was observed in rats at 90 minutes post-dose when compared to initial values at 0 minutes. A dose-related increase in mean blood insulin concentrations of 45% ($p<0.01$), 50% ($p<0.01$) and 54% ($p<0.01$) for low, mid and high doses respectively was observed at 90 minutes post-dose when compared to initial values at 0 minutes (Table 1).

[11] Streptozotocin is a naturally occurring chemical that is particularly toxic to the insulin-producing beta cells of the pancreas in mammals.
[12] Insulin-dependent diabetic model
[13] Non-insulin dependent diabetic model
[14] Tolbutamide is used to treat type II (non-insulin-dependent) diabetes (formerly 'adult-onset'), particularly in people whose diabetes cannot be controlled by diet alone. Tolbutamide lowers blood sugar by stimulating the pancreas to secrete insulin and helping the body use insulin efficiently.

Table 1: Effects of stevioside administered twice daily on mean blood (plasma) glucose concentrations in normal rats at specific times post-dose

Stevioside dose (mg/kg bw)	mg/dl 0 (min)*	mg/dl 60 (min)	mg/dl 90 (min)	mg/dl 120 (min)
0	108	106	105	105
1	98	88 ($p<0.05$)	80 ($p<0.05$)	85 ($p<0.05$)
2	105	80 ($p<0.01$)	75 ($p<0.01$)	80 ($p<0.01$)
10	105	75 ($p<0.01$)	65 ($p<0.01$)	70 ($p<0.01$)

* Estimated from graphically-presented data

Effects of stevioside administered twice daily on mean blood (plasma) insulin concentrations in normal rats at specific times post-dose

Stevioside dose (mg/kg bw)	µg/l 0 (min)*	µg/l 60 (min)	µg/l 90 (min)	µg/l 120 (min)
0	0.6	0.65	0.6	0.6
1	0.6	0.8 ($p<0.05$)	1.1 ($p<0.01$)	0.9 ($p<0.01$)
2	0.6	0.9 ($p<0.05$)	1.2 ($p<0.01$)	1.05 ($p<0.01$)
10	0.6	1.1 ($p<0.01$)	1.3 ($p<0.01$)	1.1 ($p<0.05$)

* Estimated from graphically-presented data

STZ-induced diabetic rats

A statistically significant dose-related maximum reduction in mean blood glucose of 11% ($p<0.01$), 13% ($p<0.01$) and 22% ($p<0.01$) at low, mid and high doses respectively was observed in rats at 120 minutes post-dose when compared to initial values at 0 minutes (Table 2). When stevioside was administered over a longer period (1 to 15 days) mean blood glucose concentrations were significantly reduced from day 1 post-dose with maximum reductions of 11% ($p<0.01$), 13% ($p<0.01$) and 20% ($p<0.01$) at low, mid and high doses respectively, observed at day 10 post-dose when compared to initial values at 0 days (Table 3).

Table 2: Effects of stevioside administered twice daily on mean blood (plasma) glucose concentrations in STZ-induced diabetic rats at specific times post-dose

Stevioside dose (mg/kg bw)	mg/dl 0 (min)*	mg/dl 60 (min)	mg/dl 90 (min)	mg/dl 120 (min)
0	415	415	415	415
1	415	380 ($p<0.05$)	380 ($p<0.05$)	370 ($p<0.01$)
2	415	380 ($p<0.05$)	375 ($p<0.05$)	360 ($p<0.01$)
10	415	350 ($p<0.01$)	340 ($p<0.01$)	325 ($p<0.01$)

* Estimated from graphically-presented data

Table 3: Effects of stevioside administered twice daily on mean blood (plasma) glucose concentrations in STZ-induced diabetic rats at specific times post-dose

Stevioside dose (mg/kg bw)	mg/dl 0 (days)*	mg/dl 1 day	mg/dl 5 days	mg/dl 10 days	mg/dl 15 days
0	415	415	415	415	415
1	415	390 ($p<0.05$)	380 ($p<0.05$)	370 ($p<0.01$)	370 ($p<0.01$)
2	415	380 ($p<0.05$)	370 ($p<0.05$)	360 ($p<0.01$)	360 ($p<0.01$)
10	415	360 ($p<0.01$)	340 ($p<0.01$)	330 ($p<0.01$)	330 ($p<0.01$)

* Estimated from graphically-presented data

Fructose-induced diabetic rats

A statistically significant dose-related maximum reduction in mean blood glucose of 12% ($p<0.01$), 15% ($p<0.01$) and 23% ($p<0.01$) at low, mid and high doses respectively was observed in rats at day 15 post-dose when compared to initial values at 0 days (Table 4).

Table 4: Effects of stevioside administered twice daily on mean blood (plasma) glucose concentrations in fructose-induced diabetic rats at specific times post-dose

Stevioside dose (mg/kg bw)	**mg/dl 0 (days)***	**mg/dl 1 day**	**mg/dl 5 days**	**mg/dl 10 days**	**mg/dl 15 days**
0	170	170	180	185	185
1	170	170	165 ($p<0.05$)	160 ($p<0.01$)	150 ($p<0.01$)
2	170	160	158 ($p<0.05$)	150 ($p<0.01$)	145 ($p<0.01$)
10	170	150 ($p<0.05$)	140 ($p<0.01$)	135($p<0.01$)	130 ($p<0.01$)

* Figures obtained from extrapolation from a graph of results

Glucose tolerance test

A dose-dependent reduction in blood glucose concentrations were observed in normal wistar rats treated with stevioside with a maximum reduction from 210 mg/dl (5 minutes post IV injection) to 75 mg/dl ($p<0.01$) at doses of 10 mg/kg bw at 90 minutes post IV injection of glucose. In the controls, blood glucose concentrations increased to a maximum value after 5 minutes IV injection of glucose.

PEPCK mRNA and protein concentrations in STZ-induced diabetic rats

Dose-dependent significant reductions were observed in mean PEPCK protein (24%, 42% or 47%) and mRNA expression (30%, 53% or 81%) at low, mid and high doses, respectively.

This study indicated that stevioside lowered blood glucose concentrations in normal and diabetic induced rats in a dose-dependent manner with maximum reductions observed at 90 minutes post-dose at doses of 10 mg/kg bw/day with no further reductions noted (in STZ-induced rats) when the period of blood sampling was extended to 15 days. A dose-dependent increase in insulin concentrations in normal rats was observed; however, the authors did not study insulin concentrations in diabetic-induced rats.

Stevioside counteracted the rise in blood glucose expected with a glucose-tolerance test; whereas, control rats administered vehicle alone demonstrated rapid and high concentrations of blood glucose within 5 minutes post-dose. The authors suggested that the mechanism of action of stevioside may be to regulate blood glucose concentrations by decreasing PEPCK gene expression in the liver to decrease gluconeogenesis leading to a decrease in hyperglycaemia in diabetic-induced rats.

Ferreira EB, Neves F & Da Costa MAD (2006) Comparative effects of Stevia rebaudiana leaves and stevioside on glycaemia and hepatic gluconeogenesis. Planta Med 72: 691-696

This study compared the effects of whole stevia leaves to stevioside on glycaemia and hepatic gluconeogenesis in rats. Stevia (dried powdered leaves from *S. rebaudiana* obtained from Steviafarma, Brazil; purity not stated) and stevioside/rebaudioside mixture (extracted from dried powdered leaves from *S. rebaudiana* obtained from Steviafarma, Brazil, purity not stated) were orally administered to groups of male Wistar rats (weight 220g, aged and numbers not stated) by gavage in a water vehicle at doses of 0 or 20 mg/kg bw/day (stevia powder) and 0 or 5.5 mg/kg bw/day (stevioside/rebaudioside mixture) for 15 days. At day 15, and following 15h of fasting, rats were killed and blood collected (site of collection not stated) and analysed for glucose concentrations. Hepatic gluconeogenesis was measured by liver perfusion experiments and in isolated hepatocytes. AUC values were calculated for three gluconeogenic substrates; L-alanine, L-glutamine and L-Lactate. Glucose, urea, pyruvate and L-lactate production from L-alanine, glucose and pyruvate production from L-lactate and glucose production from L-glutamine were measured in perfused livers. Isolated hepatocytes were incubated with L-alanine, L-lactate, L-glutamine, glycerol or no substrate and glucose production measured. The activity of peroxisome proliferator-activated gamma receptors[15] (PPARγ), which are mediators of insulin sensitivity, were examined in rats fasted for 15h.

Following oral doses of stevia leaves at 20 mg/kg bw/day a significant reduction in mean blood glucose from 94.10±2.99 (controls) to 67.83±5.7 mg/dL ($p<0.05$) occurred. In contrast, oral doses of stevioside/rebaudioside mixture increased mean blood glucose from 86.30±4.64 (controls) to 91.50±6 mg/dL. The AUC for glucose was significantly reduced from 6.08±0.73 (controls) to 3.45±0.39 ($p<0.05$) following oral doses of stevia powder when L-alanine was the substrate, with no significant effects on the AUC for urea, pyruvate or L-lactate.

The AUC was increased for pyruvate from 3.3±0.5 (controls) to 6.75±0.98 ($p<0.05$) and decreases in glucose from 10.81±1.19 (controls) to 6.5±0.95 ($p<0.05$) with L-lactate as substrate and a decreased AUC for glucose from 24.82±1 (controls) to 16.93±1 ($p<0.05$) with L-glutamine as substrate. Glucose production from glycerol was not affected by treatment with stevia powder. In contrast, the authors state that glucose production was not decreased in isolated liver perfusion studies (results not shown) or in isolated hepatocytes following treatment with stevioside. Stevia and or stevioside did not affect PPARγ receptor activity; whereas a 4.8-fold increase in PPARγ transcriptional activity with the positive control pioglitazone was observed.

These results suggested that whole stevia powder but not stevioside decreased glucose concentrations in fasted rats and that this may occur by reduction of hepatic gluconeogenesis. In addition, inhibition of the two key enzymes pyruvate carboxylase (PC) and phosphoenol PEPCK was proposed by the authors as a mechanism by which glucose may be reduced by oral doses of stevia in rats but the decreased glucose production did not appear to be mediated by PPARγ activation (Ferreira et al 2006).

[15] PPAR-gamma is the main target of the drug class of thiazolidinediones (TZDs), used in diabetes mellitus and other diseases that feature insulin resistance

Human studies

Barriocanal LA, Palacios M, Benitez G et al (2006) Lack of pharmacological effect of steviol glycosides as a sweetener in humans. Studies on repeated exposures in normotensive and hypotensive individuals and Type 1 and Type 2 diabetes. Unpublished report

Barriocanal LA, Palacios M, Benitez G et al (2008) Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and in type 1 and type 2 diabetics. Regulatory Toxicology & Pharmacology. doi: 10.1016/j.yrtph.2008.02.006

A randomised, double-blind, placebo-controlled study assessed the effects of stevioside on blood glucose, blood pressure (BP) and other biochemical parameters in Type 1 and 2 diabetics, and non-diabetics. Seventy-six volunteers were divided into 3 groups: (i) 16 male and female subjects with type 1 diabetes (DM1) (aged 20 to 60 years; body mass index (BMI) of 20-35 kg/m^2); (ii) 30 male and female subjects with type 2 diabetes (DM2) (aged 40 to 70 years; BMI of 20-35 kg/m^2; and (iii) 30 male and female subjects without diabetes and with normal/low-normal BP concentrations (BP of ≤120/80 mm Hg; aged 20 to 60 years; BMI of 20-35 kg/m^2). Half of the subjects in each group received capsules containing 250 mg steviol glycoside (obtained from Steviafarma Industrial, Brazil; purity ≥ 92%) three times/day for 3 months, while the other half received a placebo. At the start and end of the study period, blood glucose, glycated haemoglobin (HBA_{1c}), BP, insulin (for DM2 and low/normal BP subjects) and a range of other clinical chemistry parameters were measured (total cholesterol, HDL, LDL, triglycerides, electrolytes and creatin phosphokinase concentrations, creatinine, ALT, AST and γGT). Body weight, height, BMI and waist circumference were also measured at the beginning and end of the study period. Blood glucose, BP and body weight were measured every two weeks during the 3-month study period.

There was no treatment-related effect on BP, blood glucose, insulin, glycated haemoglobin (HBA_{1c}) or any of the other measured parameters. Statistically significant ($p<0.05$) differences in baseline (i.e. pre-treatment) systolic BP and triglycerides between treated and placebo subjects in Group 1 were not attributable to treatment because treatment had not yet begun. Similarly, significantly higher ($p<0.05$) glucose concentrations in placebo subjects from Group 1 post-treatment relative to baseline were also not attributable to treatment. On this basis, ingestion of steviol glycosides by both diabetics and non-diabetics at 250 mg, three times/day for three months was without evidence of any adverse effects.

Ferri LAF, Alves-Do-prado W, Yamada SS et al (2006) Investigation of the anti-hypertensive effect of oral crude stevioside in patients with mild hypertension. Phytotherapy Research 20: 732-736

A randomised, double blind, placebo-controlled study investigated the anti-hypertensive effect of a crude stevioside/rebaudioside A mixture (extracted from dried S. rebaudiana leaves, Brazil; purity not stated) on previously untreated mild hypertensive patients. Crude stevioside/rebaudioside capsules were administered to 18 patients in 2 divided doses at 0 (placebo phase; 4 weeks) or 3.75 mg/kg bw/day (phase 1; 7 weeks), 7.5 mg/kg bw/day (phase 2; 11 weeks) or 15 mg/kg bw/day (phase 3; 6 weeks). To be included in the study, the patient's diastolic blood pressure (BP) needed to be in the range 80-99 mm Hg and the systolic 120-159 mm Hg.

Three patients were excluded from the study due to high blood pressure readings and one because of an arrhythmia, with 14 patients entering phase 1, 2 or 3. BP was measured biweekly and in addition, body mass index (BMI) was calculated and an ECG performed after each phase. Blood and urine were collected at the end of each phase for measurement of standard haematology and a range of blood chemistry parameters.

No adverse clinical effects were reported in patients and the haematology and blood chemistry, BMI and ECG profiles were normal. Statistically significant reductions (10-11%) in mean diastolic BP for treated groups' at all three dose concentrations were observed; however, this was also observed in placebo controls. There was a dose-related reduction in mean systolic BP in the treated groups at low (4%), mid (10%) and high (12%) doses and reductions in placebo control values (1-7%) without any dose-response relationship (Table 1).

Table 1: Mean systolic and diastolic blood pressures (mm Hg) before and after treatment phases (1, 2 and 3) with crude stevioside extract for 6 subjects/group

	Phase	**0**	**1 (3.75 mg/kg bw/day)**	**2 (7.5 mg/kg bw/day)**	**3 (15 mg/kg bw/day)**
Stevioside	Systolic	140±13	134±14	126±8*	123±12
	Diastolic	94±8	85±5*	84±5*	84±8*
Placebo	Systolic	133±12	128±5	132±6	124±6
	Diastolic	94±8	86±3*	83±5*	82±4*

*$p<0.05$ compared with phase 0

At doses up to 15 mg/kg bw/day crude stevioside preparations did not reduce BP in mildly hypertensive humans. The lack of a clear affect may be attributable to the limited number of subjects used, the purity of stevioside and/or rebaudioside A or the specific doses used.

Part 4 – Evaluation of supplementary mechanistic studies

Chen TH, Chen SC, Chan P et al (2005) Mechanism of the hypoglycaemic effect of stevioside a glycoside of Stevia rebaudiana. Planta Med. 71: 108-113

Oral doses of stevioside lowered blood glucose concentrations in normal and diabetic induced rats in a dose-dependent manner. Maximum reductions occurred at 90 minutes post-dose at 10 mg/kg bw/day with no further reductions noted when the period of blood sampling was extended to 15 days. Significant reductions in PECK mRNA and protein concentrations also occurred. The authors suggested that a possible mechanism of action of stevioside may be to regulate blood glucose concentrations by decreasing PEPCK gene expression in the liver to decrease gluconeogenesis, leading to a decrease in hyperglycaemia in diabetic-induced rats.

Ferreira EB, Neves F & Da Costa MAD (2006) Comparative effects of Stevia rebaudiana leaves and stevioside on glycaemia and hepatic gluconeogenesis. Planta Med. 72: 691-696

Stevioside orally administered at doses of 5.5 mg/kg bw/day for 15 days had no effect on fasting blood glucose concentrations; whereas, stevia powder at doses of 20 mg/kg bw/day decreased glucose concentrations in fasted rats. Reduction of hepatic gluconeogenesis via inhibition of the two key enzymes PC and PEPCK were proposed by the authors as a mechanism by which glucose may be reduced by oral doses of stevia powder in rats (Ferreira et al 2006).

Hong J, Chen L & Jeppesen PB (2006) Stevioside counteracts the α-cell hypersecretion caused by long-term palmitate exposure. Am. J. Physiol. Endocrin. Metab. 290: E416-E422

Long-term exposure to fatty acids impairs β-cell function in type 2 diabetics but little is known about effects on α-cells. A study evaluated the effect of stevioside (source and purity not stated) on palmitate-induced effect on clonal α-TC1-6 cells derived from an adenoma in transgenic mice (which secrete only glucagon without detectable insulin) following culture with 18 mM glucose with 0.25, 0.5 or 1 mM palmitate in the presence or absence of stevioside at concentrations of 10^{-8} to 10^{-6}M. After 72h, glucagon secretion and concentration, triglyceride concentration and changes in gene expression (acetyl-CoA, carboxylase-1, carnitine palmitoyltransferase, glucagon, peroxisome proliferator-activated gamma receptor, stearoyl-CoA desaturase and sterol regulatory element-binding protein-1c) in α-TC1-6 cells were evaluated. The results suggested that exposure of α-cells to fatty acids resulted in glucagon hypersecretion and triglyceride accumulation. The authors proposed that stevioside was able to reduce the release of glucagon, possibly by enhanced expression of genes involved in fatty acid metabolism leading to increased mRNA expressions of carnitine palmitoyltransferase, PPARγ and stearoyl-CoA desaturase.

Abudula R, Jeppesen PB, Rolfsen SED et al (2006) Rebaudioside A potently stimulates secretion from isolated mouse islets: studies on the dose, glucose and calcium-dependency. Metabolism 53: 1378-1381

This study examined whether rebaudioside A affected insulin and glucose concentrations *in vitro*. Islet of Langerhan cells obtained from adult female NMRI mice were incubated with rebaudioside A (purity >95%) at concentrations from 10^{-10} to 10^{-6} mol/L in the presence of glucose at 3.3 or 16.7 mmol/L for 60 minutes and the insulin release measured.

A second part of the study involved islet cells placed into perifusion chambers with either 3.3, 6.6, 11.1 or 16.7 mmol/L glucose (10 to 30 minutes) in the presence or absence of rebaudioside A at concentrations of 10^{-10} mol/L. A concentration-dependent increase in insulin secretion in the presence of glucose (16.7 mmol/L) was observed with a maximum response at rebaudioside A concentrations of 10^{-10} mol/L ($p<0.01$) relative to control values (glucose 16.7 mmol/L).

Rebaudioside A increased the insulin release at glucose concentrations of 11.1 mmol/L or higher ($p<0.05$), whereas no effect was observed at normal or low glucose concentrations (3.3 or 6.6 mmol/L). This study suggested that rebaudioside A stimulates insulin secretion from isolated mouse islets in a concentration and glucose dependent manner. At normal blood glucose concentrations no effects on insulin release was observed.

Chen J, Jeppesen PB, Abudula R et al (2006) Stevioside does not cause increased basal insulin secretion or β-cell desensitisation as does sulphonylurea, glibenclamide: studies in vitro. Life Sciences 78: 1748-1753.

This study examined stevioside and its effects on basal insulin secretion (BIS) and glucose stimulated insulin secretion (GSIS). Isolated mouse islets from NMRI mice were exposed to a range of glucose concentrations (3.3, 5.5 or 16.7 mM) for 1h following pre-treatment with stevioside or glibenclamide[16] (GB) for either 2h or 24h. A significant ($p<0.001$) glucose-dependent increase in BIS (3-fold) was observed after 2h pre-treatment with GB. In contrast no significant changes were observed in BIS after pre-treatment with stevioside. A significant ($p<0.001$) increase in GSIS was observed after 24h pre-treatment with concentrations of stevioside between 10^{-7} to 10^{-5} M in the presence of high concentrations (16.7 mM) of glucose. Pre-treatment with GB (10^{-7} M) for 24h significantly ($p<0.001$) increased the BIS at concentrations of glucose of 3.3 mM but decreased GSIS at concentrations of glucose of 16.7 mM. In contrast stevioside (10^{-7} M) and GLP-1 (10^{-7} M) did not stimulate BIS but increased GSIS at concentrations of glucose of 16.7 mM. This study suggested that pre-treatment with stevioside for either 2 or 24h does not increase BIS but increases GSIS after 24h pre-treatment.

Wong KL, Lin JW & Liu JC (2006) Antiproliferative effect of isosteviol on angiotension-II-treated rat aortic smooth muscle cells. Pharmacology 76: 163-169

This study investigated the effects of isosteviol (a metabolite of steviol) on rat aortic smooth muscle cells; in particular, whether isosteviol inhibits angiotension-II-induced cell proliferation and endothelin-1-secretion both of which have been implicated in the pathogenesis of chronic vascular disease. Rat smooth muscle cells obtained from the aortas of Sprague-Dawley rats were pre-incubated with isosteviol (obtained by acid hydrolysis of stevioside in the laboratory, purity 99.8%) at 0, 1, 10 or 100 μmol/L for 30 minutes and then with or without addition of angiotension II (100 nmol/L) for 24h. ^{3}H-thymidine (5 μCi/mL) was then added to measure the synthesis of new DNA and endothelin-1 secretion examined.

[16] Glibenclamide is a sulphonylurea drug that is used in oral therapy for type 2 diabetes in humans. GB simulates the effect of glucose in eliciting insulin release

The level of reactive oxygen species was measured by pre-incubating smooth muscle cells with 2,7-dichlorofluorescin diacetate (DCF-DA) a redox-sensitive fluorescent dye at a concentration of 30 μmol/L before addition of isosteviol (0, 1, 10 or 100 μmol/L for 30 minutes) or angiotension II (100 nmol/L) as a means of examining the presence of excess reactive oxygen species (ROS) as a possible initiator of atherosclerotic events. Separate experiments were conducted in which smooth muscle cells were pre-treated with isosteviol (100 μmol/L) and the antioxidants N-acetylcysteine (10 mmol/L) and diphenylene iodonium (DPI; 10 μmol/L) and then stimulated with and without angiotension II (100 nmol/L) for 1h; and in the presence of a positive control (H_2O_2; 100 μmol/L) without angiotension II addition.

Graphical presentation of results showed a statistically significant ($p<0.05$) concentration-dependent decrease in angiotension-II-induced proliferation (as observed by the reduction in ^{3}H-thymidine incorporation in smooth muscle cells) and inhibition of angiotension-II-induced endothelin-1-secretion was observed at all doses of isosteviol. Similarly, significant reductions ($P<0.05$) in angiotension-II-induced ROS was observed. Pre-treatment with antioxidants also significantly reduced angiotension-II-induced ROS species comparable to control concentrations of isosteviol only; and the positive control elicited a significance increase in ROS in the absence of angiotension II. This study suggested that isosteviol inhibits angiotension-II-induced cell proliferation and endothelin-1-secretion via reductions in ROS generation.

Part 5 – Evaluation of supplementary toxicity studies

Toyoda K, Matsui H, Shoda T, Uneyama C, Takada K & Takahashi M (1997) Assessment of the carcinogenicity of stevioside in F344 rats. Food and Chemical Toxicology 35:597-603

Experimental: Stevioside (sourced from Stevia Industrial Association, Japan; 95.6% purity) was admixed in the diet at concentrations of 0, 2.5 or 5% and fed *ad libitum* to groups of fifty F344/DuCrJ rats per sex for 104 weeks. All rats were then placed on a basal diet for an additional 4 weeks. These dietary concentrations of stevioside were based on the subchronic toxicity study of Aze et al (1991). Rats were sourced from Charles River Japan Inc (Kanagawa, Japan), were 5-weeks old and weighed approximately 90-120 g at the commencement of dosing. Rats were observed daily for mortalities and clinical signs. Bodyweight was recorded weekly for the first 8 weeks and thereafter every 4 weeks. Food consumption was measured every 4 weeks. Blood samples were collected at sacrifice for analysis of a limited number of haematology parameters (WBC, RBC, Hb and Hct). Rats sacrificed *in extremis* and those surviving to week 108 were necropsied and the standard range of tissues and organs collected for macroscopic and histopathological examination; except for those rats where advanced autolysis had occurred. The following organs were weighed: brain, salivary glands, lungs, heart, spleen, liver, kidneys, adrenals, testes and ovaries. Results were statistically analysed by: analysis of variance (ANOVA) followed by Dunnett's Test or Scheffe's test (body and organ weights, survival time and food consumption; Fisher's Exact Test (mortality rates, incidences of neoplastic and non-neoplastic lesions); and cumulative chi-square test (severity of chronic nephropathy).

Results: Based on bodyweight and food consumption data, the authors calculated that the mean doses of stevioside were 0, 969±308 and 1997±617 mg/kg bw/day at 0, 2.5 and 5%, respectively, in males and 0, 1120±285 and 2387±508 mg/kg bw/day at 0, 2.5 and 5%, respectively, in females. There were no treatment-related clinical signs or effects on survival time. Final survival was significantly lower in high-dose males relative to the controls (60 *versus* 78%, respectively). However, the authors attributed this result to the rapid onset of large granular lymphocyte leukaemia resulting in more rats being sacrificed or dying during the last few weeks of the study. While the incidence of this leukaemia was elevated relative to the control (12.2, 18.8 and 23.4% at 0, 2.5 and 5% dietary levels, respectively), it was not statistically significant or outside the normal range for age and sex-matched rats of the same strain from the performing laboratory (16.0-33.3%). On this basis the lower final survival in high-dose males is not considered treatment-related.

Graphically-presented data indicated that there was a slight treatment-related depression in bodyweight gain in both sexes, which became more obvious in the second half of the study. Overall bodyweight gain in males was 2.3 and 4.4% lower than the controls at dietary levels of 2.5 and 5%, respectively, while it was 2.4 and 9.2% lower, respectively, in females. There was no indication that this finding was statistically significant. Absolute bodyweights of high-dose rats at week 104 were significantly lower than the controls [5% lower in males ($p<0.05$) and 14% lower in females ($p<0.01$)]. There was no treatment-related effect on food consumption. No food conversion efficiency data were provided. Given that stevioside is a non-caloric sweetener, the authors concluded that the reduced bodyweight gains and overall bodyweight is consistent with those expected from energy restriction. On this basis, the effects on bodyweight/gain are not considered toxicologically significant and are consistent with those seen in a more recent study (see below).

At the highest dose, significantly lower ($p<0.05$) absolute kidney weights (both sexes), left ovary weight (females) and relative brain weight (females) were determined. In the absence of any histopathology of these organs, these results are attributable to the reduced bodyweight gain of these groups. There were no treatment-related macroscopic or histopathological abnormalities and no evidence that stevioside had any carcinogenic activity.

The NOEL in rats following 2-years of repeated dietary exposure to stevioside was 1997 mg/kg bw/day in males and 2387 mg/kg bw/day in females, based on the absence of any toxicologically-significant effects at these doses. It should be noted that JECFA interpreted the decreased survival and bodyweight gain in high-dose males as treatment-related and toxicologically significant and therefore set the NOEL for this study at 973 mg/kg bw/day.

Nikiforov AI & Eapen AK (2008) A 90-day oral (dietary) toxicity study of rebaudioside A in Sprague-Dawley rats. International Journal of Toxicology 27: 65-80

This study was reportedly conducted in accordance with OECD and US Food and Drug Administration (FDA) Test Guidelines and principles of Good Laboratory Practice (GLP). The study was performed at WIL Research Laboratories, Ashland, Ohio, USA.

Experimental: Rebaudioside A (CAS RN 58543-16-1; sourced from Stevian Biotechnology, Malaysia; 99.5% purity;) was admixed in the diet and fed *ad libitum* to groups of 20 Crl:CD Sprague-Dawley rats per sex at target doses of 0, 500, 1000 or 2000 mg/kg bw/day for 90, 91, 92 or 93 days. The dose selection was based on an unpublished 14-day range finding study performed by the same laboratory in addition to uncited published literature for related steviol glycosides. Rats were sourced from Charles River Laboratories (Raleigh, North Caroline, USA) and were approximately 6 weeks old at the start of treatment. The body weight ranges of males and females were 185-237 and 121-170 g, respectively. Throughout the treatment period, rats were housed individually under standard conditions.

Rats were observed daily for mortalities and clinical signs, with a detailed physical examination performed weekly, beginning at least one week prior to treatment. Bodyweight was recorded at least weekly, commencing approximately two weeks before treatment. Food consumption was recorded weekly. A functional observational battery (FOB) and an assessment of locomotor activity were performed on 10 rats/sex/group at week 12. The standard range of haematology, clinical chemistry and urinalysis parameters were analysed in samples collected from 10 rats/sex/group at weeks 2, 5 and 13 (termination). Following sacrifice, all rats were necropsied and the standard range of tissues and organs collected for macroscopic and histopathological examination, and organ weight measurement. Results were statistically analysed by one-way analysis of variance (ANOVA) followed by Dunnett's Test, or by a Fisher's Exact Test.

Results: Analytical levels of the test compound in the feed were 90-105% compliant with nominal levels. Based on bodyweight and food consumption data, the authors calculated that the mean dose of rebaudioside A were 0, 517, 1035 and 2055 mg/kg bw/day, respectively, for males and 0, 511, 1019 and 2050 mg/kg bw/day, respectively, for females.

There were no mortalities in any group and no treatment-related clinical signs. The mean absolute bodyweight of high-dose males tended to be lower than the controls at most sampling intervals (5, 2, 5 and 7% at weeks 1, 2, 4 and 8, respectively), with the mean terminal body weight (week 13) of high-dose males significantly lower ($p<0.05$; 9%) than the controls.

The food conversion efficiency (bodyweight gained as a percentage of feed consumed) of this same group was also significantly lower ($p<0.01$;~4%) than the controls at weeks 1, 4 and 8, while food consumption was unaffected by treatment. A similar effect did not occur in high-dose females. On the basis of these findings it is likely that the lower bodyweight of high-dose males is attributable to a reduced caloric intake due to the relatively high proportion of rebaudioside A in the diet (up to 36 g/kg) rather than to any direct effect of the test compound. Therefore the apparently treatment-related effect on absolute bodyweight and food conversion efficiency in high-dose males is not considered toxicologically-significant.

The FOB and motor activity assessment were unremarkable. There were no treatment-related effects on any haematology, clinical chemistry or urinalysis parameters noting that there were some incidental statistically significant differences between treated and control groups [e.g. significantly elevated ($p<0.05$) mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) at every dose]. However, these were considered unrelated to treatment because of the absence of a dose-response relationship and as there was no consistency between sexes or over time. There were no treatment-related macroscopic or microscopic abnormalities. A reported increase in uterine clear fluid and dilatation of the uterus in high-dose females were stated by the authors to be consistent with the changes associated with a normal oestrus cycle and therefore unrelated to treatment. Absolute organ weights were unremarkable. Relative adrenal weights were significantly elevated ($p<0.01$) in males at 1000 and 2000 mg/kg bw/day (0.013 ± 0.0018 and 0.013 ± 0.0014 *versus* 0.011 ± 0.0016 in the control) but in the absence of a dose-response relationship was not considered treatment-related.

The NOEL in rats following 90-days of repeated dietary exposure to rebaudioside A was 2050 mg/kg bw/day (or 680 mg/kg bw/day expressed as steviol using a conversion factor of 33%; relative molecular weight of steviol/rebaudioside A = 318/966), based on the absence of any toxicologically-significant effects at this dose.

Attachment 3

Dietary Exposure Assessment

Executive Summary

A dietary exposure assessment was undertaken by FSANZ to estimate dietary exposure to steviol glycosides. Food consumption data from the 1995 Australian and 1997 New Zealand National Nutrition Surveys were used for the exposure assessments. The population groups assessed were the Australian population (2 years and above), the New Zealand population (15 years and above) and children (2-6 years for Australia only).

After the Draft Assessment, the Applicant requested some minor changes to the permissions included in the draft variation to Standard 1.3.1. The dietary exposure assessment reflects the amended levels of use requested by the Applicant. Amendments from the Draft Assessment include new requests for permission in peanut butter, fancy breads, formulated beverages and an increase in the level of use requested for bubble and chewing gum and hard boiled confectionery.

The Applicant provided FSANZ with information on proposed levels of use for steviol glycosides for specific food groups and the expected proportion of products in each food group in which sugar or intense sweeteners would be replaced by steviol glycosides over 20 years of availability to food suppliers. Based on this information, dietary exposure assessments were conducted for a *sugar replacement scenario* (Scenario One).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 63rd meeting estimated the intake of steviol glycosides would likely be 20-30% of total sugar replacement in foods (World Health Organization, 2004). Therefore, dietary exposure assessments were conducted for a *30% market share* scenario (Scenario Two) based on this assumption.

Estimated dietary exposures were compared with the reference health standard, an Acceptable Daily Intake (ADI) of 0–4 mg/kg bw/day, proposed by FSANZ.

For both scenarios, the major contributors to steviol glycosides dietary exposure for Australians aged 2 years and above and for New Zealanders aged 15 years and above were predicted to be formulated beverages, tabletop sweeteners and carbonated soft drinks. For Australian children aged 2-6 years, the major contributors were fruit and vegetable juices and products, formulated beverages, carbonated soft drinks, breakfast cereals, mueslis and muesli bars, and flavoured milks and yoghurts for both scenarios. Beverages were predicted to be major contributors to steviol glycosides exposures, because they are consumed in large volumes.

For both the *sugar replacement* model (Scenario 1) and the *30% market share* model (Scenario 2), estimated mean and 90th percentile exposures for all population groups assessed were at or below the ADI. For the *sugar replacement* scenario, Australian children aged 2-6 years had estimated 90th percentile dietary exposures to steviol glycosides at 100% ADI.

1. Background

Steviol glycosides are high intensity sweeteners 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 Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its 63rd meeting estimated the intake of steviol glycosides to be 2-5 grams per day based on a 100% sugar replacement scenario (World Health Organization, 2004). However, the Committee agreed that exposures would more likely be 20-30% of this value.

The Applicant provided FSANZ with information on proposed concentration of use of steviol glycosides for specific food groups and the expected proportion of products in each food category after 20 years of use.

The foods and the proposed concentrations for the use of steviol glycosides in Australia and New Zealand provided by the Applicant, are listed in Table 1.

1.1 Dietary exposure assessment provided by the Applicant

The Applicant did not submit a dietary exposure assessment for steviol glycosides to allow FSANZ to determine any conclusions about the likely exposure to steviol glycosides as a food additive. Therefore, FSANZ conducted a dietary exposure assessment for Australian and New Zealand population groups. The mean dietary exposures and high consumer (90th percentile) dietary exposures were assessed.

Table 1: Proposed uses of Steviol glycosides in foods provided by the Applicant

	Food Group	Typical sugar* content (%)	Type of Food product or amount of sugar* replaced	Milligrams of steviol glycosides per 100 g product	Uptake after 20 years (% of category likely to include some steviol glycosides)
Dairy Products	Milk Products – flavoured	5 – 7	All sugar* replaced	11.5	100
	Yoghurts – flavoured	8 – 11	All sugar* replaced	17.6	100
	Ice confection - liquid	7	All sugar* replaced	11.5	100
	Ice creams	7 – 17	Some sugar* replaced	6.4	40
	Ice creams reduced & low fat	7 – 17	Low-fat low-joule product	20.8	66
	Ice confection reduced & low fat	7 – 17	Low-fat low-joule product	20.8	66
Fruit and Vegetables and Products	Fruit & Veg in vinegar - beetroot	10	All sugar* replaced	16.0	100
	Low joule chutneys, jams etc	NS	Low-fat low-joule product	45.0	50
	Fruit & Veg preparations – tomato sauces etc	25 – 30	Some sugar* replaced	20.8	30
	Soy milks plain	4	All sugar* replaced	6.4	100
	Soy milks flavoured	8 – 11	All sugar* replaced	17.5	100
	Peanut butter	NS	NS	10.0	NS
Confectionery	Chocolate & cocoa – carbohydrate modified lines	NS	Low-fat low-joule product	55.0	100
	Sugar confectionery – carbohydrate modified lines	NS	Low-fat low-joule product	110.0	100
	Low joule chewing gum	NS	Low-fat low-joule product	110.0	50
Cereals	Processed cereals – breakfast	0 – 35	Some sugar* replaced	25.0	50

Table 1: Proposed uses of Steviol glycosides in foods, as provided by the Applicant (cont'd)

	Food Group	Typical sugar* content (%)	Type of Food product or amount of sugar* replaced	Milligrams of steviol glycosides per 100 g product	Uptake after 20 years (% of category likely to include some steviol glycosides)
Bakery Products	Biscuits – sweet (excl. choc. Coated)	15 – 25	Some sugar* replaced	16.0	50
	Cakes & muffins	15 – 25	Some sugar* replaced	16.0	20
	Slices	20 – 30	Some sugar* replaced	16.0	30
	Pastries – sweet only	5 – 20	Some sugar* replaced	8.0	20
	Fancy breads	NS	NS	16.0	NS
Tabletop Sweeteners	Tabletop sweeteners	NS	All sugar* replaced	40,000	50
	Tabletop sweeteners – liquids	NS	All sugar* replaced	8,000	50
	Tabletop sweeteners – portion size	NS	All sugar* replaced	40,000	50
Non-alcoholic Beverages	Fruit & Vegetable juices	3	All sugar* replaced	5.0	30
	Low joule fruit & veg drinks	NS	Low-fat low-joule product	12.5	80
	Cordials	10	Some sugar* replaced	4.8	33
	Diet cordials	NS	Low-fat low-joule product	9.6	80
	Carbonated non-cola soft drinks	10	Some sugar* replaced	4.8	66
	Diet carbonated non-cola soft drinks	NS	Low-fat low-joule product	16.0	50
	Cola type drinks – carbonated	10	Some sugar* replaced	4.8	33
	Diet cola type drinks – carbonated	NS	Low-fat low-joule product	16.0	50
	Brewed soft drink	10	All sugar* replaced	16.0	100
	Herbal tea	NS	NS	10.0	NS
	Coffee substitutes beverage	NS	NS	10.0	NS

Table 1: Proposed uses of Steviol glycosides in foods, as provided by the Applicant (cont'd)

	Food Group	Typical sugar⁂ content (%)	Type of Food product or amount of sugar⁂ replaced	Milligrams of steviol glycosides per 100 g product	Uptake after 20 years (% of category likely to include some steviol glycosides)
Non-alcoholic Beverages (cont'd)	Coffee-based mixes beverage	NS	NS	10.0	NS
	Formula meal replacements and formulated supplementary foods	NS	NS	17.5	NS
	Formulated supplementary sports foods	NS	NS	17.5	NS
	Formulated beverages	NS	NS	16.0	NS
Desserts	Desserts	15	Some sugar⁂ replaced	15.0	30
	Desserts – dairy only	8 – 12	Some sugar⁂ replaced	15.0	50
	Jelly – low joule only	NS	Low-fat low-joule product	26.0	75
	Custard powder etc	4 – 7	Some sugar⁂ replaced	8.0	50
Mueslis and Muesli Bars	Cereal products – sugared mueslis	15 – 25	Some sugar⁂ replaced	12.5	50
	Breakfast and muesli bars	20 – 30	Some sugar⁂ replaced	12.5	50
Sauces, Toppings, Mayonnaises & Dressings	Gravy & Sauces – sweetened only	5 – 20	Some sugar⁂ replaced	12.5	50
	Mayonnaises & salad dressings	0 – 25	Some sugar⁂ replaced	16.0	50
	Toppings only	20 – 50	Some sugar⁂ replaced	32.0	50
Soups	Soup	NS	NS	10.0	NS

* per 100 mL made up drink
⁂Assumed that "Sugar" refers to added sugars
NS –Not supplied by the Applicant

2. Dietary exposure assessment

2.1 What is dietary exposure assessment?

Dietary modelling is a tool used to estimate exposures to food chemicals from the diet as part of the risk assessment process.

To estimate dietary exposure to food chemicals, records of what foods people have eaten are needed along with information on how much of the food chemical is in each food. The accuracy of these exposure estimates depend on the quality of the data used in the assessment. Sometimes, not all of the data required are available or there is uncertainty about the accuracy of the data. Therefore, assumptions are made either about the foods eaten or about chemical levels, based on previous knowledge and experience. The models are generally set up according to international conventions for food chemical exposure estimates. However, each modelling process requires decisions to be made about how to set the model up and what assumptions to make; a different decision may result in a different answer. Therefore, FSANZ documents clearly all such decisions and assumptions to enable the results to be understood in the context of the data available and so that risk managers can make informed decisions.

The dietary exposure assessment was conducted using dietary modelling techniques that combine food consumption data with steviol glycosides concentration data to estimate the exposure to steviol glycosides from the diet. The dietary exposure assessment was conducted using FSANZ's dietary modelling computer program, DIAMOND.

Dietary exposure = food chemical concentration x food consumption amount

The exposure was estimated by combining usual patterns of food consumption, as derived from national nutrition survey (NNS) data, with proposed levels of use of steviol glycosides in foods.

2.2 Dietary survey data

DIAMOND contains dietary survey data for both Australia and New Zealand; the 1995 NNS from Australia that surveyed 13,858 people aged 2 years and above, and the 1997 New Zealand NNS that surveyed 4,636 people aged 15 years and above. Both of the NNSs used a 24-hour food recall methodology. It is recognised that these survey data have several limitations. For a complete list of limitations see Section 4: *Limitations of the dietary exposure assessment*.

2.3 Additional food consumption data or other relevant data

The 1995 and 1997 NNSs did not report any consumption of formulated beverages. Market share data were therefore required to enable dietary modelling to be conducted. Using German data on the percentage of the soft drinks market held by functional soft drinks (4.1%), FSANZ assumed that formulated beverages will replace 5% of the non-alcoholic beverages market (excluding milk) (Leatherhead Food International, 2003). The foods that may be replaced by formulated beverages included tea and coffee, cordials, carbonated drinks, fruit juices, fruit juice drinks, sports drinks, bottled water and tap water (when used as a beverage or to make up a beverage).

2.4 Population groups assessed

The dietary exposure assessment was conducted for both Australian and New Zealand populations. An assessment was conducted for the whole population (2 years and above for Australia; 15 years and above for New Zealand), as well as for children aged 2-6 years (Australia only). Dietary exposure assessments were conducted for the whole population as a proxy for lifetime exposure. An exposure assessment was conducted for children aged 2-6 years because children generally have higher dietary exposures due to their smaller body weight and the fact that they consume more food per kilogram of body weight compared to adults. They also consume many of the foods proposed to contain steviol glycosides, such as cordials, processed cereal and meal products, biscuits and cakes and fruit and vegetable juice products.

It is important to note that, while children aged 2-6 years have been assessed as a separate group, this group has also been included in the dietary exposure assessment for the whole population for Australia.

2.5 Steviol glycosides concentration levels

The levels of steviol glycosides in foods that were used in the dietary exposure assessment were derived from information provided by the Applicant. The foods and proposed levels used for the dietary exposure assessment are shown in Table 2.

Concentrations of steviol glycosides were assigned to food groups using DIAMOND food classification codes. These codes are based on the Australian New Zealand Food Classification System (ANZFCS) used in Standard 1.3.1 Food Additives (for example, 14.1.2.1 represents fruit and vegetable juices). The foods proposed by the Applicant to contain steviol glycosides (as shown in Table 1) were matched to the most appropriate ANZFSC code(s) for dietary modelling purposes.

Where the Applicant provided a range of possible concentrations, the highest level in the range was used for calculating the estimated exposures in order to assume the worst-case scenario.

2.6 Scenarios for dietary exposure assessment

For the purpose of assessing this application, dietary exposures to steviol glycosides were calculated for a *sugar replacement* and a *30% market share* scenario.

2.6.1 Scenario 1: Sugar replacement scenario

The Applicant stated that steviol glycosides can be used in conjunction with sugar or other sweeteners and will replace some or all of the sweeteners now used. The actual levels of steviol glycosides used will vary between individual manufacturers and products. The Applicant provided FSANZ with information on proposed concentrations of steviol glycosides for specific food groups and the expected uptake of steviol glycosides for those foods by the food industry (as a percentage uptake after 20 years of use). Refer to Table 1 for full details. It was assumed that formulated beverages would account for an additional 5% uptake after 20 years for all non-alcoholic beverages (excluding milk and milk based beverages).

For these non-alcoholic beverages, it was also assumed that, where a non-alcoholic beverage group was estimated to have a 100% market uptake of steviol glycosides after 20 years, 95% of the market was the non-alcoholic beverage and 5% was formulated beverage. Based on these concentrations, dietary exposure assessments were conducted for Scenario One (*sugar replacement scenario*).

Where the Applicant stated that steviol glycosides would be used in the intense sweetened versions of the food and where consumption data existed for intense sweetened versions, the steviol glycosides concentrations were assigned to that subgroup only. e.g. 14.1.3.6 Soft drinks, artificially sweetened. Where consumption data only existed for sugar sweetened versions of a food and a market share value has been provided to specify the percentage of products in that food group that would contain steviol glycosides, the market share value was used in conjunction with the specified concentration to derive a weighted concentration for the exposure assessment. e.g. steviol glycosides concentration in biscuits was 160 mg/kg, the market share 50%; therefore the steviol glycosides concentration used in the exposure assessment was 80 mg/kg.

2.6.2 Scenario 2. 30% market share scenario

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 63rd meeting estimated the intake of steviol glycosides to be between 2-5 grams per day based on a 100% sugar replacement scenario (World Health Organization, 2004). However, the Committee agreed that exposures would more likely be 20-30% of this value. For all non-alcoholic beverages (excluding milk and milk based beverages), it was assumed that formulated beverages would account for an additional 5% uptake. Based on this, dietary exposure assessments were conducted for Scenario Two (*30% market share* scenario). The concentration for each food used in the *30% market share* scenario was calculated by multiplying the concentration assigned to the food group from Table 1 (mg of steviol glycosides/100 g product) by 0.3.

Table 2: Food groups and steviol glycosides concentration used in DIAMOND for the purpose of estimating dietary exposure

DIAMOND Food Code	Food Name	Steviol glycosides concentration (mg/kg)	
		Scenario 1 Sugar replacement*	**Scenario 2** 30% market share
1.1.2	Liquid milk products and flavoured liquid milk	115	35
1.2.2	Fermented milk products and rennetted milk products	176	53
3.0.1	Ice confection sold in liquid form	115	35
3.1	Ice cream	26	19
3.1.1	Ice cream reduced & low fat	137	62
3.1.2.1	Ice confection reduced & low fat	137	62
3.1.2.2	Ice confection, artificially sweetened	137	62
4.3.3.4	Canned Beetroot only	160	48
4.3.4.1	Chutneys, low joule jam & low joule spreads	225	135
4.3.6	Fruit and vegetable preparations inc pulp	62	62
4.3.6.3	Peanut butter	100	30
4.3.8.4	Soy beverages flavoured	175	53
4.3.8.5	Soy beverages plain	65	19
5.1.2	Chocolate products, artificially sweetened	550	165

DIAMOND Food Code	Food Name	Steviol glycosides concentration (mg/kg)	
		Scenario 1 Sugar replacement*	**Scenario 2** 30% market share
5.2.1.1	Bubble & chewing gum, artificially sweetened	550	330
5.2.3.1	Hard boiled confectionary, CHO modified	1,100	330
6.2.1	Custard powder	40	24
6.3	Processed cereal and meal products.	125	75
7.1.2	Fancy breads	160	48
7.2.1	Sweet biscuits (excluding chocolate coated & filled)	80	48
7.2.2	Cakes & muffins	32	48
7.2.3	Slices	48	48
7.2.4	Pastries	16	24
11.4	Table top sweeteners	200,000	120,000
11.4.1	Tabletop sweeteners, liquid preparation	4,000	2,400
11.4.2	Tabletop sweeteners – tablets or powder or granules packed in portion sized packages	200,000	120,000
13.3	Formula meal replacements & supplementary sports foods	175	53
13.4	Formulated supplementary sports foods	175	53
14.1	Non-alcoholic beverages	8	8
14.1.2.1	Fruit and vegetable juices	23#	23#
14.1.2.2	Fruit and vegetable juices products	108#	46#
14.1.3.1	Brewed soft drinks	160#	56#
14.1.3.2	Soft drinks, cola type	24#	22#
14.1.3.3	Soft drinks, non-cola type	40#	22#
14.1.3.4	Cordial only	24#	22#
14.1.3.6	Soft drinks, artificially sweetened	88#	56#
14.1.3.7	Cordials, Artificially sweetened	85#	37#
14.1.3.8	Kola type drinks - sugar sweetened	24#	22#
14.1.3.9	Kola type drinks - artificially sweetened	88#	56#
14.1.5.3	Herbal tea	103#	38#
14.1.5.6	Coffee substitute beverages	103#	38#
14.1.5.7	Coffee-based mixes beverage	103#	38#
20.2.1.1	Desserts, dairy only	75	45
20.2.1.2	Desserts	45	45
20.2.1.3	Desserts, artificially sweetened	75	45
20.2.1.4	Jelly, artificially sweetened only	195	78
20.2.4.1	Gravy & sauces only	63	38
20.2.4.2	Mayonnaise & salad dressings only	80	48
20.2.4.3	Toppings only	160	96
20.2.5.6	Pastry dishes (sweet)	16	24
20.2.9	Soup	100	30
20.3.1	Cereal products (commercial)	63	38

Note: the s*ugar replacement* scenario incorporates the expected proportion of products in each food group in which sucrose or intense sweeteners would be replaced by steviol glycosides over 20 years of availability to food suppliers.
* adjusted to reflect potential market share as predicted by the applicant
# adjusted to reflect market share for formulated beverages within the category

2.7 How were the estimated dietary exposures calculated?

A detailed explanation of how the estimated dietary exposures were calculated can be found in Appendix 1.

2.8 Assumptions in the dietary exposure assessment

The aim of the dietary exposure assessment was to make as realistic an estimate of dietary exposure as possible when only proposed concentration levels were available. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the dietary exposure assessment did not underestimate exposure.

Assumptions made in the dietary exposure assessment include:

- all the foods within the group contain steviol glycosides at the levels specified in Table 2. Unless otherwise specified, the maximum proposed concentration of steviol glycosides in each food category has been used;
- consumption of foods as recorded in the NNS represent current food consumption patterns;
- consumers do not alter their food consumption habits besides substituting non-steviol glycosides containing products with steviol glycosides containing products;
- consumers do not increase their consumption of foods/food groups upon foods/food groups containing steviol glycosides becoming available;
- formulated beverages account for an additional 5% of the non-alcoholic beverages (excluding milk and milk based beverages) market;
- for non-alcoholic beverages (excluding milk and milk based beverages), it was also assumed that where a non-alcoholic beverage was estimated to have a 100% market uptake after 20 years, 95% of the market was the non-alcoholic beverage and 5% was formulated beverage;
- 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 e.g. biscuits used in cheesecakes;
- there are no reductions in steviol glycosides concentrations from food preparation or due to cooking; and
- for the purpose of this assessment, it is assumed that 1 mL is equal to 1 g for all liquid and semi-liquid foods (e.g. milk, yoghurt).

These assumptions are likely to lead to a conservative estimate for steviol glycoside dietary exposure.

3 Results

3.1 Estimated dietary exposures to steviol glycosides

The dietary exposure assessment for steviol glycosides was conducted for the Australian population (2 years and above) and the New Zealand population (15 years and above), as well as for children aged 2-6 years (Australia only). Dietary exposures to steviol glycosides were calculated for:

- Scenario One (*sugar replacement* scenario);
- Scenario Two (*30% market share* scenario).

3.1.1 Scenario 1. Sugar replacement scenario

The estimated dietary exposures for steviol glycosides are shown in Figure 1 (full results in Table A2.1 in Appendix 2).

Australia – 2 years and above:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 57 mg/day and 107 mg/day, respectively.

Australia – 2-6 years:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 40 mg/day and 74 mg/day, respectively.

New Zealand – 15 years and above:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 40 mg/day and 74 mg/day, respectively.

3.1.2 Scenario 2. 30% market share scenario

The estimated dietary exposures for steviol glycoside are shown in Figure 2 (full results in Table A2.2 in Appendix 2).

Australia – 2 years and above:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 37 mg/day and 63 mg/day, respectively.

Australia – 2-6 years:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 24 mg/day and 41 mg/day, respectively.

New Zealand – 15 years and above:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 29 mg/day and 49 mg/day, respectively.

Estimated mean and 90th percentile dietary exposures to steviol glycosides for the Australian population (2 years and above) were higher than those for the New Zealand population (15 years and above) for the *sugar replacement* scenario. This may be due to different food consumption patterns (food types and/or amounts) between the two countries.

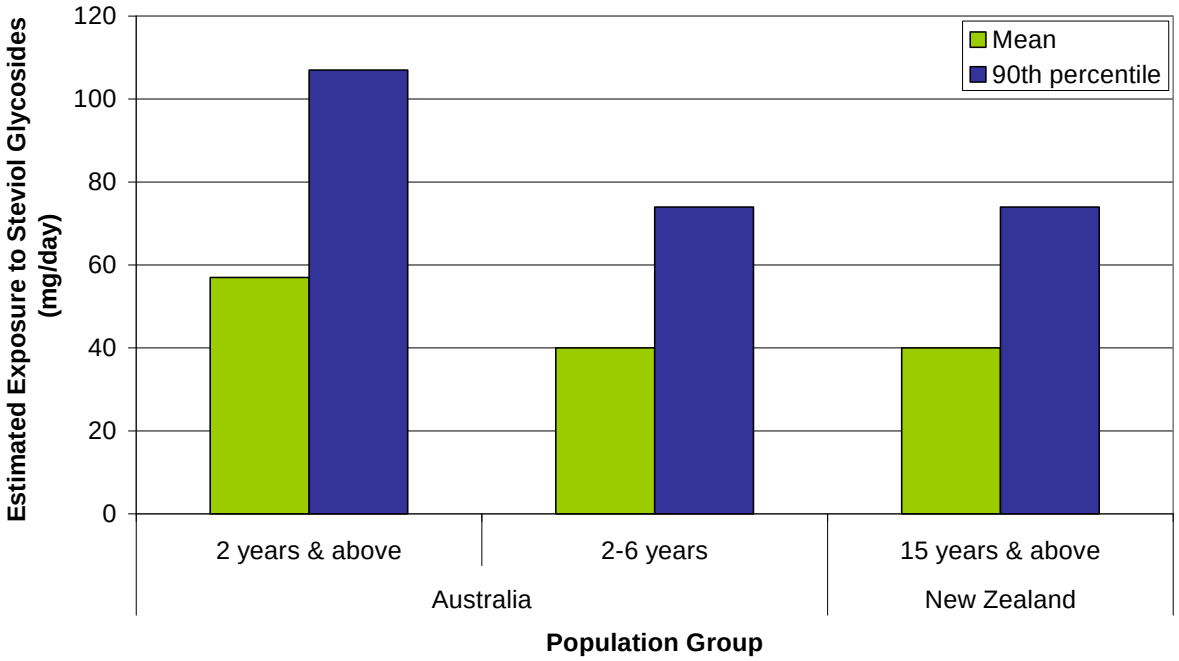

Figure 1: Estimated mean and 90th percentile dietary exposures (mg/day) for consumers of steviol glycosides for the Australian and New Zealand population groups (sugar replacement scenario)

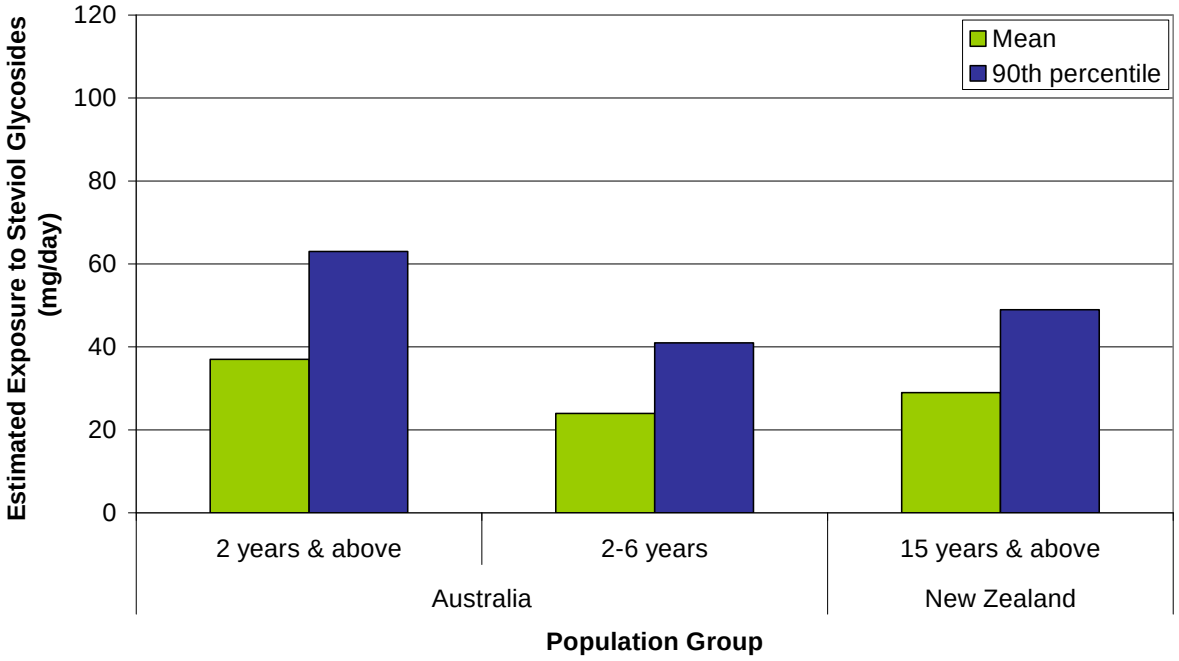

Figure 2: Estimated mean and 90th percentile dietary exposures (mg/day) for consumers of steviol glycosides for the Australian and New Zealand population groups (30% market share scenario)

3.2 Major contributing foods to total estimated dietary exposures

3.2.1 Scenario 1. Sugar replacement scenario

A full list of all the food groups and their contributions to steviol glycosides dietary exposure can be found in Table A2.3 in Appendix 2. The major contributors (≥5%) are shown in Figure 3 for Australians aged 2 years and above, Figure 4 for Australians aged 2-6 years and Figure 5 for New Zealanders aged 15 years and above.

Australia – 2 years and above:
The major contributors (≥5%) to total steviol glycosides dietary exposures were formulated beverages (21%), tabletop sweeteners (16%), carbonated soft drinks (14%), flavoured milks & yoghurts (9%) and fruit & vegetable juices & products (9%).

Australia – 2-6 years:
The major contributors (≥5%) to total steviol glycosides dietary exposures were fruit & vegetable juices & products (23%), flavoured milks and yoghurts (14%), formulated beverages (11%), carbonated soft drinks (9%), breakfast cereals, mueslis and muesli bars (7%) and ice creams and ice confections (6%).

New Zealand – 15 years and above:
The major contributors (≥5%) to total steviol glycosides dietary exposures were formulated beverages (29%), tabletop sweeteners (15%), carbonated soft drinks (12%) and bakery products (9%).

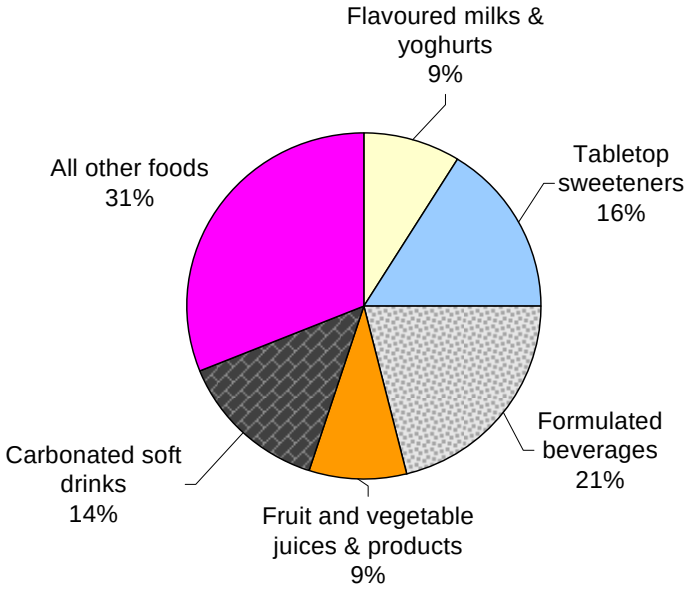

Figure 3: Major contributors to steviol glycosides dietary exposures for Australians aged 2 years and above (sugar replacement scenario)[17]

[17] Note: The percent contribution of each food group is based on total steviol glycosides exposures for all consumers in the population groups assessed. Therefore the total steviol glycosides exposures differ for each population group and each scenario.

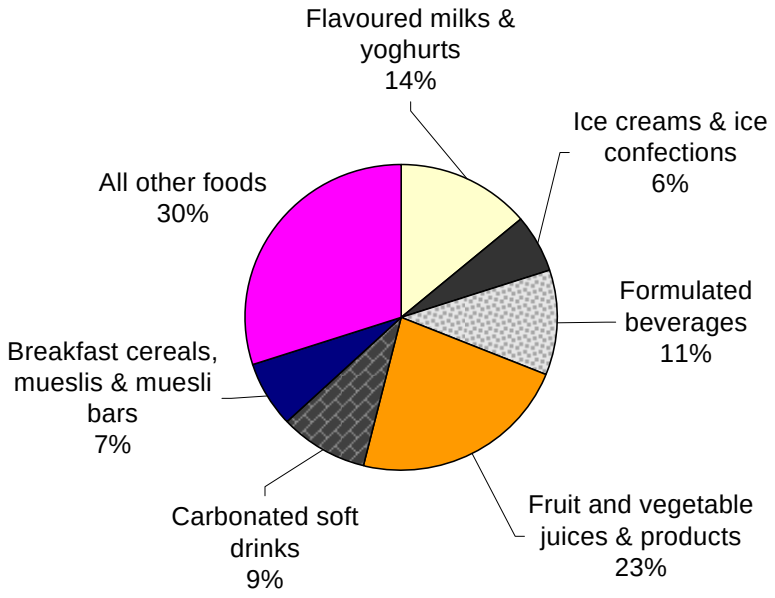

Figure 4: Major contributors to steviol glycosides dietary exposures for Australia – 2-6 years (sugar replacement scenario) .[18]

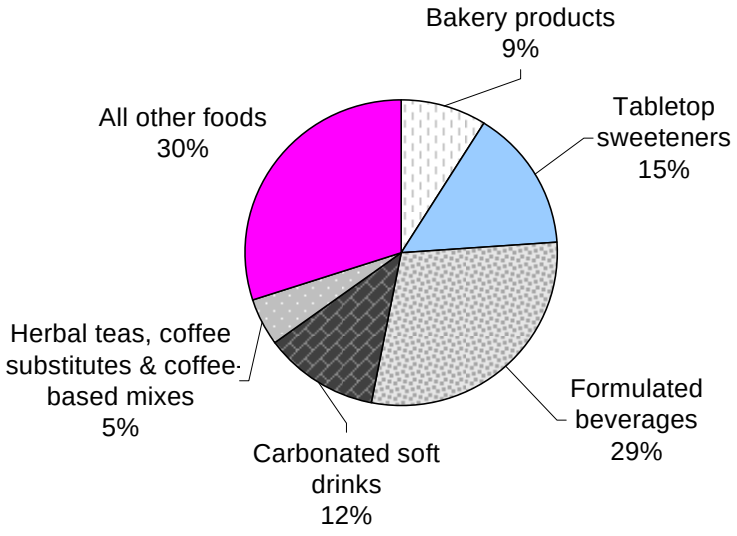

Figure 5: Major contributors to steviol glycosides dietary exposures for New Zealand - 15 years and above (sugar replacement scenario).[2]

[18] Note: The percent contribution of each food group is based on total steviol glycosides exposures for all consumers in the population groups assessed. Therefore the total steviol glycosides exposures differ for each population group and each scenario.

3.2.2 Scenario 2. 30% market share scenario

A full list of all the food groups and their contributions to total steviol glycosides dietary exposures can be found in Table A2.4 in Appendix 2. The major contributing foods (≥5%) for Scenario Two (*30% market share* scenario) are shown in Figure 6 for Australians aged 2 years and above, Figure 7 for Australians aged 2-6 years and Figure 8 for New Zealanders aged 15 years and above.

Australia – 2 years and above:
The major contributors (≥5%) to total steviol glycosides dietary exposures were formulated beverages (32%), tabletop sweeteners (15%), carbonated soft drinks (14%) and fruit and vegetable juices & products (8%).

Australia – 2-6 years:
The major contributors (≥5%) to total steviol glycosides dietary exposures were fruit and vegetable juices & products (22%), formulated beverages (17%), carbonated soft drinks (9%), breakfast cereals, mueslis and muesli bars (7%), flavoured milks and yoghurts (7%), and bakery products (5%).

New Zealand – 15 years and above:
The major contributors (≥5%) to total steviol glycosides dietary exposures were formulated beverages (41%), tabletop sweeteners (12%), carbonated soft drinks (11%), and bakery products (9%).

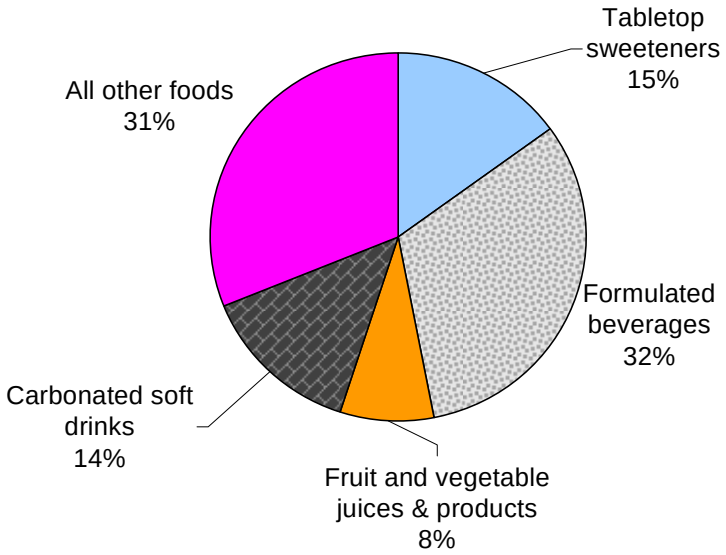

Figure 6: Major contributors to steviol glycosides dietary exposures for Australians aged 2 years and above (30% market share scenario)[19]

[19] Note: The percent contribution of each food group is based on total steviol glycosides exposures for all consumers in the population groups assessed. Therefore the total steviol glycosides exposures differ for each population group and each scenario.

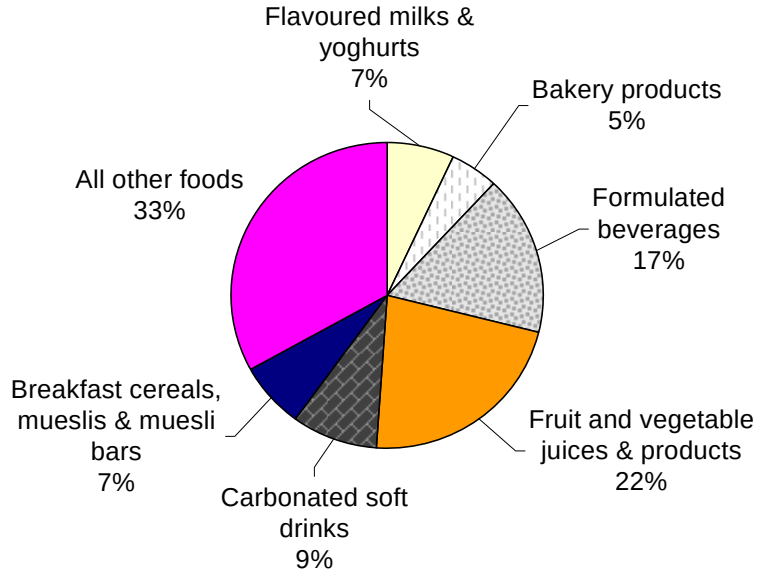

Figure 7: Major contributors to steviol glycosides dietary exposures for Australians aged 2-6 years (30% market share scenario)[4]

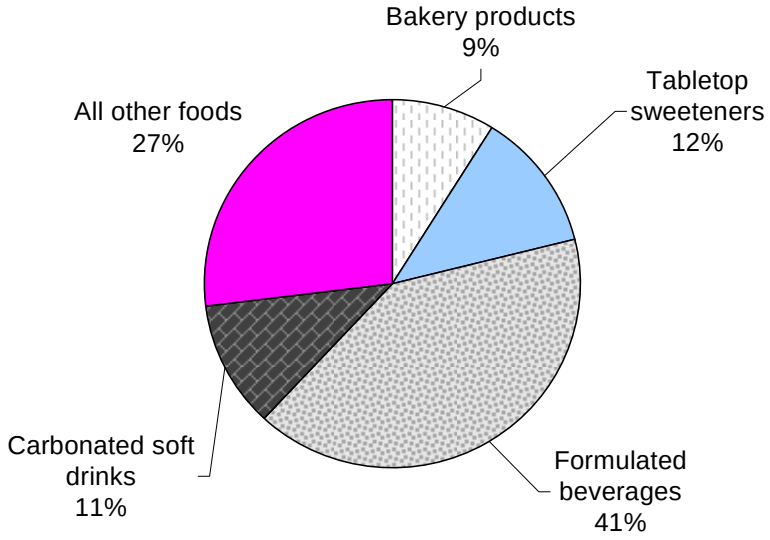

Figure 8: Major contributors to total steviol glycosides dietary exposures for New Zealanders aged 15 years and above (30% market share scenario) [20]

[20] Note: The per cent contribution of each food group is based on total steviol glycosides exposures for all consumers in the population groups assessed. Therefore the total steviol glycosides exposures differ for each population group and each scenario.

For both scenarios, the major contributors to steviol glycosides dietary exposures for Australians aged 2 years and above and for New Zealanders aged 15 years and above were predicted to be formulated beverages, tabletop sweeteners and carbonated soft drinks. For Australian children aged 2-6 years, the major contributors were fruit and vegetable juices and products, formulated beverages, carbonated soft drinks, breakfast cereals, mueslis and muesli bars, and flavoured milks and yoghurts for both scenarios. Beverages were predicted to be major contributors to steviol glycosides exposures, most likely due to the large volume of these products consumed.

4 Limitations of the dietary exposure assessment

Dietary modelling based on 1995 or 1997 NNS food consumption data provides the best estimate of actual consumption of a food and the resulting estimated dietary exposure of a chemical for the population. However, it should be noted that the NNS data do have limitations. These limitations relate to the age of the data and the changes in eating patterns that may have occurred since the data were collected. Generally, consumption of staple foods such as fruit, vegetables, meat, dairy products and cereal products, which make up the majority of most people's diet, is unlikely to have changed markedly since 1995/1997 (Cook *et al*., 2001). However, there is uncertainty associated with the consumption of foods that may have changed in consumption since 1995/1997, or that have been introduced to the market since 1995/1997.

A limitation of estimating dietary exposure over a period of time associated with the dietary modelling is that only 24-hour dietary survey data were available, and these tend to over-estimate habitual food consumption amounts for high consumers. FSANZ dietary exposure assessments report 90th percentile exposures rather than 95th percentile exposures in order to minimise this limitation.

Daily food consumption amounts for occasionally consumed foods based on 24 hour food consumption data would be higher than daily food consumption amounts for those foods based on a longer period of time. This specifically affects the food groups in this assessment such as sauces, toppings, mayonnaises and salad dressings.

Over time, there may be changes to the ways in which manufacturers and retailers make and present foods for sale. Since the data were collected for the Australian and New Zealand NNSs, there have been significant changes to the Food Standards Code to allow more innovation in the food industry. As a consequence, another limitation of the dietary modelling is that some of the foods that are currently available in the food supply were either not available or were not as commonly available in 1995/1997.

While the results of national nutrition surveys can be used to describe the usual intake of groups of people, they cannot be used to describe the usual intake of an individual (Rutishauser, 2000). In particular, they cannot be used to predict how consumers will change their eating patterns as a result of an external influence such as the availability of a new type of food.

FSANZ does not apply statistical population weights to each individual in the NNSs in order to make the data representative of the population. This prevents distortion of actual food consumption amounts that may result in an unrealistic intake estimate.

Maori and Pacific Islanders were over-sampled in the 1997 New Zealand NNS so that statistically valid assessments could be made for these population groups. As a result, there may be bias towards these population groups in the dietary exposure assessment because population weights were not used.

As steviol glycosides are not currently permitted to be added to foods in Australia or New Zealand, it is difficult to predict the concentrations of steviol glycosides that will be used in foods, and the proportion of food groups containing steviol glycosides. The dietary exposure assessment may cover more foods than would actually contain steviol glycosides, should permission for use be granted.

5 Comparison with the Acceptable Daily Intake

In order to determine if the level of exposure to steviol glycosides will be a public health and safety concern, the estimated dietary exposures were compared to a reference health standard, the Acceptable Daily Intake (ADI). The ADI is defined as an estimate of the amount of a chemical that can be ingested daily over a lifetime without appreciable risk to health (WHO, 2001). An ADI of 4 mg/kg body weight (bw) (expressed as steviol equivalents), was set by FSANZ and used in this assessment (see attachment 2 for further details).

5.1. Scenario 1. *Sugar replacement* scenario

The estimated dietary exposures for steviol glycosides for the *sugar replacement* scenario, as compared to the ADI are shown in Figure 6 (full results in Table A3.1 in Appendix 3). Estimated mean and the 90th percentile exposures for the Australian and New Zealand population groups assessed were at or below the ADI.

Australia – 2 years and above:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 25% of the ADI and 50% of the ADI, respectively.

Australia – 2-6 years:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 55% of the ADI and 100% of the ADI, respectively.

New Zealand – 15 years and above:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 15% of the ADI and 25% of the ADI, respectively.

5.1.2 Scenario 2. *30% market share* scenario

The estimated dietary exposures to steviol glycosides for the *30% market share* scenarios, as compared with the ADI, are shown in Figure 7 (full results in Table A3.2 in Appendix 3). Estimated mean and 90th percentile exposures for all population groups assessed were at or below the ADI.

Australia – 2 years and above:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 15% of the ADI and 30% of the ADI, respectively.

Australia – 2-6 years:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 35% of the ADI and 55% of the ADI, respectively.

New Zealand – 15 years and above:
Estimated mean and 90th percentile exposures for consumers of steviol glycosides were 10% of the ADI and 20% of the ADI, respectively.

Australian children aged 2-6 years had the highest estimated mean and 90th percentile dietary exposures to steviol glycosides (as a %ADI) for both the *sugar replacement* and *30% market share* scenarios. Children generally have higher dietary exposures due to their smaller body weight and the fact that they consume more food per kilogram of body weight compared to adults.

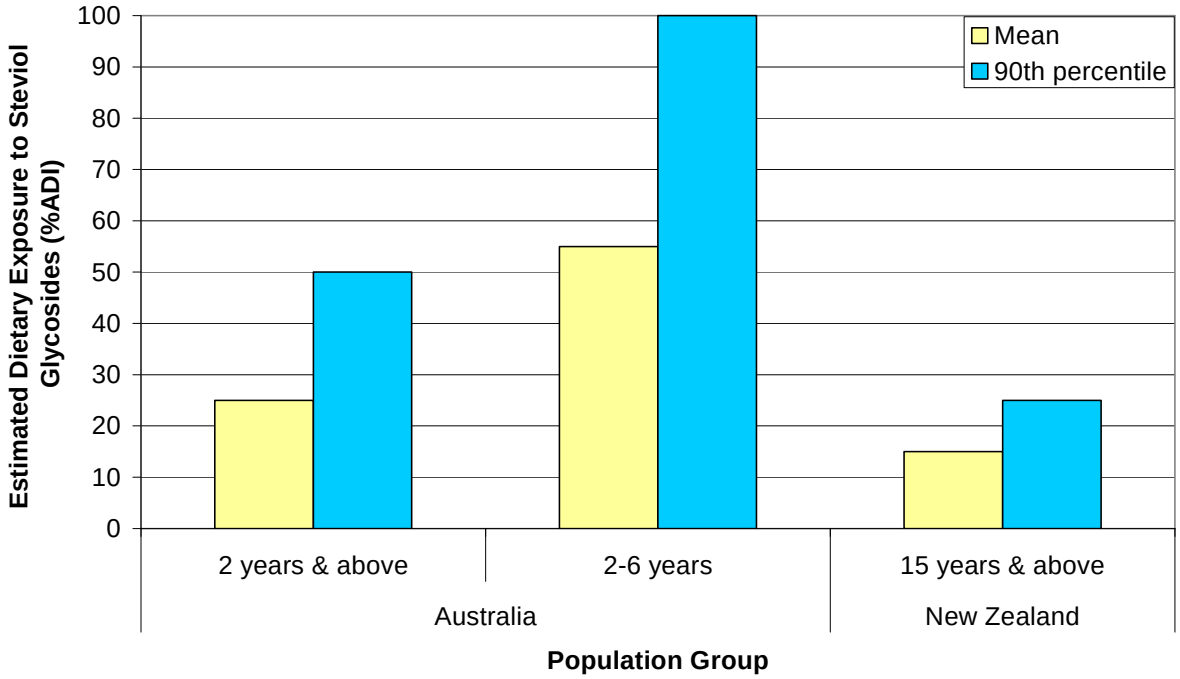

Figure 6: Estimated dietary exposures to steviol glycosides, for the sugar replacement scenario, as a percentage of the ADI

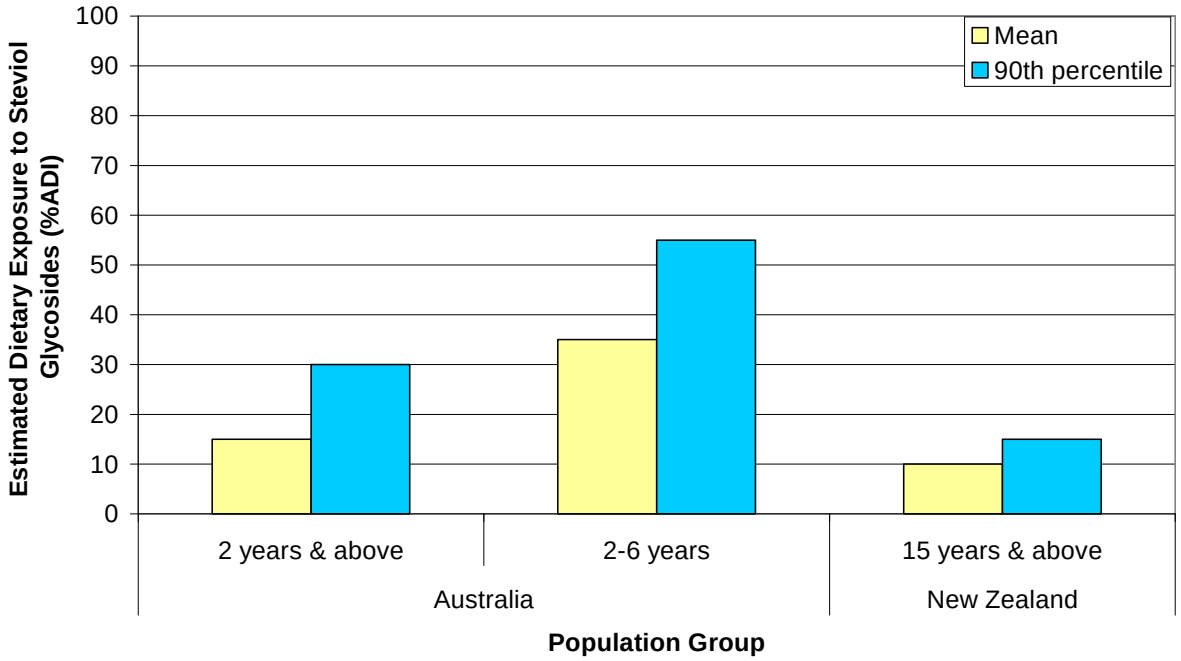

Figure 7: Estimated dietary exposures to steviol glycosides for the 30% market share scenario, as a percentage of the ADI

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Rutishauser, I. (2000) *Getting it right:- how to use the data from the 1995 National Nutrition Survey*. Commonwealth of Australia, Canberra.

WHO (2001) *Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1956-2003) (First through fifty-seventh meetings) Internet Edition*. ILSI Press International Life Sciences Institute, Washington DC.

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Appendix 1

How were the estimated dietary exposures calculated?

Steviol glycosides are used as a sweetener and can be used as a sugar replacement in a range food groups. The exposure to steviol glycosides was calculated for each individual in the National Nutrition Survey (NNS) using his or her individual food records from the dietary survey.

The DIAMOND program allows steviol glycosides concentrations to be assigned to food groups. The DIAMOND program multiplies the specified concentration of steviol glycosides by the amount of food that an individual consumed from that group in order to estimate the exposure to steviol glycoside from each food. Once this has been completed for all of the foods specified to contain steviol glycosides, the total amount of steviol glycosides consumed from all foods is summed for each individual. Population statistics (mean and high percentile exposures) are then derived from the individuals' ranked exposures.

Where estimated dietary exposures are expressed per kilogram of body weight, each individual's total dietary exposure from all foods is divided by their own body weight, the results ranked, and population statistics derived. A small number of NNS respondents did not provide a body weight. These respondents are not included in calculations of estimated dietary intakes that are expressed per kilogram of body weight.

Where estimated exposures are expressed as a percentage of the reference health standard, each individual's total exposure from all foods is calculated as a percentage of the reference health standard (either using the total exposures in units per day or units per kilogram of body weight per day, depending on the units of the reference health standard), the results are then ranked, and population statistics derived.

Food consumption amounts for each individual take into account where each food in a classification code is consumed alone and as an ingredient in mixed foods. For example, milk consumed as a glass of milk, milk in a coffee, and milk in a sauce or custard are all included in the consumption of milk. Where a higher level food classification code (e.g. 7.2 Biscuits, cakes and pastries) is given a steviol glycosides concentration, as well as a sub-category (e.g. 7.2.4 pastries), the consumption of the foods in the sub-classification is not included in the higher level classification code.

In DIAMOND, all mixed foods in classification codes 20 and 21 have a recipe. Recipes are used to break down mixed foods into component ingredients that are in classification codes 1-14. The data for consumption of the ingredients from the recipe are then used in models and multiplied by steviol glycosides concentrations for each of the component ingredients. This only occurs if the *Mixed food* classification code (classification code 20) is not assigned its own steviol glycosides permission. If the *Mixed foods* classification is assigned a steviol glycosides concentration, the total consumption of the mixed food is multiplied by the proposed level and the recipes are not used for that food group.

When a food that does not have a recipe is classified in two food groups in classification codes 1-14, and these food groups are assigned different permissions, DIAMOND will assume the food is in the food group with the highest assigned steviol glycosides level to assume a worst-case scenario.

If the food groups have the same permitted steviol glycosides level, DIAMOND will assume the food is in the food group that appears first, based numerically on the ANZFCS.

In DIAMOND, hydration factors are applied to some foods to convert the amount of food consumed in the dietary survey to the equivalent amount of the food in the form to which a food chemical permission is given. For example, consumption figures for cordial concentrates are converted into the equivalent quantities of cordial prepared ready to consume.

Percentage contributions of each food group to total estimated exposures are calculated by summing the exposures for a food group from each individual in the population group who consumed a food from that group and dividing this by the sum of the exposures of all individuals from all food groups containing steviol glycosides, and multiplying this by 100.

Appendix 2

Complete information on dietary exposure assessment results

Table A2.1: Estimated dietary exposures to steviol glycosides for the *sugar replacement* scenario

Country	Population group	No. of consumers of steviol glycosides	Consumers* as a % of respondents#	Estimated consumer dietary exposure to steviol glycosides	
				Mean (mg/day)	90th percentile (mg/day)
Australia	2 years and above	13,851	99.9	57	107
	2-6 years	987	99.8	40	74
New Zealand	15 years and above	4,634	99.9	40	74

Total number of respondents for Australia: 2 years and above = 13,858, 2-6 years = 989; New Zealand: 15 years and above = 4,636. Respondents include all members of the survey population whether or not they consumed a food that contains steviol glycosides.
* Consumers only – This only includes the people who have consumed a food that contains steviol glycosides.

Table A2.2: Estimated dietary exposures to steviol glycosides for the *30% market share* scenario

Country	Population group	No. of consumers of steviol glycosides	Consumers* as a % of respondents#	Estimated consumer dietary exposures to steviol glycosides (mg/day)	
				Mean	90th percentile
Australia	2 years and above	13,851	99.9	37	63
	2-6 years	987	99.8	24	41
New Zealand	15 years and above	4,634	99.9	29	49

Total number of respondents for Australia: 2 years and above = 13,858, 2-6 years = 989; New Zealand: 15 years and above = 4,636. Respondents include all members of the survey population whether or not they consumed a food that contains steviol glycosides.
* Consumers only – This only includes the people who have consumed a food that contains steviol glycosides.

Table A2.3: Major contributing foods to steviol glycosides dietary exposures for Australia and New Zealand, for different population groups (*sugar replacement* scenario)

Food Code	Food Name	% Contribution to Steviol Glycosides Exposures		
		Australia		New Zealand
		2 yrs & above	2-6 years	15 yrs & above
1.1.2	Liquid milk products and flavoured liquid milk	4	4	1
1.2.2	Fermented milk products and rennetted milk products	4	10	2
3.0.1	Ice confection sold in liquid form	<1	2	-
3.1	Ice cream	<1	1	<1
3.1.1	Ice cream reduced & low fat	1	3	<1
3.1.2.1	Ice confection reduced & low fat	<1	-	-
4.3.3.4	Canned beetroot only	<1	<1	<1
4.3.4.1	Low joule chutneys, low joule jam & low joule spreads	<1	<1	<1
4.3.6	Fruit and vegetable preparations including pulp	1	1	<1
4.3.6.3	Peanut butter	<1	<1	<1
4.3.8.4	Soy beverages flavoured	<1	<1	<1
4.3.8.5	Soy beverages plain	<1	1	<1
5.1.2	Chocolate products, intensely sweetened	<1	<1	-
5.2.1.1	Bubble & chewing gum, intensely sweetened	<1	<1	<1
5.2.3.1	Hard boiled confectionary, CHO modified	<1	<1	-
6.2.1	Custard powder	<1	-	-
6.3	Breakfast cereals	4	6	4
7.1.2	Fancy breads	3	2	4
7.2.1	Sweet biscuits	1	2	2
7.2.2	Cakes & muffins	<1	<1	2
7.2.3	Slices	<1	<1	<1
7.2.4	Pastries	<1	<1	<1
11.4.1	Tabletop sweeteners, liquid preparations	<1	-	<1
11.4.2	Tabletop sweeteners, tablets or powder or granules packed in portion sized packages	16	2	15

Table A2.3: Major contributing foods to steviol glycosides dietary exposures for Australia and New Zealand, for different population groups (s*ugar replacement* scenario) (cont'd)

Food Code	Food Name	% Contribution to Steviol Glycosides Exposures		
		Australia		New Zealand
		2 yrs & above	2-6 years	15 yrs & above
13.3	Formula meal replacements & supplementary food	<1	<1	<1
13.4	Formulated supplementary sports foods	-	-	<1
14.1	Formulated beverages	21	11	29
14.1.2.1	Fruit and vegetable juices (fruit juices only)	3	6	2
14.1.2.2	Fruit and vegetable juices products	6	17	3
14.1.2.3	Coconut milk, cream & syrup	-	-	-
14.1.3.1	Brewed soft drinks	3	<1	-
14.1.3.2	Soft drinks, cola type	3	2	4
14.1.3.3	Soft drinks, non-cola type	3	4	5
14.1.3.4	Cordial only	3	11	3
14.1.3.6	Soft drinks, intensely sweetened	5	2	3
14.1.3.7	Cordials, intensely sweetened	1	2	<1
14.1.5.3	Herbal tea	3	-	4
14.1.5.6	Coffee substitutes beverage	1	<1	1
14.1.5.7	Coffee-based mixes beverage	<1	-	<1
20.2.1.1	Desserts, dairy	<1	1	<1
20.2.1.2	Desserts	<1	<1	<1
20.2.1.3	Desserts intensely sweetened	-	-	-
20.2.2.2	Jelly intensely sweetened	<1	<1	<1
20.2.4.1	Gravy & sauces	1	<1	3
20.2.4.2	Mayonnaise & salad dressings	<1	<1	<1
20.2.4.3	Toppings	<1	<1	<1
20.2.5.6	Pastry dishes (sweet)	<1	<1	<1
20.2.9	Soup	4	2	3
20.3.1	Muesli and muesli bars	<1	<1	<1

Table A2.4: Major contributors to total steviol glycosides dietary exposures for Australia and New Zealand, for different population groups (*30% market share* scenario)

Food Code	Food Name	% Contribution to Steviol Glycosides Exposures		
		Australia		New Zealand
		2 yrs & above	2-6 years	15 yrs & above
1.1.2	Liquid milk products and flavoured liquid milk	2	2	<1
1.2.2	Fermented milk products and rennetted milk products	2	5	<1
3.0.1	Ice confection sold in liquid form	<1	1	-
3.1	Ice cream	<1	2	<1
3.1.1	Ice cream reduced & low fat	<1	2	<1
3.1.2.1	Ice confection reduced & low fat	<1	-	-
4.3.3.4	Canned beetroot only	<1	<1	<1
4.3.4.1	Low joule chutneys, low joule jam & low joule spreads	<1	<1	<1
4.3.6	Fruit and vegetable preparations including pulp	2	2	1
4.3.6.3	Peanut butter	<1	<1	<1
4.3.8.4	Soy beverages flavoured	<1	<1	<1
4.3.8.5	Soy beverages plain	<1	<1	<1
5.1.2	Chocolate products, intensely sweetened	<1	<1	-
5.2.1.1	Bubble & chewing gum, intensely sweetened	<1	<1	<1
5.2.3.1	Hard boiled confectionary, CHO modified	<1	<1	-
6.2.1	Custard powder	<1	-	-
6.3	Breakfast cereals	4	6	3
7.1.2	Fancy breads	1	<1	2
7.2.1	Sweet biscuits	1	2	2
7.2.2	Cakes & muffins	2	2	4
7.2.3	Slices	<1	<1	<1
7.2.4	Pastries	<1	<1	<1
11.4.1	Tabletop sweeteners, liquid preparations	<1	-	<1
11.4.2	Tabletop sweeteners, tablets or powder or granules packed in portion sized packages	15	2	12
13.3	Formula meal replacements & supplementary food	<1	<1	<1

Food Code	Food Name	% Contribution to Steviol Glycosides Exposures		
		Australia		New Zealand
		2 yrs & above	2-6 years	15 yrs & above
13.4	Formulated supplementary sports foods	-	-	<1
14.1	Formulated beverages	32	17	41
14.1.2.1	Fruit and vegetable juices (fruit juices only)	4	10	3
14.1.2.2	Fruit and vegetable juices products	4	12	2
14.1.3.1	Brewed soft drinks	1	<1	-
14.1.3.2	Soft drinks, cola type	5	3	5
14.1.3.3	Soft drinks, non-cola type	3	4	4
14.1.3.4	Cordial	4	16	4
14.1.3.6	Soft drinks, intensely sweetened	5	2	3
14.1.3.7	Cordials, intensely sweetened	<1	2	<1
14.1.5.3	Herbal tea	2	-	2
14.1.5.6	Coffee substitutes beverage	<1	<1	<1
14.1.5.7	Coffee-based mixes beverage	<1	-	<1
20.2.1.1	Desserts, dairy	<1	1	<1
20.2.1.2	Desserts	<1	<1	<1
20.2.1.3	Desserts intensely sweetened	-	-	-
20.2.2.2	Jelly intensely sweetened	<1	<1	<1
20.2.4.1	Gravy & sauces	1	<1	3
20.2.4.2	Mayonnaise & salad dressings	<1	<1	<1
20.2.4.3	Toppings	<1	<1	<1
20.2.5.6	Pastry dishes (sweet)	<1	<1	<1
20.2.9	Soup	2	<1	1
20.3.1	Muesli and muesli bars	<1	<1	<1

Appendix 3

Complete information on risk characterisation

Table A3.1: Estimated dietary exposures to steviol glycosides, as a percentage of ADI for the *sugar replacement* scenario

Country	Population group	No. of consumers of steviol glycosides	Consumers* as a % of respondents#	Estimated consumer dietary exposures to steviol glycosides (%ADI*)	
				Mean	90th percentile
Australia	2 years and above	13,851	99.9	25	50
	2-6 years	987	99.8	55	100
New Zealand	15 years and above	4,634	99.9	15	25

Total number of respondents for Australia: 2 years and above = 13,858, 2-6 years = 989; New Zealand: 15 years and above = 4,636. Respondents include all members of the survey population whether or not they consumed a food that contains steviol glycosides.
* Consumers only – This only includes the people who have consumed a food that contains steviol glycosides.
* ADI (Acceptable Daily Intake) of 4 mg/kg bw/day proposed by FSANZ.

Table A3.2: Estimated dietary exposures to steviol glycosides, as a percentage of ADI for the *30% market share* scenario

Country	Population group	No. of consumers of steviol glycosides	Consumers* as a % of respondents#	Estimated consumer dietary exposures to steviol glycosides (%ADI*)	
				Mean	90th percentile
Australia	2 years and above	13,851	99.9	15	30
	2-6 years	987	99.8	35	55
New Zealand	15 years and above	4,634	99.9	10	15

Total number of respondents for Australia: 2 years and above = 13,858, 2-6 years = 989; New Zealand: 15 years and above = 4,636. Respondents include all members of the survey population whether or not they consumed a food that contains steviol glycosides.
* Consumers only – This only includes the people who have consumed a food that contains steviol glycosides.
* ADI (Acceptable Daily Intake) of 4 mg/kg bw/day proposed by FSANZ.

Attachment 4

Summary of submissions to Draft Assessment Report

Submitter	Comments
Stevia Australia P/L	Strongly supports the Application. Stevia Australia P/L has Federal Government support through a grant from the New Industries Development Program to assist in starting this industry. Believes this industry will greatly help Australia's obesity problem.
Centre for Plant & Water Science, Central Queensland University (the Applicant)	Welcomes the overall conclusions of the Report and recommendation to allow the use of steviol glycosides in a wide variety of foods. Would prefer unlimited approval (Schedule 2 food additive), but acknowledge that some limits may be required to manage potential (albeit remote) high intakes of steviol glycosides. Advantages of Option 2 include providing a greater opportunity for innovation, greater potential for mixing of sweeteners to reduce exposure to single sweeteners and maximise potential for health professionals to target any health benefits for particular individuals. **Risk Assessment** • Adoption of ADI of 4 mg/kg bw/day (expressed as steviol) is supported. • Dietary modelling indicated high consuming children are unlikely to exceed the ADI and average consumers were substantially below the ADI, assuming there will never be full substitution of sugar by steviol glycosides. Provided data on gross sugar consumption in Australia. If all sugar used in Australia (up to 40 kg/head/year) was substituted by steviol glycosides, the average intake of steviol glycosides would be 3.3–3.5 mg/kg bw/day (as steviol – 85% of the ADI). As actual uptake of steviol glycosides is likely to be less than 30%, the potential average uptake of steviol glycosides would likely only be 1 mg/kg bw/day. • Appears to be over estimate of steviol glycoside consumption via tea and coffee products. Most tea and coffee packs will not contain steviol glycosides. The intention was to enable only herbal teas and substitutes for coffee to contain steviol glycosides. • In view of low expected uptake of steviol glycosides, relative to the ADI of 4 mg/kg bw/day, requests consideration of allowing the use of steviol glycosides in additional products (as requested in the original Application). • Believes that extending the range of products containing steviol glycosides is more likely to increase the number of people consuming some steviol glycosides rather than increase the amount consumed by already moderate/high consuming individuals.

Submitter	Comments
	It is expected that main determinant of dietary exposure to steviol glycosides will be the rate of adoption of products containing some steviol glycosides rather than the maximum percentage content of steviol glycosides being used in the food or beverage. • Provided a number of suggested amendments to the draft variations. The requested amendments include new requests for permission in peanut butter, fancy breads, formulated beverages and an increase in the level of use requested for bubble and chewing gum and hard boiled confectionery. **Terminology** • Still confusion regarding terminology. Suggests Final Assessment Report should make it very clear that reference to steviol glycosides, in terms of the ADI and the draft variations to the Code, is expressed as steviol. **Specifications** • No reference to specifications for steviol glycosides in main part of Report. Reference to tentative JECFA specification in Attachments to the Report. • Applicant provided a suggested specification, which varies slightly from the JECFA specification. Suggests minimum steviol glycoside content of 90% compared to JECFA's 95% and should include all measured glycosides. • It is suggested that 95% purity will exclude a large portion of the current world supply of steviol glycosides. Generally, 95% purity is achieved only by reprocessed glycosides powder, often associated with the separation of rebaudioside A or with enzymatic modification. • Most health and safety trials have used commercially available 85-95% pure product.
International Association for Stevia Research	Submitted a literature review on the pharmacological effects of steviol glycosides. Supports the approval of steviol glycosides, however does not state whether Option 2 or 3 is preferred. Is concerned that FSANZ may have overestimated the supposed sweetness of stevia, and that the suggested permissions may not be sufficient to provide manufacturers with the appropriate levels of sweetness. Suggests that additional research on the metabolism of steviol glycosides and long-term effects in animals and humans should be conducted in order to establish a higher ADI.
Cargill Incorporated	Supports Application, and highlighted that the Report only included a conversion factor for stevioside to steviol. Suggested that it should include conversion factors for other steviol glycosides to steviol, particularly rebaudioside A. Provided a number of conversion factors – stevioside (40%), rebaudioside A (33%), rebaudioside C (33%) and dulcoside A (40%).

Submitter	Comments
	Also provided some input around use levels for food applications where Cargill has experience, particularly the use of rebaudioside A. Suggests that a number of the use rates indicated in the draft variation (using only the 40% conversion rate) will fall short of what is required to provide optimal sweetness to those products.
Food Technology Association of Victoria	Supports Option 2. Provided comment on appropriate categorisation of soy-based beverages in the draft variation. Suggests a new sub-category to be called 'Soy Based Beverages' and numbered 4.3.9, following 4.3.8 – 'Other fruit and vegetable based products'. Soybeans are classified as a legume vegetable in Standard 1.4.2, Schedule 4 therefore, soy based beverage was considered to more appropriately placed with Section 4 – Fruits and Vegetables of Schedule 1 of Standard 1.3.1.
Queensland Health	Supports Option 2 on the understanding that there is no alternative view expressed by the Scientific Panel of food additives, flavourings, processing aids and materials in contact with food and follows a request by the European Commission (expected in second half of 2007). Questions whether the use of steviol glycosides is justified in foods that are not low joule. Analysis of stevioside in simple matrices such as beverages is fairly easy. However, there is a range of steviol glycosides and pure standards of each of them would need to be obtained for enforcement purposes. It is not known how easy analysis of the compounds in more complex matrices would be. Considers that soybean beverage should be considered as a vegetable juice. However, recognises the need to obtain views from other stakeholders to ensure any change to a listing in a food category does not impact significantly on industry.
Calorie Control Council	Supports the Application. Highlighted the Council's long standing support for the approval of a wide variety of safe alternative sweeteners. Suggests FSANZ might wish to consult with industry representatives in Australia and New Zealand concerning the proposed levels of use for steviol glycosides – with some concern expressed that the proposed levels might not be adequate for their intended uses. State that the approval of steviol glycosides could provide an additional tool to address concerns about obesity and related conditions.

Submitter	Comments
New Zealand Food Safety Authority	Supports Option 2, subject to a favourable outcome at the 68th JECFA meeting. Suggests it would be prudent to take into account and refer to JECFA's evaluation of steviol glycosides in the Final Assessment. The inclusion of tabletop sweeteners permitted to contain steviol glycosides, under Option 2, will enable products manufactured in New Zealand that are currently sold as dietary supplements to be sold under the Code. With regard to the categorisation of soybean beverages, pointed out a discussion paper presented at 39th meeting of the Codex Committee on Food Additives in April 2007 proposing that soybean beverages be placed into a new sub-category under 06.8 Soybean Products. NZFSA has no objection with soy beverages being placed under 14.1.2.2 Fruit and Vegetable Juice Products in Schedule 1 of Standard 1.3.1 as proposed under the heading Soybean Beverage (plain or flavoured) – noting that additives in Schedules 2, 3 and 4 are permitted in this category.
Dietitians Association of Australia	Qualified support of Option 2. Notes that there is only a temporary ADI as determined by JECFA and it may be prudent for FSANZ to wait until JECFA has made a recommendation on an ADI at the June 2007 meeting. In addition, the Scientific Panel of food additives, flavourings, processing aids and materials in contact with food is expected to provide an opinion in the second half of 2007. Recommends that changes to Standard 1.3.1 be delayed until these reports have been considered.
DIC International (Australia)	Supports Option 2. Parent company in Japan has been one of the major manufacturers of stevia for the Japanese market for the last 20 years. DIC Australia has been importing stevia for a number of years for use in Therapeutic Goods Administration regulated over the counter (OTC) supplements. Agree with proposed ADI of 4 mg/kg bw/day. Refers to a clinical study conducted in 2006 in response to outstanding issues raised by JECFA at their 63rd meeting. In over 20 years of manufacturing and selling stevia sweeteners in the Japanese market DIC have not become aware of any adverse health effects or complaints from their clients. State that stevia can fulfil consumers' desire for natural products and is a good alternative to other sweeteners.

Submitter	Comments
Coca-Cola South Pacific	Supports FSANZ's view. Requests clarification regarding the expression of steviol glycosides in drafting. Quantities of steviol glycosides should be clearly expressed as steviol equivalents. Permissions proposed in the Report for beverages may be too low to produce beverage products with complete sugar replacement. Use levels for partial sugar replacement in beverages is also considered too low to permit development of many future beverage products. Recommend steviol glycosides also be approved for use in formulated beverages. Suggests that dietary exposure assessment scenario based on complete replacement of sugar is unrealistic and unnecessarily conservative. The 30% total sugar replacement scenario is more realistic and produced results similar to intake assessment conducted for Coca-Cola by Professor Andrew Renwick. Suggests there is room beneath the ADI for inclusion of higher permissions of steviol glycosides for beverages.
Australian Beverages Council Ltd	Supports Option 2. Recommends clarifying in the draft regulation that steviol glycosides are expressed as steviol equivalents. Also suggest that use levels provided by the Applicant may be too low to produce beverage products with complete sugar replacement. Suggests that all beverage categories where non-nutritive sweeteners are currently permitted should be included in the permissions for steviol glycosides. This may allow for innovation of new beverage products without the need to seek further regulatory changes in the future (that is, amending Schedule 1 of Standard 1.3.1).
Functional Biology Laboratory KULeuven	Provided a published study entitled 'Metabolism of Stevioside by Healthy Subjects', by Geuns, J.M.C. et al (Exp Biol Med 232:164173, 2007). In the risk assessment FSANZ had referred to this study as an unpublished report, it has since been published. Suggested clarification of the molecular mass of stevioside as 804, rather than 809 or 805 as mentioned in FSANZ's risk assessment.
Stevian Biotechnology Corporation Sdn Bhd	Support FSANZ preferred position (Option 2). Request FSANZ recognise 'glycosylated stevia extract' and/or 'enzymatically glycosylated stevia extract' as one of the recognised steviol glycosides. This would reflect the use of enzymatic modification to produce steviol glycosides that is undertaken by Korean and Japanese manufacturers. Provided data to indicate enzymatically-modified stevia is heat stable, acid stable and does not adversely interact with vitamins in foods; and may assist in delaying the degradation rate of vitamin C.

Submitter	Comments
Department of Human Services Victoria	Does not support Option 2 at this stage. Support at Final Assessment depends on how issues identified will be addressed. 1. Not approved in US or EU and not GRAS status. FSANZ did not address the reasons/situation in sufficient detail to allay DHS concerns. 2. Draft Assessment indicated that Japan has used Steviol glycosides for 30 years but provided no further details for comparison with this Application – specifically, the precise types of steviol glycosides consumed, the quantities consumed (particularly by young children)and an indication of ADIs and max permitted levels in foods and beverages in Japan. 3. No definitions in the draft variation of what steviol glycosides have to be – unclear which specific compounds are planned to be used in Australia and New Zealand (nor the compounds used in other countries) – not expressed in the draft variation. 4. Less than satisfied that FSANZ progressed Application before JECFA and AFC considerations were finalised. Requests Application be delayed until these reports have been published and considered to enable a thorough risk assessment to be conducted. 5. Concern that FSANZ obtained further literature from the Applicant (unpublished report) and devised an ADI for Australia and New Zealand based on this data. No opportunity for the data to be scrutinized independently of FSANZ and the Applicant. DHS not prepared to accept the ADI on two grounds: 6. FSANZ is not the body charged with drafting ADIs for Australia; and 7. Believe the process followed by FSANZ in developing the ADI is unacceptable. Feel that there was no opportunity for the data to be scrutinised independently of FSANZ. Are not prepared to rely on the FSANZ assessment without that scrutiny.
Cadbury Schweppes Pty Ltd	Supports approval of steviol glycosides in a broad range of products. Suggests that proposed permission levels (draft variations to Standard 1.3.1) for beverages and confectionery may be too low to optimise the use of steviol glycosides.
Sanitarium Health Foods	Supports Option 3 on the basis that the use of the additive would be self limiting for a number of technological reasons. Accepts Option 2 is more likely to be accepted as no significant consumer group exceeds the ADI. Requests that the Final Assessment clarify whether the ADI applies to steviol only or to steviol glycosides, or to steviol equivalents. Suggests that if Option 2 is taken, soy bean beverages should be classified under item 14.1.2.2, however the category ‘soy bean beverage (plain or flavoured)’ should be amended to ‘soy, pulse and/or cereal based beverage (plain or flavoured)’, with attendant changes to the qualifying statements. Requests permission for the use of steviol glycosides in additional food categories, in particular in formula meal replacements and formulated supplementary foods.

Submitter	Comments
	Also request broadening the permission in spreads from low-joule spreads to all spreads.
Australian Food & Grocery Council	Supports Option 3. Considers that there is no evidence of public health and safety concerns with the proposed use of steviol. Given that FSANZ acknowledges the dietary modelling in this case is likely to have over-estimated exposure, and that it will take time before market share reaches the projected 30% penetration, AFGC believes that Option 3 should be considered. This Option is consistent with minimum effective regulation and would provide benefit to consumers in being able to choose foods lower in energy.
New South Wales Food Authority	Does not support the further progression of the Application until the report from JECFA has been included in the assessment process. Does not believe that all issues pertinent to the further progression of this Application have been adequately addressed in the Report. Requests that FSANZ seek information from the EU and FDA on why steviol glycosides are not approved for use in food. Unable to find information pertaining to the fate of rebaudioside A, rebaudioside C, dulcoside or the other 6 glycosides in the human digestive system, noting that only the rate of stevioside in the human digestive system is reported on. NSWFA reserves judgment as to the safety of steviol glycosides in the human digestive system until all components of steviol glycosides likely to appear in food have been assessed. Unable to find a reference to the OCS or JECFA for the proposed ADI of 4 mg/kg bw for steviol glycosides in the DAR. NSWFA unsure of the source of this figure, although notes it is double the temporary ADI set by JECFA. Concerned that FSANZ has not reported results from long-term studies to support the findings with respect to the proposed ADI in the Report. Questions why information from countries who have had long standing approvals for the use of steviol glycosides in food have not been included in the FSANZ risk assessment. Unsure whether the 30% substitution figure use by FSANZ to estimate dietary exposure is comparable to the expected uptake in foods as listed on page 7 of the Report. Suggests FSANZ model at the temporary ADI rather than the 4 mg/kg bw in case JECFA's report does not support the 4 mg/kg bw figure. Concerned that comment with respect to intakes greater than the acute reference dose for steviol glycosides in children aged 2-6 years, and all other age groups, has not been provided in the Report. Currently considering opinion on appropriate classification for soy beverages.