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

3-07
23 May 2007

DRAFT ASSESSMENT REPORT

APPLICATION A540

STEVIOL GLYCOSIDES AS INTENSE SWEETENERS

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 4 July 2007
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

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 *Stevia rebaudiana*. They are 250-300 times sweeter than sucrose and have been used for several years in a number of countries as sweeteners for a range of food products. 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.

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 bw/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 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, 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.

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. The estimated exposure for high consumers (children aged 2-6 years) marginally exceeded the ADI[2]. However, this estimate is based on very conservative assumptions and when a dietary exposure estimate was undertaken with concentrations of steviol glycosides that reflect a more realistic level of use[3], it was estimated that dietary exposure for high consumers (children aged 2-6 years) was only 50% 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.

The Initial Assessment Report was circulated for a round of public comment from the 7 December 2005 until 1 February 2006. Sixteen submissions were received of which fourteen submissions supported the progression of the Application and two suggest FSANZ defer a decision until further information on the pharmacological effects of this sweetener (as required by FAO/WHO) are received.

[1] The most common names used for steviol glycosides are stevia, stevioside and sometimes stevia extract and stevia sugar
[2] Based on a full sugar replacement scenario
[3] A 30% market share uptake scenario

These matters are addressed in this Draft Assessment Report.

Submissions are now invited on this Draft Assessment Report to assist FSANZ to complete the Final Assessment.

Purpose

The Applicant seeks approval for the use of steviol glycosides (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.

Preferred Approach

Amend Schedule 1 of Standard 1.3.1 to permit the use of steviol glycosides in a specified range of foods at restricted levels.

Reasons for Preferred Approach

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 safety 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

The Initial Assessment Report was circulated for a round of public comment from the 7 December 2005 until 1 February 2006. Sixteen submissions were received and a summary of these is attached to this report. FSANZ has taken the submitters' comments into account in preparing the Draft Assessment of this application.

Public submissions will be invited on this Draft Assessment Report.

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INVITATION FOR PUBLIC SUBMISSIONS

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation/s to the Code for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment of this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 10 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions need to be received by FSANZ by 6pm (Canberra time) 4 July 2007.

Submissions received after this date will not be considered, unless agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ website and will apply to all submitters.

While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Management Officer at the above address or by emailing slo@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

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 *Stevia 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 one usually being steviol glycosides. 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 steviol glycosides content.

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. This opinion is expected to be provided in the second half of 2007.

Stevia 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 *Stevia 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 *Stevia rebaudiana*) as intense sweeteners/flavour enhancer 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, slice 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

Although Stevia (whole leaves and powder) is not prohibited in Standard 1.4.4 Prohibited and Restricted Plants and Fungi of the Australia New Zealand Food Standards Code, previous consultations with State/Territory Jurisdictions considered Stevia to be a novel food. Therefore, for Stevia to be legally sold in Australia and New Zealand an application must be made seeking permission to use Stevia as a novel food under Standard 1.5.1 – Novel Foods.

While Stevia is regarded as a novel food, an extract of Stevia might be regarded as either a novel food or a food additive. If the intent of using an extract were to achieve a technological purpose such as sweetening a food, then FSANZ considers that it would be regarded as a food additive and requires an express permission under Standard 1.3.1.

1.2.1 Previous Applications seeking permissions to use stevioside

1.2.1.1 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.

1.2.1.2 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-Novel Foods or Standard 1.3.1-Food Additives. 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 there was no data 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 deemed to be withdrawn on 23 December 2002.

2. The Problem

Standard 1.3.1 requires that food additives undergo a pre-market risk assessment through an application to FSANZ before being offered for sale in Australia and New Zealand.

Steviol glycosides are being requested as a new intense sweetener (food additive) for 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 safety 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 could be used at rates of up to 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 10 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

FSANZ has considered the following questions at Draft Assessment:

- 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 Safety assessment

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 and mechanistic studies have been reviewed to determine whether it may alter the safety factor used to establish the temporary 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.

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

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 factors that were 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. (**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).

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 that would be using steviol glycosides instead of sugar or other intense sweeteners after 20 years. Based on this information, dietary exposure assessments were conducted for a 'sugar replacement scenario' (Scenario One).

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). Based on this assumption, dietary exposure assessments were conducted for a '30% market share scenario' (Scenario Two). Estimated dietary exposures were compared with the reference health standard, an Acceptable Daily Intake (ADI) of 0 - 4 mg/kg bw, proposed by FSANZ (**Attachment 3**).

Estimated dietary exposure to steviol glycosides (Scenario 1)

Population group	**Mean consumers mg/kg bw/day (% ADI)**	**95th percentile consumers mg/kg bw/day (% ADI)**
2 years and above (Australia)	0.7 (20%)	2.2 (55%)
2-6 years (Australia)	1.6 (40%)	4.5 (115%)
15 years and above (New Zealand)	0.3 (8%)	1.0 (25%)

Estimated dietary exposure to steviol glycosides (Scenario 2)

Population group	Mean consumers mg/kg bw/day (% ADI)	95th percentile consumers mg/kg bw/day (% ADI)
2 years and above (Australia)	0.6 (15%)	1.5 (40%)
2-6 years (Australia)	0.9 (25%)	2.0 (50%)
15 years and above (New Zealand)	0.5 (15%)	1.1 (30%)

For the sugar replacement model (Scenario 1), estimated mean exposures for consumers of steviol glycosides for all population groups assessed were below the ADI. Estimated 95th percentile exposures for consumers of steviol glycosides were also below the ADI, except for 95th percentile exposure for children aged 2-6 years (Australia only) (115% of the ADI). For the market share model (Scenario 2), estimated mean and 95th percentile exposures for all population groups assessed were below the ADI.

For both scenarios, table top sweeteners were predicted to be a major contributor to steviol glycosides dietary exposure due to their high concentration of steviol glycosides. They were not a major contributor for children 2-6 years because not many respondents in this age group consumed table top sweeteners. For these age groups beverages such as cordials, fruit & vegetable juice products, processed cereal and meal products and fermented and rennetted milk products, liquid milk products and flavoured liquid milk were the major contributors of dietary exposure to steviol glycosides most likely due to the large volume of these products consumed.

5.3 Risk characterisation

FSANZ has concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day. The adequacy of the existing database and a new study in humans provides a basis for revising the uncertainty factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore 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.

The data support the safety of steviol glycosides at the level of intake that would be achieved by addition of steviol glycosides to a range of foods at the levels proposed by the Applicant. Although, in scenario 1 the ADI is exceeded for specific consumers (Australia only), the market share exposure model (which would reflect a more realistic exposure estimate) indicates that the highest consumption estimated for steviol glycosides for Australian aged 2-6 years was at 50% of the ADI. However, 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).

In addition, mean exposure which is a better representation of potential 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 animal studies using a safety factor (x100) there are additional existing inbuilt safeguards if exposure is at or the ADI is marginally exceeded.

In conclusion, high consumers (children aged 2-6 years) in scenario 1 marginally exceeded the ADI. However, 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

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.

The Food Technology Assessment (**Attachment 4**) concludes that the use of steviol glycosides as an intense sweetener and flavour enhancer in a range of foods is technologically justified.

RISK MANAGEMENT

The Risk Characterisation identified the potential for the 95th percentile of consumers of steviol glycosides to exceed the ADI in the 2-6 years age group. Despite the conservative nature of the dietary modelling, imposing limits on use levels of steviol glycosides, if approved, has been considered as a Risk Management strategy.

6. Options

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.

Food additives used in Australia and New Zealand are required to be listed in Standard 1.3.1 – Food Additives. 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:

6.1 Option 1 – do not approve 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.

6.2 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.

6.3 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.

7. Impact Analysis

7.1 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.

7.2 Benefit Cost Analysis

7.2.1 Option 1 – Not permit the use of steviol glycosides as a food additive

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.

7.2.2 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.

- There is no perceived impact on government agencies.

7.2.3 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.

7.3 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 95th 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

8. Communication and Consultation Strategy

FSANZ has applied a basic communication strategy to this application. This involves advertising the availability of assessment reports for public comment in the national press and making the reports available on the FSANZ website. We will issue a media release drawing journalists' attention to the matter.

The Applicant and individuals and organisations who make submissions on this Application will be notified at each stage of the Application.

If the FSANZ Board approves the Final Assessment Report, we will notify 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.

9. Consultation

9.1 Consultation on the Initial Assessment Report

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

Sixteen submissions were received during this period and a summary of these is included in **Attachment 5** to this Report. 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.

FSANZ has taken the submitters' comments into account in preparing the Draft Assessment of this application. The major issues raised are discussed here.

9.1.1 FSANZ should defer assessment until after JECFA has evaluated additional studies on potential pharmacological effects of steviol glycosides

9.1.1.1 FSANZ Response

The Applicant has supplied additional studies on the potential pharmacological effects of steviol glycosides and the Risk Assessment has included consideration of these studies. Refer to section 5 of this Draft Assessment Report and Attachment 2 for further details.

9.1.2 Clarification of FSANZ Novel Food Reference Group opinion

One submitter suggested that clarification be sought from the FSANZ Novel Food Reference Group (NFRG) regarding the status of stevia.

9.1.1.1 FSANZ Response

The NFRG (an internal FSANZ group) has previously provided an opinion on the status of stevia with respect to the definitions of 'non-traditional food' and 'novel food' in Standard 1.5.1 – Novel Foods of the Code. The NFRG was of the opinion that the crushed leaves of stevia be considered a novel food in Australia and New Zealand, but that stevia extract and stevioside would be more appropriately regulated as a food additive. Therefore, the opinion of the NFRG is that:

- if the crushed leaves of stevia are intended to be used or added to food without further extraction then they are considered a novel food; and
- if the extract of stevia is intended to be added to food as an intense sweetener it would be considered a food additive, which is consistent with the request for approval in this Application

9.2 Consultation on the Draft Assessment Report

The views of submitters in response to the Draft Assessment Report will assist in the development of the Final Assessment. Therefore further public comment is sought on the Draft Assessment Report, including the proposed draft variations to the Code (**Attachment 1**). In particular FSANZ would appreciate comments and advice on the following issue.

In the proposed draft variations to the Code, a permission for the use of steviol glycosides in soybean beverages has been placed in food category 14.1.2.2 in Schedule 1 of Standard 1.3.1 – Fruit and vegetable juice products. Additives in Schedules 2, 3 and 4 are permitted for this food category. It is questioned whether a soybean beverage should be considered as a vegetable juice. Comment is sought on this matter.

In the Codex General Standard for Food Additives (GSFA), 12.9.1 covers soybean protein products (12.9.1.1 – soybean beverage), 12.0 covers salts, spices, soups, sauces, salads, protein products (including soybean protein products) and fermented soybean products. The corresponding food category in the Code is 12 – salt and condiments (12.6 vegetable protein products). Colours in Schedule 4 must not be added to 12.6 vegetable protein products. Based on the different categorisation of soybean beverages in the GSFA as compared with the proposed draft variations to the Code, comment is sought on the following: In which food category should soy based beverages be listed and food additive permissions be given in Schedule 1 of Standard 1.3.1?

9.3 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, it is proposed to notify 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.

CONCLUSION

10. Conclusion and Preferred Option

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.

Preferred Approach

Amend Schedule 1 of Standard 1.3.1 to permit the use of steviol glycosides in a specified range of foods at restricted levels.

10.1 Reasons for Preferred Approach

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 safety 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.

11. Implementation and Review

It is proposed that the draft variation come into effect on the date of gazettal.

ATTACHMENTS

1. Draft variation or standard to the *Australia New Zealand Food Standards Code*
2. Risk Assessment Report
3. Dietary Exposure Assessment Report
4. Food Technology Report
5. Summary of issues raised in public submissions to the Initial Assessment Report

ATTACHMENT 1

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

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	115	mg/kg

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

960	Steviol glycosides	176	mg/kg

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

960	Steviol glycosides	64	mg/kg

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

960	Steviol glycosides	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	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	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	450	mg/kg

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

960	Steviol glycosides	208	mg/kg

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

960	Steviol glycosides	550	mg/kg

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

960	Steviol glycosides	550	mg/kg

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

960	Steviol glycosides	450	mg/kg

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

960	Steviol glycosides	250	mg/kg

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

960	Steviol glycosides	160	mg/kg

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

960	Steviol glycosides	GMP

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

960	Steviol glycosides	GMP

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

960	Steviol glycosides	GMP

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

960	Steviol glycosides	50	mg/kg

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

soy bean beverage (plain or flavoured)

960	Steviol glycosides	65	mg/kg	Plain soy bean beverage only
960	Steviol glycosides	175	mg/kg	Flavoured soy bean beverage only

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

960	Steviol glycosides	125	mg/kg	

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

960	Steviol glycosides	160	mg/kg	

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

960	Steviol glycosides	160	mg/kg	Clause 4 limits do not apply

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

960	Steviol glycosides	100	mg/kg	

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

960	Steviol glycosides	80	mg/kg	

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

960	Steviol glycosides	260	mg/kg	

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

960	Steviol glycosides	150	mg/kg	dairy and fat based dessert products only

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

960	Steviol glycosides	320	mg/kg	

Attachment 2

Risk Assessment

EXECUTIVE SUMMARY

Steviol glycosides[5] 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 and mechanistic studies have been reviewed to determine whether it may alter the safety factor used to establish the temporary 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 factors that were 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

Stevia rebaudiana is an herb belonging to the chrysanthemum family which grows as a small shrub in parts of Paraguay and Brazil. The leaves of *Stevia 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.

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

Steviol glycosides are extracted from the leaves of *Stevia rebaudiana* Bertoni with hot water, followed by solvent purification of the water-soluble extract (Nabors, 2001; Hanson and 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 and 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 *Stevia rebaudiana*

- **Analogue**	- **Approximate concentration (w/v)**	- **Sweetness relative to sugar**
- Stevioside	- 5-10	- X300
- Rebaudioside A	- 2-4	- X250-450
- Rebaudioside B	- No data	- X300-350
- Rebaudioside C	- 1-2	- X50-120
- Rebaudioside D	- No data	- X250-400
- Rebaudioside E	- No data	- X150-300
- Rebaudioside F	- No data	- No data
- - Dulcoside A	- 0.4-0.7	- X50-120
- Steviolbioside	- No data	- X100-125
- 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).

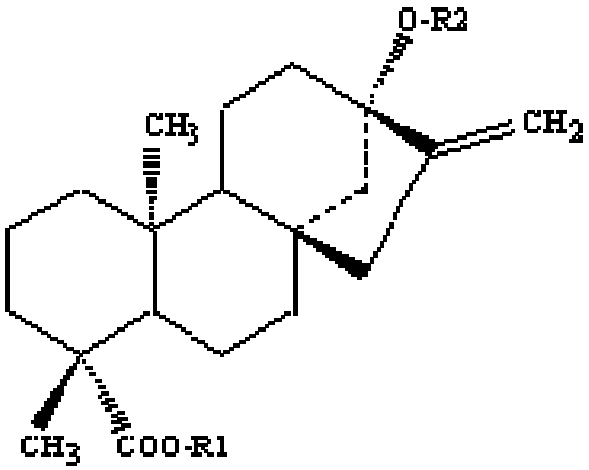

Figure 1: Diagram of steviol skeleton

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[6] (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.

A temporary ADI of 2 mg/kg body weight/day, 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.

JECFA will be considering both the additional safety data and new information on specifications at the 65th meeting in June 2007.

[6] Baded on 60 kg adult weights

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 and mechanism of action. The data assessed was provided from the Applicant (unpublished studies) and from published papers. The full assessments of the supplementary data are at Parts 2-4.

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 2003a&b; Geuns and 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 2006a&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 1977) 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 and 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[7] 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 and 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). Following a single oral dose of stevioside, steviol was also detected in the plasma of rats at 8h, peaking at 24h 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[8] 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) or in type 1 or 11 diabetics (Barriocanal et al 2006) 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).

[7] Steviol solubility <0.001%
[8] Based on 70 kg adult weights

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).

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.

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 and mechanism of action.

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 2006a&b). 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) 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) 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 and 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 and Pietta, 2004; Geuns et al 2006a&b) 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 and Pietta, 2004; Geuns et al 2006a&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[9] or glucagon reductions (Jeppeson et al 2002 and 2003; Lailerd et al 2004, Chen et al 2005, Hong et al 2006) or by decreased PEPCK[10] gene expression in the liver leading to a decrease in gluconeogenesis in diabetes-induced rats (Chen et al 2005). 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) 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).

[9] 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

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

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). 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[11] 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). 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).

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

However, the recent results in the study by Barriocanal et al (2006) 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) 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). 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.

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 factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required.

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 NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study, has been established. At the next highest dose equivalent to 2000 and 2400 mg/kg bw/day in males and female rats, respectively reduced bodyweight gain and survival were observed.

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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±5mm 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±21.5mm 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±7.3mm 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. Experienti 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 and 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 (steviobioloside, 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. (Sung, 2002)

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 and 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 and 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±9.2mg 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 7 h 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. (2006). Identification of steviol glucuronide in human urine. J Agric Food Chem 5: 2794-2798.

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 aminotransferae/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).

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[12] (STZ) induced diabetic rats (IDDM[13] model) and in a diabetic model induced by feeding rats with 60% fructose (NIDDM[14] model) for 2-weeks. Tolbutamide[15] (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).

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).

[12] Streptozotocin is a naturally occurring chemical that is particularly toxic to the insulin-producing beta cells of the pancreas in mammals.
[13] Insulin-dependent diabetic model
[14] Non-insulin dependent diabetic model
[15] 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[16] (PPARγ), which are mediators of insulin sensitivity, were examined in rats fasted for 15-h.

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.

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

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).

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.

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 subjects were divided into 3 groups: (i) 16 male and female subjects, aged 20 to 60 years, a body mass index (BMI) between 20 and 35 kg/m^2 with type 1 diabetes (DM1); (ii) 30 male and female subjects aged 40 to 70 years, BMI between 20 and 35 kg/m^2 with type 2 diabetes (DM2); and (iii) 30 male and female subjects aged 20 to 60 years, BMI between 20 and 35 kg/m^2 without diabetes and normal/low-normal BP concentrations (BP of ≤120/80 mm Hg). Half of the subjects in each group were allocated to placebo and half were treated with stevioside capsules (obtained from Steviafarma Industrial, Brazil purity 92%) at doses of 250 mg three times/day for 3 months. At the start and end of the study period, blood glucose, BP, insulin (for DM2 and low/normal BP subjects) and a range of other clinical chemistry parameters were measured (total cholesterol, HDL, LDL, triglycerides, electrolyte and creatinphosphokinase concentrations, creatinine, ALT, AST and γGT . Blood glucose and weight were measured every 2 weeks during the 3-month period. No significant differences were observed in mean blood glucose or insulin concentrations, BP or other clinical chemistry parameters between control and treated subjects for all three groups with the exception of significantly increased ($p<0.05$) glucose concentrations in placebo controls for DM1 at the end of the treatment period (3 months), compared to the baseline concentrations. However, the blood glucose concentrations in patients receiving placebo treatment in the DM1 group at the start of treatment were already elevated compared to baseline concentrations for the stevioside-treated groups which is not considered to be treatment related.

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[17] (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.

[17] 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.

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).

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 that would be using steviol glycosides instead of sugar or other intense sweeteners after 20 years. 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) on it 63rd meeting estimated the intake of steviol glycosides would likely to be 20-30% of total sugar replacement in foods (World Health Organization, 2004). Based on this assumption, dietary exposure assessments were conducted for a '30% market share scenario' (Scenario Two). Estimated dietary exposures were compared with the reference health standard, an Acceptable Daily Intake (ADI) of 0 - 4 mg/kg bw, proposed by FSANZ.

For both the sugar replacement model (Scenario 1) and the market share model (Scenario 2), estimated mean and 95th percentile exposures for all population groups assessed were below the ADI except for the 95th percentile dietary exposure for Australian children aged 2-6 years (115% of the ADI).

For both scenarios, table top sweeteners were predicted to be a major contributor to steviol glycosides dietary exposures due to their high concentration of steviol glycosides. They were not a major contributor for children 2-6 years because not many respondents in this age group consumed table top sweeteners. Beverages, such as fruit & vegetable juice products and coffee, coffee substitutes, tea, herbal infusions and similar products, were also predicted to be major contributors, most likely due to the large volume of these products consumed.

1. Background

Steviol glycosides are high intensity sweeteners 250-300 times that of 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. Steviol glycosides are not currently permitted to be added to foods in the Code.

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, as not all sugar would be replaced.

The Applicant provided FSANZ with information on proposed concentration of use for steviol glycosides for specific food groups and the expected proportion of products in each food using steviol glycosides category after 20 years. Information was provided both within the application and upon request for more detailed data.

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

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

Food Group	Typical sugar content (%)	Type of Food product or amount of sugar replaced	Grams of steviol glycosides/100 gm product	Uptake after 20 years -% of product category likely to include some steviol glycosides
Milk Products – flavoured	5 – 7	All sugar replaced	0.0115	100
Yoghurts – flavoured	8 – 11	All sugar replaced	0.0176	100
Ice confection - liquid	7	All sugar replaced	0.0115	100
Ice creams	7 – 17	Some sugar replaced	0.0064	40
Ice creams reduced & low fat	7 – 17	Low-fat low-joule product	0.0208	66
Ice confection reduced & low fat	7 – 17	Low-fat low-joule product	0.0208	66
Fruit & Veg in vinegar - beetroot	10	All sugar replaced	0.0160	100
Low joule chutneys, jams etc	NA	Low-fat low-joule product	0.0450	50
Fruit & Veg preparations – tomato sauces etc	25 – 30	Some sugar replaced	0.0208	30
Soy milks plain	4	All sugar replaced	0.0064	100
Soy milks flavoured	8 – 11	All sugar replaced	0.0175	100
Chocolate & cocoa – diabetic lines	NA	Low-fat low-joule product	0.0550	100
Sugar confectionery – diabetic lines	NA	Low-fat low-joule product	0.0550	100
Low joule chewing gum	NA	Low-fat low-joule product	0.0450	50
Processed cereals – breakfast	0 – 35	Some sugar replaced	0.0250	50
Biscuits – sweet (excl. choc. coated)	15 – 25	Some sugar replaced	0.0160	50
Cakes & muffins	15 - 25	Some sugar replaced	0.0160	20
Slices	20 - 30	Some sugar replaced	0.0160	30
Pastries – sweet only	5 - 20	Some sugar replaced	0.0080	20
Tabletop sweeteners	NA	All sugar replaced	40.00	50
Tabletop sweeteners - liquids	NA	All sugar replaced	0.800	50
Tabletop sweeteners - portion size	NA	All sugar replaced	40.00	50
Fruit & Vegetable juices	3	All sugar replaced	0.0050	30

Food Group	Typical sugar content (%)	Type of Food product or amount of sugar replaced	Grams of steviol glycosides/100 gm product	Uptake after 20 years -% of product category likely to include some steviol glycosides
Low joule fruit & veg drinks	NA	Low-fat low-joule product	0.0125	80
Cordials	10	Some sugar replaced	0.0048	33
Diet cordials	NA	Low-fat low-joule product	0.0160	80
Carbonated non-cola soft drinks	10	Some sugar replaced	0.0048	66
Diet carbonated non-cola soft drinks	NA	Low-fat low-joule product	0.0160	50
Cola type drinks – carbonated	10	Some sugar replaced	0.0048	33
Diet cola type drinks - carbonated	NA	Low-fat low-joule product	0.0160	50
Brewed soft drink	10	All sugar replaced	0.0160	100
Coffees, teas & infusions	0	All sugar replaced	0.010*	5
Desserts	15	Some sugar replaced	0.0150	30
Desserts – dairy only	8 - 12	Some sugar replaced	0.0150	50
Jelly – low joule only	NA	Low-fat low-joule product	0.0260	75
Custard powder etc	4 - 7	Some sugar replaced	0.0080	50
Cereal products – sugared mueslis	15 – 25	Some sugar replaced	0.0125	50
Breakfast and muesli bars	20 – 30	Some sugar replaced	0.0125	50
Gravy & Sauces – sweetened only	5 - 20	Some sugar replaced	0.0125	50
Mayonnaises & salad dressings	0 - 25	Some sugar replaced	0.0160	50
Toppings only	20 - 50	Some sugar replaced	0.0320	50

* per 100 ml made up drink
NA –Not available from the Applicant

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 (95th percentile) dietary exposures were assessed.

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 along with information on how much of the food chemical is in each food is required. The accuracy of these exposure estimates depend on the quality of the data used in the dietary models. 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 model 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 food chemical concentration data to estimate the exposure to the food chemical 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

No further information was required or identified for the purpose of refining the dietary exposure estimates for this application.

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 on 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 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 below 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 a 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 'market share' scenario.

2.6.1 Scenario 1.Sugar replacement scenario

The Applicant states that steviol glycosides can be used in conjunction with sugar or other sweeteners and will replace some or all of the sweeteners now used. Also, 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). These concentrations were converted to mg/kg concentrations for use in the DIAMOND program. 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.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. Based on this, dietary exposure assessments were conducted for Scenario Two ('30% market share scenario'). The concentration for each food used in the market share scenario was calculated by multiplying the concentration assigned to the food group from Table 1 (grams of steviol glycosides/100 gm 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	34.5
1.2.2	Fermented milk products and rennetted milk products	176	52.8
3.0.1	Ice confection sold in liquid form	115	34.5
3.1	Ice cream	25.6	19.2
3.1.1	Ice cream reduced & low fat	137.28	62.4
3.1.2	Ice confection	0	0
3.1.2.1	Ice confection reduced & low fat	137.28	62.4
3.1.2.2	Ice confection, artificially sweetened	137.28	62.4
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	62.4	62.4
4.3.6.1	Chilli paste	0	0
4.3.6.2	Mustard	0	0
4.3.6.3	Peanut butter	0	0
4.3.8.2	Soy beverages plain	64	19.2
4.3.8.4	Soy beverages flavoured	175	52.5
5.1.2	Chocolate products, artificially sweetened	550	165
5.2.1	Bubble gum and chewing gum	0	0
5.2.1.1	Bubble & chewing gum, artificially sweetened	225	135
5.2.3.1	Hard boiled confectionary, CHO modified	550	165
6.2.1	Custard powder	40	24
6.3	Processed cereal and meal products.	125	75
7.2.1	Biscuits	80	48
7.2.1.1	Biscuits, savoury	0	0
7.2.2	Cakes & muffins	32	48
7.2.3	Slices	48	48
7.2.4	Pastries	16	24
7.2.8	Chocolate coated and filled biscuits	0	0
11.4	Table top sweeteners	200000	120000
11.4.1	Tabletop sweeteners, liquid preparation	4000	2400
11.4.2	Tabletop sweeteners – tablets or powder or granules packed in portion sized packages	200000	120000
14.1.2.1	Fruit and vegetable juices	15	15
14.1.2.2	Fruit and vegetable juices products	100	37.5
14.1.2.3	Coconut milk, cream & syrup	0	0
14.1.3.1	Brewed soft drinks	160	48

DIAMOND Food Code	Food Name	Steviol glycosides concentration (mg/kg)	
		Scenario 1 'Sugar replacement'	**Scenario 2** '30% market share'
14.1.3.2	Soft drinks, cola type	15.84	14.4
14.1.3.3	Soft drinks, non-cola type	31.68	14.4
14.1.3.4	Cordial only	15.84	14.4
14.1.3.6	Soft drinks, artificially sweetened	80	48
14.1.3.7	Cordials, Artificially sweetened	128	48
14.1.3.8	Kola type drinks - sugar sweetened	15.84	14.4
14.1.3.9	Kola type drinks - artificially sweetened	80	48
14.1.5	Coffee, coffee substitutes, tea, herbal infusions and similar products	5	30
20.2.1	Desserts	45	45
20.2.1.1	Desserts, dairy only	75	45
20.2.1.2	Desserts, artificially sweetened	75	45
20.2.1.3	Jelly only	0	0
20.2.1.4	Jelly, artificially sweetened only	195	78
20.2.2	Cereal products (commercial)	62.5	37.5
20.2.4.1	Gravy & sauces only	62.5	37.5
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

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 always select the products containing steviol glycosides;
- consumers do not alter their food consumption habits besides to substitute 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;
- all steviol glycosides present in food is absorbed by the body;
- 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 millilitre is equal to 1 gram 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 glycoside

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 95th percentile exposures for consumers of steviol glycosides were 0.7 mg/kg bw/day and 2.2 mg/kg bw/day, respectively.

Australia – 2-6 years:
Estimated mean and 95th percentile exposures for consumers of steviol glycosides were 1.6 mg/kg bw/day and 4.5 mg/kg bw/day, respectively.

New Zealand - 15 years and above:
Estimated mean and 95th percentile exposures for consumers of steviol glycosides were 0.3 mg/kg bw/day and 1.0 mg/kg bw/day, respectively.

3.1.2 Scenario 2.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 95th percentile exposures for consumers of steviol glycosides were 0.6 mg/kg bw/day and 1.5 mg/kg bw/day, respectively.

Australia – 2-6 years:
Estimated mean and 95th percentile exposures for consumers of steviol glycosides were 0.9 mg/kg bw/day and 2.0 mg/kg bw/day, respectively.

New Zealand - 15 years and above:
Estimated mean and 95th percentile exposures for consumers of steviol glycosides were 0.5 mg/kg bw/day and 1.1 mg/kg bw/day, respectively.

Of the population groups assessed, Australians aged 2 -6 years had the highest exposure (in mg/kg bw) to steviol glycosides. As discussed in Section 2.4, 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.

Estimated mean and 95th 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 Scenario 1 'Sugar replacement'. The Australian population aged 2 years and above includes children aged 2-6 years who have higher steviol glycosides dietary exposures in comparison to the population group aged 2 years and above. The inclusion of children aged 2-6 years is a possible reason why the Australian population (aged 2 years and above) have higher estimated dietary exposures to steviol glycosides in comparison to the New Zealand population (aged 15 years and above).

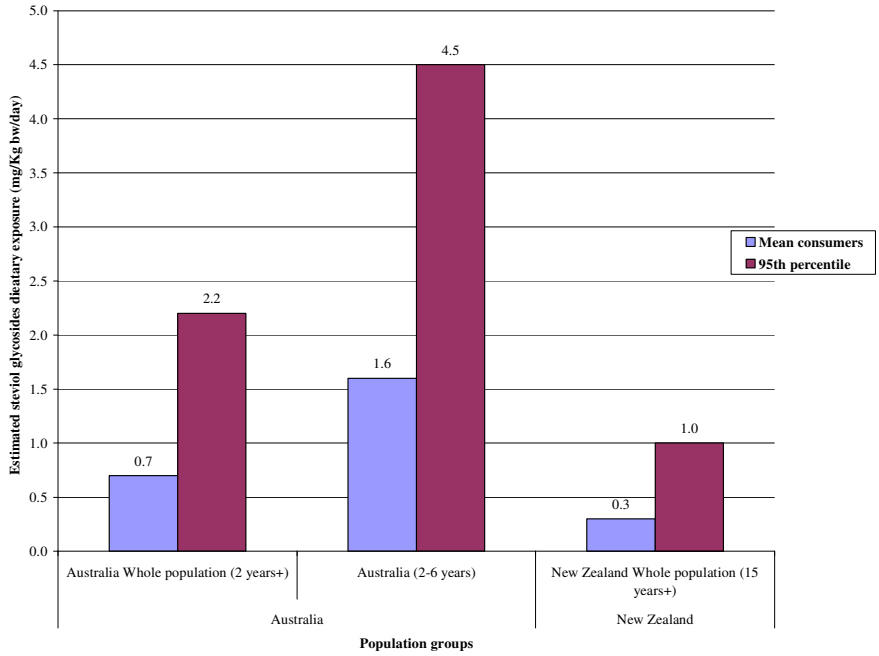


Figure 1: Estimated mean and 95th percentile dietary exposures (mg/kg bw/day) for consumers of steviol glycosides for the Australian and New Zealand population groups (Sugar replacement scenario)

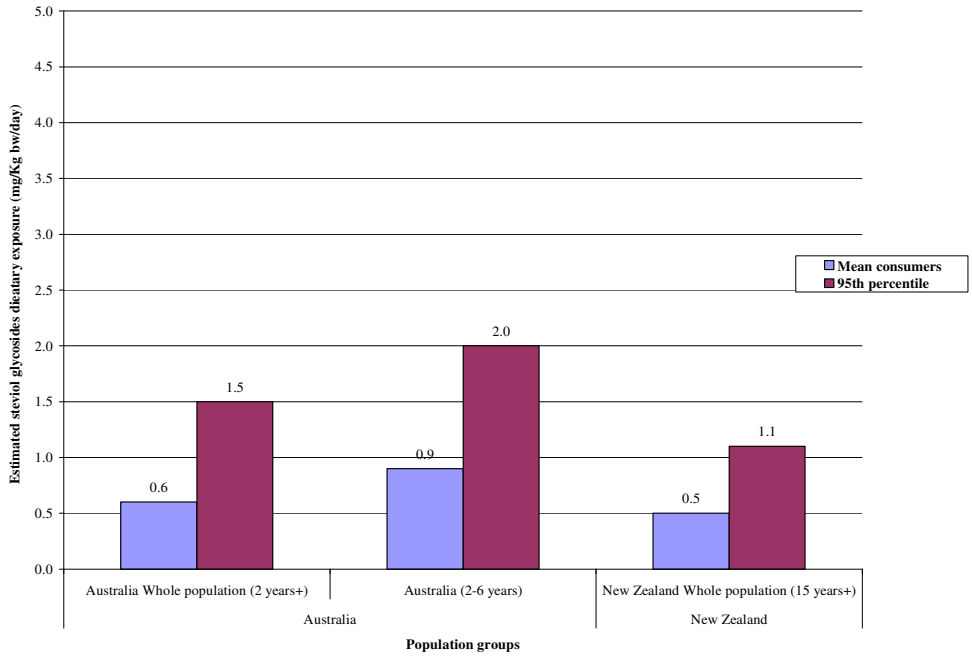


Figure 2: Estimated mean and 95th percentile dietary exposures (mg/kg bw/day) for consumers of steviol glycosides for the Australian and New Zealand population groups (Market share scenario)

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 total 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 tabletop sweeteners – tablets or powder or granules packed in portion sized packages (23%), coffee, coffee substitutes, tea, herbal infusions and similar products (9%), fruit & vegetable juice products (8%), soft drinks, artificially sweetened (7%), liquid milk products and flavoured liquid milk (6%), fermented milk products and rennetted milk products (6%) and processed cereal & meal products (6%).

Australia – 2-6 years:
The major contributors (≥5%) to total steviol glycosides dietary exposures were fruit & vegetable juice products (22%), fermented milk products and rennetted milk products (13%), processed cereal & meal products (9%), cordials (9%), liquid milk products and flavoured liquid milk (6%) and fruit & vegetable juices (5%).

New Zealand - 15 years and above:

The major contributors (≥5%) to total steviol glycosides dietary exposures were tabletop sweeteners – tablets or powder or granules packed in portion sized packages (24%), coffee, coffee substitutes, tea, herbal infusions and similar products (13%), processed cereal & meal products (6%), soft drinks, non-cola type (6%), soft drinks, artificially sweetened (5%) and gravy & sauces only (5%).

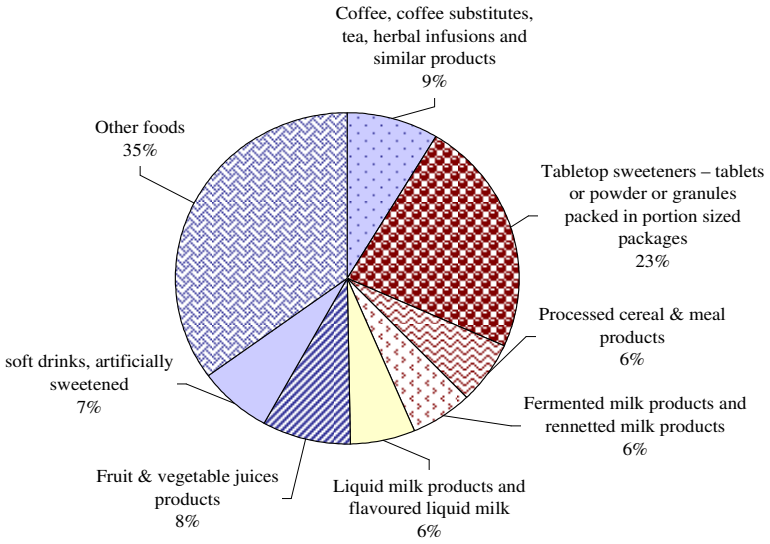


Figure 3: Major contributors to total steviol glycosides dietary exposures for Australia - 2 years and above (Sugar replacement scenario)[18]

[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.

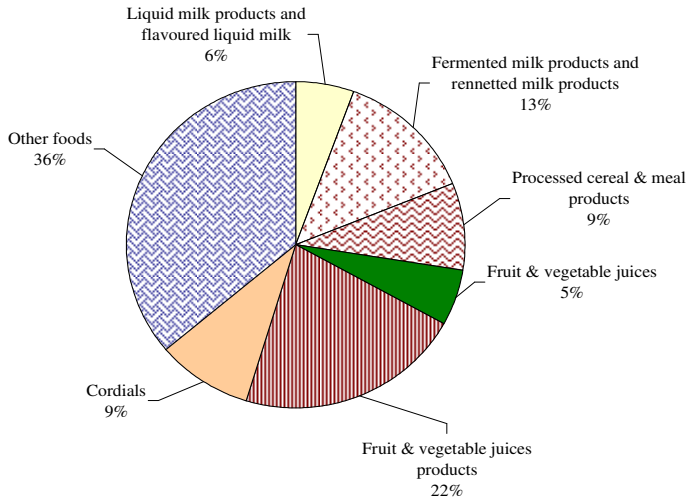


Figure 4: Major contributors to total steviol glycosides dietary exposures for Australia – 2-6 years (Sugar replacement scenario) .[19]

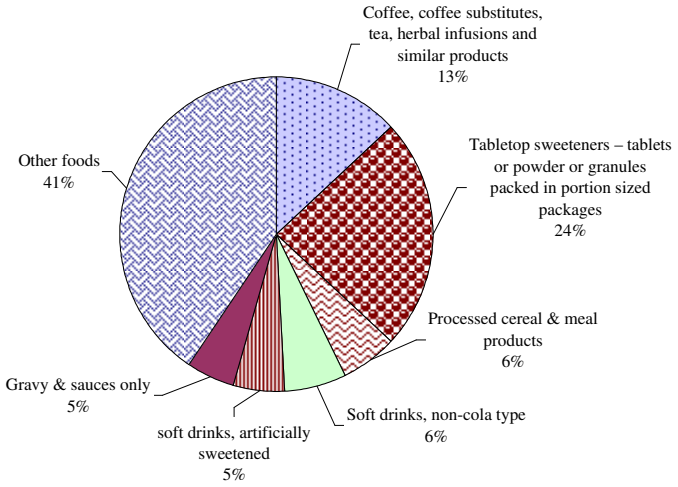


Figure 5: Major contributors to total steviol glycosides dietary exposures for New Zealand - 15 years and above (Sugar replacement scenario).[20]

[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.

[20] 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.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 coffee, coffee substitutes, tea, herbal infusions and similar products (50%) and tabletop sweeteners – tablets or powder or granules packed in portion sized packages (14%).

Australia – 2-6 years:
The major contributors (≥5%) to total steviol glycosides dietary exposures were cordials (15%), fruit & vegetable juice products (15%), fruit & vegetable juices (10%), processed cereal & meal products (9%) and fermented milk products and rennetted milk products (7%).

New Zealand - 15 years and above:
The major contributors (≥5%) to total steviol glycosides dietary exposures were coffee, coffee substitutes, tea, herbal infusions and similar products (58%) and tabletop sweeteners – tablets or powder or granules packed in portion sized packages (11%).

Figure 6: Major contributors to total steviol glycosides dietary exposures for Australia - 2 years and above (Market share scenario)[21]

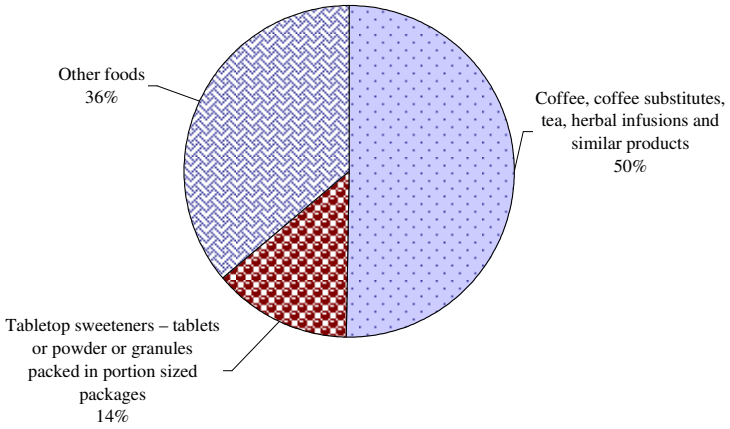
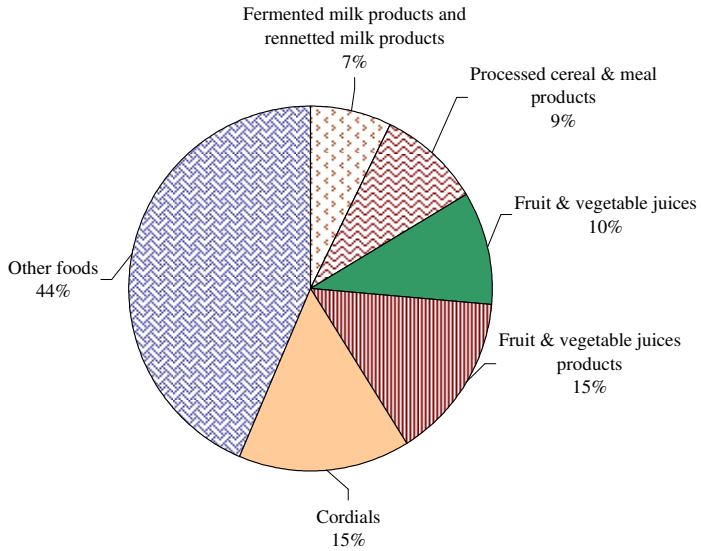
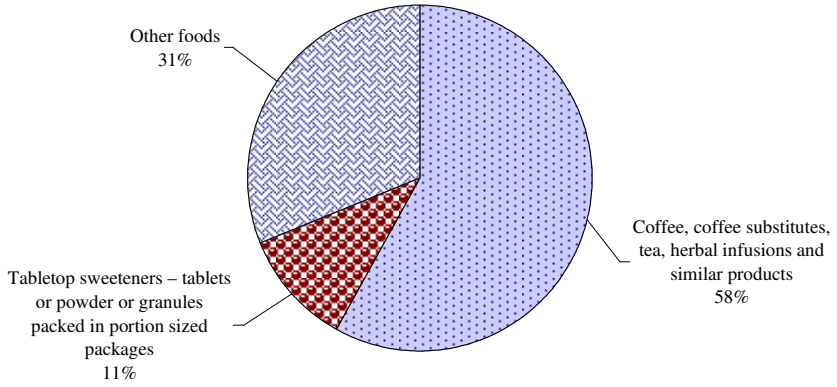


Figure 7: Major contributors to total steviol glycosides dietary exposures for Australia – 2-6 years (Market share scenario)[1]



[21] 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 8: Major contributors to total steviol glycosides dietary exposures for New Zealand - 15 years and above (Market share scenario)[22]



For both scenarios, table top sweeteners were a major contributor due to their high concentration of steviol glycosides. They were not a major contributor for children 2-6 years because not many respondents in this age group consumed table top sweeteners in the 1995 NNS. Beverages, such as fruit & vegetable juice products and coffee, coffee substitutes, tea, herbal infusions and similar products, were also major contributors, 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 does have its 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.

[22] 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.

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. Therefore, predicted high percentile exposures are likely to be higher than actual high percentile exposures over a lifetime.

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 in food what concentrations of steviol glycosides will be used, and the proportion of food groups contain steviol glycosides. The dietary exposure assessment may cover more foods than those that would actually contain steviol glycosides, should permission for use be granted.

5 Risk characterisation

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 the reference health standard. FSANZ has undertaken a review and considered that the 200-fold safety factor applied to the current temporary Acceptable Daily Intake (ADI) (2 mg/kg bw/day) set by JECFA in 2005 (World Health Organization, 2004) can be reduced to the standard 100-fold safety factor on the basis of the results of the new studies in humans.

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 (World Health Organisation 2001). An ADI of 0 - 4 mg/kg bw/day, was set by FSANZ and used in this assessment.

5.1 Comparison of the estimated dietary exposures with the reference health standard

5.1.1 Scenario 1.Sugar replacement scenario

The estimated dietary exposures for steviol glycosides for sugar substitution scenarios, as compared to the ADI are shown in Figure 6 (full results in Table A3.1 in Appendix 3). Estimated mean and the 95th percentile exposures for the Australian population group assessed were below the ADI except for the 95th percentile dietary exposure for Australian children aged 2-6 years.

Australia - 2 years and above:
Estimated mean and 95th percentile exposures for consumers of steviol glycosides were 20% of the ADI and 55% of the ADI, respectively.

Australia – 2-6 years:
Estimated mean and 95th percentile exposures for consumers of steviol glycosides were 40% of the ADI and 115% of the ADI, respectively.

New Zealand - 15 years and above:
Estimated mean and 95th percentile exposures for consumers of steviol glycosides were 8% of the ADI and 25% of the ADI, respectively.

5.1.2 Scenario 2.Market share scenario

The estimated dietary exposures for steviol glycosides for the market share scenario, as compared to the ADI are shown in Figure 7 (full results in Table A3.2 in Appendix 3). Estimated mean and 95th percentile exposures for all population groups assessed were at or below the ADI.

Australia - 2 years and above:
Estimated mean and 95th percentile exposures for consumers of steviol glycosides were 15% of the ADI and 40% of the ADI, respectively.

Australia – 2-6 years:
Estimated mean and 95th percentile exposures for consumers of steviol glycosides were 25% of the ADI and 50% of the ADI, respectively.

New Zealand - 15 years and above:
Estimated mean and 95th percentile exposures for consumers of steviol glycosides were 15% of the ADI and 30% of the ADI, respectively.

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

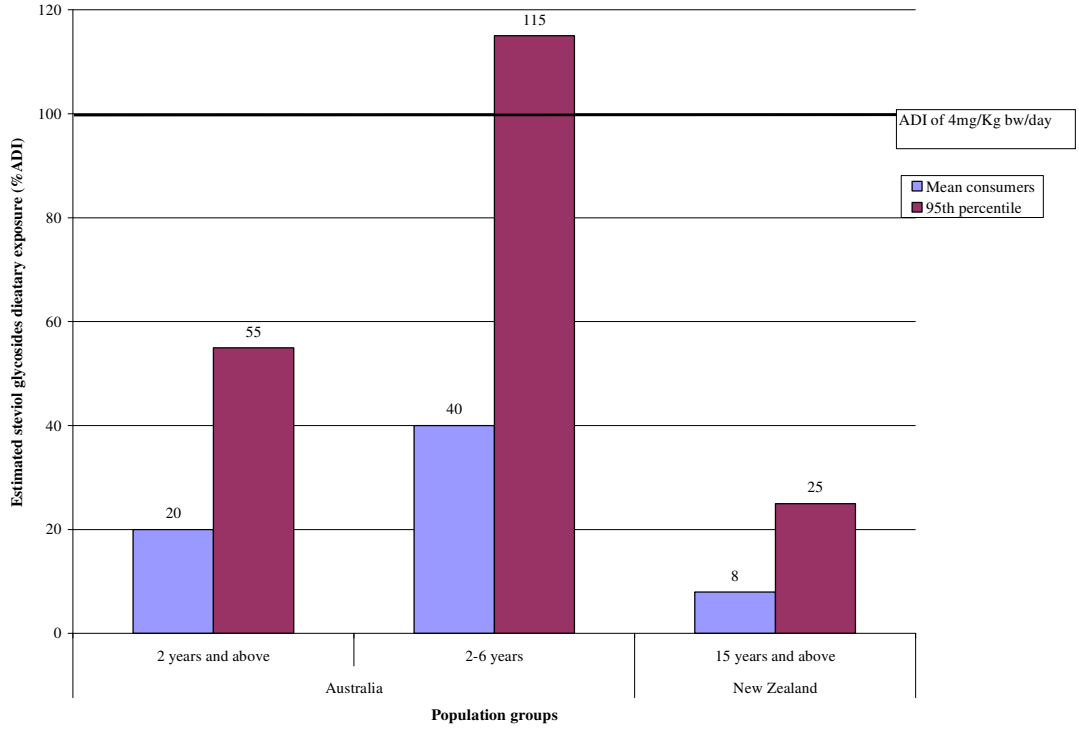
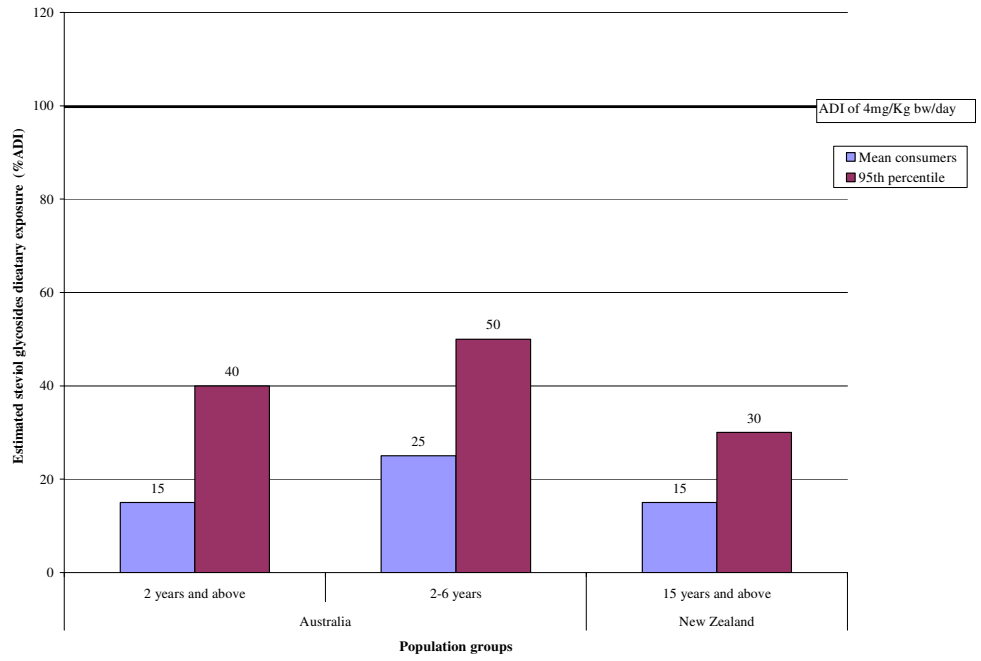


Figure 7: Estimated dietary exposures to steviol glycosides for the Market shares scenario, as a percentage of ADI



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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.

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

World Health Organization (2004) *Joint FAO/WHO Expert Committee on Food Additives Sixty-third meeting. World Health Organisation, Geneva.*

Appendix 1

How were the estimated dietary exposures calculated?

Steviol glycosides are used as a sweetener, and can be used as a sugar replacement for a range food groups. The exposure to steviol glycosides was calculated for each individual in the NNSs 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 individuals' 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 raw 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.

Complete information on dietary exposure assessment results

Table A2.1: Estimated dietary exposures to steviol glycosides for Scenario 1 ('sugar replacement scenario')

Country	Population group	Number of consumers of steviol glycosides	Consumers[♦] as a % of total respondents[#]	Estimated dietary exposures to steviol glycosides	
				Mean consumers (mg/kg bw/day)	95th percentile consumers (mg/kg bw/day)
Australia	2 years and above	13,791	99.5	0.7	2.2
	2-6 years	980	99.1	1.6	4.5
New Zealand	15 years and above	4,603	99.3	0.3	1.0

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 Scenario 2 ('market share scenario').

Country	Population group	Number of consumers of steviol glycosides	Consumers[1] as a % of total respondents[#]	Estimated dietary exposures to steviol glycosides	
				Mean consumers	95th percentile consumers
				(mg/kg bw/day)	(mg/kg bw/day)
Australia	2 years and above	13,791	99.5	0.6	1.5
	2-6 years	980	99.1	0.9	2.0
New Zealand	15 years and above	4,603	99.3	0.5	1.1

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 (Scenario 1-'sugar replacement scenario')

Country	Population group	Food Name	Contribution (%)
Australia	2 years and above	Tabletop sweeteners – tablets or powder or granules packed in portion sized packages	23
		Coffee, coffee substitutes, tea, herbal infusions and similar products	9
		Fruit & vegetable juices products	8
		Soft drinks, artificially sweetened	7
		Liquid milk products and flavoured liquid milk	6
		Fermented milk products and rennetted milk products	6
		Processed cereal & meal products	6
		Other foods	35
	2-6 years	Fruit & vegetable juices products	22
		Fermented milk products and rennetted milk products	13
		Processed cereal & meal products	9
		Cordials	9
		Liquid milk products and flavoured liquid milk	6
		Fruit & vegetable juices	5
		Other foods	36

Country	Population group	Food Name	Contribution (%)
New Zealand	15 years and above	Tabletop sweeteners – tablets or powder or granules packed in portion sized packages	24
		Coffee, coffee substitutes, tea, herbal infusions and similar products	13
		Processed cereal & meal products	6
		Soft drinks, non-cola type	6
		Soft drinks, artificially sweetened	5
		Gravy & sauces only	5
		Other foods	41

Table A2.4: Major contributors to total steviol glycosides dietary exposures for Australia and New Zealand, for different population groups (Scenario 2 -'30% market share scenario')

Country	Population group	Food Name	Contribution (%)
Australia	2 years and above	Coffee, coffee substitutes, tea, herbal infusions and similar products	50
		Tabletop sweeteners – tablets or powder or granules packed in portion sized packages	14
		Other foods	36
	2-6 years	Cordials	15
		Fruit & vegetable juices products	15
		Fruit & vegetable juices	10
		Processed cereal & meal products	9
		Fermented milk products and rennetted milk products	7
		Other foods	44
New Zealand	15 years and above	Coffee, coffee substitutes, tea, herbal infusions and similar products	58
		Tabletop sweeteners – tablets or powder or granules packed in portion sized packages	11
		Other foods	31

Complete information on risk characterisation

Table A3.1: Estimated dietary exposures to steviol glycosides, as a percentage of ADI (Acceptable Daily Intake) for Scenario 1 ('sugar replacement scenario')

Country	Population group	Number of consumers of steviol glycosides	Consumers[1] as a % of total respondents[#]	Estimated dietary exposures to steviol glycosides (%ADI)	
				Mean consumers	95th percentile consumers
				(% ADI*)	(% ADI*)
Australia	2 years and above	13,791	99.5	20	55
	2-6 years	980	99.1	40	115
New Zealand	15 years and above	4,603	99.3	8	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 (Acceptable Daily Intake) for Scenario 2 ('30% market shares scenario')

Country	Population group	Number of consumers of steviol glycosides	Consumers[1] as a % of total respondents[#]	Estimated dietary exposures to steviol glycosides (%ADI)	
				Mean consumers	95th percentile consumers
				(% ADI*)	(% ADI*)
Australia	2 years and above	13,791	99.5	15	40
	2-6 years	980	99.1	25	50
New Zealand	15 years and above	4,603	99.3	15	30

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

Food Technology Report

Introduction

Food Standards Australia New Zealand (FSANZ) received an Application (A540) from the Plant Sciences Group, Central Queensland University and Australian Stevia Mills Pty Ltd to approve the use of steviol glycosides (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 - Food Additives of the *Australia New Zealand Food Standards Code* (the Code). There are currently no permissions for steviol glycosides in the Code.

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

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

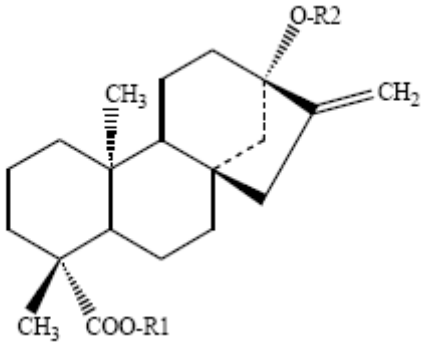
The Applicant states the main purpose of using steviol glycosides in foods is to enhance the taste and sweetness without needing to use high calorie sweeteners (such as sucrose, glucose, fructose, honey) or man-made chemical sweeteners.

Chemical Structure and Specification

Chemical structure

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

Figure 1: Steviol skeleton and R groups



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)

Glc and Rha represent, respectively, glucose and rhamnose sugar moieties.

Quantification of steviol glycosides

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

Specification

A tentative specification for steviol glycosides is included in the online edition of the Compendium of Food Additive and Flavouring Agents specifications produced by JECFA and can be found at the following URL: http://www.fao.org/ag/agn/jecfa-additives/details.html?id=898. This tentative specification is contained in one of the primary sources of specifications contained in Standard 1.3.4 – Identity and Purity of the Code. This specification has been published in the addenda 12 (2004) of the Compendium of Food Additives Specifications.

Technological justification

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

Steviol glycosides can be used in conjunction with sugar or other sweeteners and it is claimed could replace some (or all) of the sweetener now used in various food products. They could be used at rates of up to 0.004 times the rate of sugar currently used in food products. The Applicant indicates that sugar mixtures (for example, "double strength" mixtures containing both sucrose and steviol glycosides) are expected to be a major ingredient for many products. The Applicant has stated that a number of food and beverage manufacturers have expressed a strong desire to be able to use steviol glycosides in their products to meet the latent and growing consumer demand for a (versatile) natural, non-calorie sweetener.

Steviol glycosides are heat and acid stable and are therefore suitable for use in a wide range of food products, including baked and cooked products. Steviol glycosides also have a flavour enhancing effect when used in association with other sweeteners or flavours. Therefore, if steviol glycosides are added to a food, other flavours and sweeteners may be used at lower rates than required without the inclusion of steviol glycosides.

Manufacture

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

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

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

The Applicant indicates that in designing the manufacturing process to be used in Australia, it is hoped to avoid the need for an alcohol release ion exchange resin process and so maintain a water only solvent procedure. However, most steviol glycosides that will be used in Australia in the next few years are likely to be imported and have been processed using the more common ion exchange resin procedure.

Conclusion

The use of steviol glycosides as an intense sweetener and flavour enhancer in a range of foods is technologically justified. Steviol glycosides are high intensity sweeteners 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.

References

FAO (2006) *Steviol Glycosides specification*. Online Edition: "Combined Compendium of Food Additive Specifications".
URL: http://www.fao.org/ag/agn/jecfa-additives/details.html?id=898
Also found in Compendium of Food Additive Specifications Volume 1 and 2, addenda 12 (2004), FAO, Rome.

RIRDC (2006) *Stevia – An intense, natural sweetener. Laying the groundwork for a new rural industry*. Rural Industries Research and Development Corporation, Canberra.

WHO (1999) *Safety evaluation of certain food additives (stevioside)*. Food Additives Series No. 42, WHO, Geneva.

WHO (2005) *Evaluation of certain food additives and contaminants (Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives). (steviol glycosides)*. WHO Technical Report Series No. 928, WHO, Geneva.

Attachment 5

Summary of Submissions

Initial Assessment

Sixteen submissions were received in response to the Initial Assessment Report. 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.

Submitter	Comments
Complementary Healthcare Council (CHC)	The CHC has no concerns with the progression of this application.
Department of Human Services Victoria (DHS)	DHS notes steviol glycosides are not permitted for use in the EU or USA, however, are approved and used in other countries. DHS will provide further comment at the Draft Assessment stage after reviewing the toxicological and dietary modelling data.
Crop & Food Research New Zealand	Crop & Food Research New Zealand support A540 based on Initial Assessment. It is noted that safety literature has not been examined by Crop and Food Research.
DIC International (Australia) Pty. Ltd.	DIC International strongly supports A540. DIC International also provided additional information including: • history and manufacturing process of stevia; • merits and defects of stevia as a sweetener; • metabolism of stevia; • some additional Toxicological information; and • countries where stevia is approved for use.
Fonterra Brands Australia (P & B)	Fonterra Brands Australia (P & B) supports progression of A540 to Draft Assessment. Additional Comments: • Steviol glycosides would provide alternative intense sweeteners for use. • Consumer research shows interest in low caloric foods. • Suggest FSANZ may consider why this additive is not permitted for use in the US or Europe.
SA Department of Health	SA Department of Health has no objections to the progression of this application.

Submitter	Comments
New Zealand Food Safety Authority (NZFSA)	NZFSA supports A540 proceeding to Draft Assessment. Additional comments to consider in the Draft Assessment include: • only a temporary ADI has been set with JECFA waiting for further data (does this application contain the extra information requested by JECFA?); • consideration needs to be given to JECFA concerns regarding pharmacological effects particularly in relation to Type I & II diabetics; • NZFSA believe dietary modelling needs to consider exposure from table top sweeteners; • NZFSA is aware of a dietary supplement sold in NZ as 'Stevia Dietary Supplement' which contains 60 mg *Stevia rebaudiana* Bertoni extract per 1g serving; and • NZFSA suggests clarification be sough re: status of stevia from the Novel Foods Reference Group.
New Zealand Juice & Beverage Association Inc (NZJBA)	NZJBA support A540. NZJBA believe that this will extend the number of approved sweeteners available increasing consumer choice.
Australian Beverages Council Ltd (ABCL)	ABCL supports approval of steviol glycosides as a food additive. Additional comments: • Temporary JECFA ADI is based on a 200-fold safety factor assuming a mid-dose of 970 mg/kg of stevioside was the NOEL in rat carcinogenicity study. ABCL and the University of Queensland believe it to based on a NOEL of 2,000 mg/kg. Believe the ADI can safely be assessed at 4 times that set by JECFA • ABCL requests FSANZ approves a use level of steviol glycosides at 1000 ppm in water based flavoured beverages and fruit and vegetable juice products. ABC note that milk and soy containing beverages will require more stevia sweeteners because of their protein and fat contents and request amount permitted to be 1000 ppm. • ABCL suggests dietary modelling should be conservative in assumptions of market use. They suggest dietary modelling should be based on current uses of aspartame and other approved sweeteners are appropriate. • ABCL believes that JECFA's assessment of steviol glycosides replacing 20-30% of sugar is very optimistic market assessment. • ABCL believe there will be consumer benefit through controlled energy intake while enjoying food and beverages. • ABCL also notes the potential development of a new agricultural crop for Australia.

Submitter	Comments
Australian Stevia Mills	Supports application A540. Additional comments • Stevia is a safe natural alternative to artificial sweeteners • Stevia does not promote calories • Stevia is safe to use in baked products and products with varying pH. • Currently no artificial sweeteners are locally owned products, potential cash crop for Australia. Successful trials in Eastern states of Australia. • The federal government supports development of stevia through projects under RIRDC. • Potential stakeholder benefits to federal and state governments, diabetic and obese people, general public in reducing dental caries
Australian Food and Grocery Council (AFGC)	Supports A540 – Steviol glycoside as an intense sweetener.
Health & Herbs Ltd	Supports A540 – Steviol glycoside as a sweetener
Queensland Health - Environmental Health Unit	Believe FSANZ should defer further assessment until 2007 when additional studies on pharmacological effects of the sweetener (required by FAO/WHO). Suggest delay will not be significant to industry as other intense sweeteners are available. Also notes that EU and USA do not currently permit steviol glycosides.
Cadbury Schweppes	Supports A540 – Steviol glycosides as a sweetener in a broad range of products
NSW Food Authority	Recommends waiting for further toxicological data required by JECFA. Notes that the NFRG formed the view that stevia is a novel food, therefore the novelty of this food will need to be assessed
Food Technology Association of Victoria Inc	Supports A540 – Steviol glycosides as a sweetener