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**Supporting document 1**

Risk and technical assessment – Application A1172

Enzymatic production of rebaudioside D

# Executive summary

SweeGen’s application seeks permission in the Australia New Zealand Food Standards Code (the Code) for a rebaudioside D (Reb D) from a novel production method. The method is based on an enzymatic conversion process using an enzyme processing aid (UGT-A) sourced from a genetically modified (GM) strain of *Pichia pastoris*. UGT-A has been assessed and approved under application A1157.

Steviol glycosides extracted from the leaves of *Stevia rebaudiana* Bertoni, including Reb D, are already permitted for use as a food additive in the Code, with maximum permitted levels (MPLs) in a variety of food categories and at Good Manufacturing Practice (GMP) levels in tabletop sweeteners in Schedule 15. The applicant claims Reb D exhibits preferential sensory characteristics when compared to the major glycosides, being more reflective of sugar. Hence it is a useful food additive in formulations for reduced‐calorie or no‐sugar‐added products, where it is used in mixtures to replace sugar.

SweeGen’s Reb D complies with the purity specifications of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (but not the method of manufacture). The *P. pastoris* source organism for the enzyme used to produce Reb D has a long history of industrial use, is commonly used for recombinant gene expression and is not toxigenic. No major allergens are used to culture the yeast or at any other stage of the production process and sufficient information was provided concerning potential homology between the novel enzyme and known allergens for FSANZ to conclude there is no concern.

An acceptable daily intake (ADI) of 0-4 mg/kg bodyweight for steviol glycosides, expressed as steviol, was established by FSANZ in 2008 and JECFA in 2009. This ADI is appropriate for Reb D produced using an enzyme from GM *Picha pastoris* as it is chemically the same as Reb D extracted traditionally from *S. rebaudiana* Bertoni and would therefore follow the same metabolic pathway in humans. Toxicological and other relevant data published subsequent to FSANZ’s previous assessments of steviol glycosides raised no concerns regarding the safety of steviol glycosides and did not indicate a need to amend the ADI.

In conclusion, FSANZ’s hazard assessment has not identified any safety concerns associated with SweeGen’s Reb D produced using an enzyme from a GM strain of *P. pastoris*.

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# 1 Introduction

SweeGen’s application seeks permission in the Australia New Zealand Food Standards Code (the Code) for a rebaudioside D (Reb D) from a novel production method. The traditional method of extraction uses hot water extraction of the *Stevia rebaudiana* Bertoni (stevia) leaf, followed by purification and recrystallisation using methanol or ethanol. Conversely, SweeGen uses an enzymatic process that converts stevia extract into Reb D. This conversion makes use of an enzyme processing aid (UGT-A) containing uridine diphosphate glucosyltransferase (UDP-glucosyltransferase) and sucrose synthase components. The enzyme processing aid is sourced from a genetically modified (GM) strain of *Pichia pastoris* and has previously been assessed and approved under application A1157.

Schedule 3 of the Code contains specifications for steviol glycosides in S3—35, for which the SweeGen’s Reb D complies with the identity and purity (but not the method of production). There are also primary source specifications for steviol glycosides contained within section S3—2, being either S3—2(1)(b) [the FAO JECFA Monograph], S3‐2(1)(c) [the Food Chemicals Codex] or S3—2(1)(d) [European Commission Regulation No 231/2012 (EU, 2012) laying down specifications for food additives]. Specifications for steviol glycosides from these primary sources, including Reb D, indicate that the substances are extracted from the leaves of *Stevia rebaudiana* Bertoni. As such, the SweeGen’s Reb D does not comply completely with specifications in Schedule 3.

SweeGen are not requesting a change to the purity specification (≥95% steviol glycosides) or proposing an extension for the use of Reb D in additional food products nor do they propose to increase the permitted quantities of Reb D in permitted food products.

## 1.1 Objectives of the assessment

The objectives of this technical, safety and risk assessment for the enzymatic production of Reb D were to:

* determine whether the proposed new production method to produce Reb D produces an equivalent product to that obtained by the traditional extraction method from the *Stevia rebaudiana* Bertoni leaf
* determine whether SweeGen’s Reb D meets the current identity and purity requirements of the steviol glycoside specifications listed in Schedule 3
* determine whether the current acceptable daily intake (ADI) is appropriate for SweeGen’s Reb D, by assessing any recent toxicological studies and other data published subsequent to FSANZ’s most recent assessment of steviol glycosides.

# 2 Food technology assessment

## 2.1 Identity of Reb D

SweeGen’s Reb D is produced by enzymatic conversion of purified stevia leaf extract. The final product is a high purity preparation containing no less than 95% Reb D. Reb D is a minor, naturally occurring, steviol glycoside that is present in the leaves of *S. rebaudiana* Bertoni. SweeGen has established product specifications, specifically identity and purity, for Reb D that are consistent with the specifications within subsection S3—2(1) in Schedule 3 for steviol glycosides. But the important difference is these specifications all refer to extracting the steviol glycosides from the stevia leaf which is different to SweeGen’s production method. The below information has been taken from the application.

**Chemical name**: 13‐[(O‐β‐D‐Glucopyranosyl‐3‐O‐β‐D‐glucosylpyranosyl‐β‐Dglucosylpyranosyl) oxy]‐kaur‐16‐en‐18‐oic acid, 2‐O‐β‐D‐glucosylpyranosyl‐β‐D‐glucosylpyranosyl ester

**Common name**: Rebaudioside D

**Synonym**: Reb D

**Chemical formula**: C50H80O28

**Molecular weight**: 1129.15 Daltons

**CAS Number**: 63279‐13‐0



**Figure 1 Chemical structure of rebaudioside D (taken from the application, referenced from Sigma-Aldrich:** [**www.sigmaaldrich.com**](http://www.sigmaaldrich.com)**)**

## 2.2 Physical and chemical properties of Reb D

Reb D is a white to off-white powder that is only slightly soluble in water. SweeGen’s product is consistent with Reb D extracted from stevia leaves. Reb D is one of a group of compounds called steviol glycosides that share a similar molecular structure that are all extracted from the stevia leaf. Steviol glycosides include any compound containing a steviol backbone that is conjugated to any number of sugar moieties, such as glucose, rhamnose, xylose, fructose, deoxyglucose, galactose and arabinose (JECFA 2017).

## 2.3 Information on the identity of the enzyme used in the production method

The enzyme UGT-A used as a processing aid in the conversion of the stevia leaf extract to Reb D, is a fusion protein containing a UDP‐glucosyltransferase and a sucrose synthase component. The enzyme is produced from a GM strain *of P. pastoris*.

**UDP‐glucosyltransferase**

*Source (strain):* *Pichia pastoris* containing DNA sequences encoding UDP‐glucosyltransferase and sucrose synthase enzymes

*Common:* Glucosyltransferase

*EC Number:* 2.4.1.17

*Systematic Name:* UDP‐glucose β‐D‐glucosyltransferase

*CAS Number:*9030‐08‐4

**Sucrose synthase**

*Source (strain): Pichia pastoris* containing DNA sequences encoding UDP‐glucosyltransferase and sucrose synthase enzymes

*Common:* Sucrose synthase

*EC Number:* 2.4.1.13

*Systematic Name:* NDP‐glucose:D‐fructose 2‐α‐D-glucosyltransferase

*CAS Number:* 9030‐05‐1

## 2.4 Specifics of the enzymatic reactions

UDP-glycosyltransferases are involved in the transfer of a sugar residue from an activated donor molecule (*e.g.,* UDP-glucose) to an acceptor molecule (Richman *et al.,* 2005). Steviol glycoside synthesis in the *S. rebaudiana* Bertoni plant involves successive glucosylation steps starting with steviol to form steviolmonoside, followed by steviobioside, and then stevioside etc.Specifically, UDP-glucosyltransferase UGT76G1 catalyses the reaction of stevioside to form rebaudioside A (Reb A) by glucosylation at the C‐4 carboxyl group (Richman *et al.,* 2005; Humphrey *et al.,* 2006). In this reaction, the activated sugar donor (UDP-glucose) and fructose are formed from UDP and sucrose, the reaction of which is catalysed by the enzyme sucrose synthase (Humphrey *et al.,* 2006). The reaction mechanism of UDP‐glucosyltransferase and sucrose synthase to form Reb A from stevioside is shown in Figure 2 below, taken from the application. These coupled activities of UDP‐glucosyltransferase and sucrose synthase were adapted by SweeGen to efficiently produce Reb D from stevia extract (Mao *et al*., 2016a, b).



***Figure 2*** Mechanism of formation of Reb A from Stevioside with UDP-Glucosyltransferase and sucrose synthase (taken from the application, which was adapted from Wang et al. 2015)

## 2.5 Technological purpose

Steviol glycosides extracted from the leaves of *S. rebaudiana* Bertoni, including Reb D, are already permitted for use as food additives in the Code, with the International Numbering System (INS) assignation 960. The technological purpose of steviol glycosides as a food additive is that of an intense sweetener which replaces the sweetness normally provided by sugars in food, without contributing significantly to their available energy. Hence it is valuable for use in foods such as reduced‐energy or no‐added sugar products. Steviol glycosides are permitted in Schedule 15 at maximum permitted levels (MPLs) in a variety of food categories and at Good Manufacturing Practice (GMP) level (which limits the amount of substance that is added to food to the lowest possible level necessary to accomplish its desired effect) in tabletop sweeteners. The technological purpose of this particular Reb D from SweeGen does not differ from currently permitted steviol glycosides, rather it is the method of manufacture that differs.

## 2.6 Technological justification

The primary reason for developing alternative methods to the traditional extraction methods for steviol glycosides is that not all glycosides are naturally produced to the same degree in the leaves of *S. rebaudiana* Bertoni. For example, stevioside is a major glycoside present in the leaves of the plant, constituting about 5 to 10% in dry leaves (JECFA, 1999), whereas Reb D is a minor glycoside that is present at much lower levels. Some of the minor glycosides, such as Reb D, have more favourable sensory characteristics when compared to the major glycosides (e.g.stevioside, Reb A) and have taste profiles that are more reflective of sucrose. Hence the development of the new technology to produce a glycoside with preferential sensory characteristics for product development.

The sweetness equivalency to sucrose of SweeGen’s Reb D was determined to be 200 times sweeter than sucrose upon evaluation by a sensory panel. The full study report was provided with the application.

## 2.7 Manufacturing process

SweeGen uses a novel enzymatic process to manufacture its high purity Reb D (≥95%). This process makes use of an enzyme to facilitate the transfer of glucose to purified stevia leaf extract via glycosidic bonds.

The manufacturing process is divided into 2 stages, see figure 3:

Stage 1. Enzyme production

*P. pastoris* undergoes fermentation to generate the enzyme UGT-A. Following the fermentation step, the enzyme is isolated from the source microorganism.

Stage 2. Reb D production

The enzyme UGT-A is mixed with stevia extract (≥95% steviol glycosides), extracted from the leaves of *S. rebaudiana* Bertoni to generate Reb D.

The resulting Reb D undergoes a series of purification and isolation steps to produce the final high‐purity Reb D (≥95%).

SweeGen’s Reb D is currently manufactured in China and will not be manufactured in Australia or New Zealand. All materials and processing aids utilised in its manufacture are food‐grade and comply with relevant internationally‐recognised standards. The enzyme is produced by *P. pastoris*. SweeGen’s Reb D is manufactured in compliance with current Good Manufacturing Practices (cGMP).

Further details on the manufacturing process can be found on pages 17 – 19 of the application.

## 2.8 Manufacturing flow chart

Yeast A collected

Enzymes on the cell surface are loosened by homogeniser

Enzymes separated from the cells by centrifugation

Enzyme UGT-A collected

Yeast A: expressing UGT-A fusion enzyme

***Stage 1.***

***Enzyme production***

Fermentation

UGT-A mixed with reaction buffer

Stevia extract added to enable reaction

Denatured enzymes removed by centrifugation

Re-dissolve powder

Precipitate collection

Grinding and sieving

Concentrate Reb D solutions

Supernatant loaded into macroporous resin

Elute Reb D from resin

Colour removal

Cool and crystallise

**Reb D**

***Stage 2.***

***Reb D production***

After enzymatic conversion, the reaction mixture is heated to 85°C for 20 minutes

Column washed

Filtration

Dry

***Figure 3*** Schematic of the production process of SweeGen’s Reb D

## 2.9 Product specification

SweeGen’s application contains a comprehensive product specification in Table B.6.1-1 on page 23 for Reb D produced via enzymatic conversion of purified stevia leaf extract. The product specifications are consistent with the specifications in Schedule 3 for steviol glycosides in S3—35. They also comply with the assay and impurity specifications in the FAO JECFA Monograph 19 for “steviol glycosides from *Stevia rebaudiana* Bertoni” (JECFA 2017). Additional heavy metal specifications in S3—4 for cadmium and mercury are also relevant since they are not addressed in S3—35 or primary sources of specifications for steviol glycoside preparations in S3—2.

***Table 1*** Comparative Reb D Specifications

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Analysis | Specifications | | | |
| **SweeGen** | **JECFA** | **the Code (S3—35)** | **the Code (S3—4)** |
| Appearance/Description | Off-white to white powder | White to light yellow powder | White to light yellow powder |  |
| Purity (%) steviol glycosides | ≥ 95 | ≥ 95 | ≥ 95 |  |
| Solubility | Soluble in water | Freely soluble in water | Freely soluble in water |  |
| pH (1% solution) | 4.5-7.0 | 4.5-7.0 | 4.5-7.0 |  |
| Total ash (%) | ≤1 | ≤1 | <1 |  |
| Loss on drying | ≤6 | ≤6 | ≤6 |  |
| Residual ethanol (mg/kg) | <1000 | ≤5000 | ≤5000 |  |
| Residual methanol (mg/kg) | <200 | ≤200 | ≤200 |  |
| Arsenic (mg/kg) | <0.5 | ≤1.0 | ≤1.0 |  |
| Lead (mg/kg) | <0.5 | ≤1.0 | ≤1.0 |  |
| Cadmium | <0.5 | - | - | ≤1.0 |
| Mercury | <0.5 | - | - | ≤1.0 |

Certificates of analyses for five non‐consecutive batches of SweeGen’s Reb D were provided with the application (page 24) to demonstrate compliance with the defined product specifications, which meet the relevant Code’s analytical and purity requirements for steviol glycosides.

## 2.10 Analytical method for detection

The analytical methods used to confirm that SweeGen’s Reb D meets the established chemical and microbial specifications are listed in Table B.6.1‐1 on page 23 of the application. The methods are internationally recognised, such as the Association of Official Analytical Chemists [AOAC], U.S. Pharmacopeia [USP], and JECFA. The Reb D content in the final Reb D product is quantified according to the JECFA High Performance Liquid Chromatography (HPLC) method for steviol glycosides described in FAO JECFA Monograph 19 for “Steviol Glycosides from *Stevia rebaudiana* Bertoni” (JECFA 2017). Details of the HPLC method and chromatographic data were provided with the application. These showed that the SweeGen’s Reb D met and exceeded the acceptance criteria for Reb D content, purity and moisture.

Analytical methods for determining the presence of, and to quantify levels for SweeGen’s Reb D in the final food products are the same as those currently used for all steviol glycosides.

There have been analytical methods available for the detection and quantification of steviol glycosides in food since preparations of steviol glycosides have been commercialised and permitted as intense sweetener food additives. These have been based on HPLC and referred to the European Food Safety Authority (EFSA) Scientific Opinion on steviol glycosides in 2010 (EFSA 2010). Two HPLC analytical methods have been published (Geuns et al 2008, Gardana et al 2010). Such methods have been readily adapted by steviol glycosides producers for the analysis of all types of steviol glycosides from steviol glycosides preparations added to foods and beverages.

## 2.11 Production stability

SweeGen provided results of a 6‐month accelerated stability study conducted on 5 representative batches of their Reb D product, when stored at 40±2°C at a relative humidity of 75±5%. SweeGen’s Reb D was observed to be stable over the course of the accelerated stability study, based on appearance, moisture content, and percent Reb D content.

JECFA have concluded that “steviol glycosides, including steviol glycosides extract preparations containing higher levels of new glycosides, are thermally and hydrolytically stable for food use, including acidic beverages, under normal conditions of processing/storage” (JECFA, 2007).

## 2.12 Food technology conclusion

The food technology assessment concludes that SweeGen’s Reb D produced by a novel multi‐step enzymatic pathway process meets the purity specifications currently listed in the Code for steviol glycosides. SweeGen demonstrated that its particular method of production of Reb D produces a consistent product that conforms to these specifications. SweeGen’s Reb D is of high purity and contains no impurities resulting from its unique manufacturing process. Its technological purpose matches that of currently permitted Reb D preparations produced by the traditional method of hot water extraction.

# 3 Safety assessment

The enzymatic process used to convert leaf-extracted stevia to Reb D uses the enzyme UGT-A produced from a GM *Pichia pastoris* strain. This enzyme and the source microorganism have been previously assessed by FSANZ ([A1157](https://admin-www.foodstandards.gov.au/code/applications/Pages/A1157–EnzymaticproductionofRebaudiosideM.aspx)[[1]](#footnote-2)). The conclusions from this assessment were that the *P. pastoris* source organism for UGT-A has a long history of industrial use, is commonly used for recombinant gene expression and is not toxigenic. Furthermore, it would be unlikely that the enzyme or the production strain will be present in the highly purified Reb D product. No major allergens are used to culture the yeast or at any other stage of the production process and sufficient information was provided concerning potential homology between the UGT-A and known allergens for FSANZ to conclude there is no public health concern.

# 4 Hazard Assessment

## 4.1 Previous FSANZ assessments

The safety of steviol glycosides has been assessed previously by FSANZ. FSANZ first assessed steviol glycosides in 2008 (Application A540) and established an ADI of 0-4 mg/kg bw/day steviol. At that time, only ten steviol glycosides were known. FSANZ updated the hazard assessment of steviol glycosides in 2011 (Application A1037) but did not find reason to change the ADI established in 2008. Reb M was assessed and approved in 2015 (Application A1108). FSANZ expanded the definition of steviol glycosides to include all steviol glycosides found in the leaves of *S. rebaudiana* Bertoni in February 2017 (Application A1132). Most recently, the safety of Reb M produced via a similar production method to this application was assessed in Application A1157 (October 2018). A number of new studies were assessed in the course of these applications, but no evidence was found to justify a change to the ADI of 0-4 mg/kg bw/day steviol set in 2008.

Briefly, all known steviol glycosides share a common metabolic pathway, and are hydrolysed to steviol at similar rates. The number of sugar moieties in the glycosides, and the sugars present in those moieties, do not have any marked effect on the rate of hydrolysis.

Toxicokinetic data obtained from human male volunteers show that oral administration of stevioside results in a similar Cmax for plasma steviol as that found in rats administered a corresponding dosage, although Tmax occurs later in humans than in rats. In both species, steviol is metabolised by conjugation to steviol glucuronide, which in humans is predominantly excreted in the urine.

A number of genotoxicity studies have been conducted, using a range of assays. There is no evidence that steviol glycosides are genotoxic. On the basis of a two-year rat study of stevioside, JECFA concluded that there is no indication of carcinogenic potential. There is no evidence that purified steviol glycosides are likely to allergenic.

## 4.2 Characteristics of Reb D manufactured using enzymes from GM *P. pastoris*

Reb D synthesized using enzymes from GM *Pichia pastoris* is the same molecule as that extracted directly from leaves of *Stevia rebaudiana* Bertoni. The Reb D preparation has a purity of ≥95% and is compliant with the specifications of JECFA and the Code. From the information provided, there is no evidence that any major allergens are likely to be present from the manufacturing process.

## 4.3 Toxicological data

FSANZ has previously reviewed and summarized almost all the safety studies provided by the applicant in support of the application. The exceptions are the studies of Philippaert et al. (2017) and al-Dujaili et al. (2017), which are summarised below.

In vitro *and* in vivo *studies of steviol and steviol glycosides (Philippaert et al. 2017) Regulatory status: Non-GLP*

These studies were not designed as toxicity studies. The *in vitro* studies were mechanistic in nature and are not considered to be relevant to this assessment. Findings in knockout mice are not included here because they are not representative of normal physiology, however findings in a subchronic study in wild-type mice are of some relevance. The mice were males of the C57Bl/6J strain. Mice were housed individually and the experiment started when they were 7 weeks old. At 7 weeks of age, mice were fed a high fat diet (HFD) comprising pellets containing 30% saturated fat, provided *ad libitum,* and assigned to three groups (n = 8-10/group). The control group received plain water to drink and the other two groups received water containing 100 mg/L stevioside. Body weight, food consumption and blood glucose were measured weekly. A glucose tolerance test (GTT) was performed on all mice after 10 weeks (mice were 17 weeks of age), and stevioside supplementation of one of the two treatment groups was stopped, while the other group remained on the stevioside supplementation for another 5 weeks (22 weeks of age), when a GTT was again performed on all mice in the experiment. Stevioside significantly improved glucose tolerance in mice consuming HFD, but this effect disappeared in the group from which stevioside was withdrawn, and their resting glycaemia increased. Mice provided with stevioside in their drinking water gained less weight on the HFD than control mice, but this effect was also reversible after withdrawal of stevioside. No adverse effects of stevioside administration in the drinking water were reported.

The mice were obtained from Jackson Laboratories. According to the Jackson Laboratories website ([www.jax.org](http://www.jax.org)) wild-type male mice of this strain average approximately 23 g at 7 weeks and approximately 32 g at 20 weeks, but average water intake for this strain is not stated. Assuming a daily water intake of 1.5 mL/10 g bw/day (Johns Hopkins University ACUC; web.jhu.edu/animalcare/procedures/mouse.html), the mice would have consumed 0.15 mg/10 g bw/day stevioside, or 15 mg/kg bw/day.

*Crossover placebo-controlled study of stevia consumption in human volunteers (al-Dujaili et al. 2017) Regulatory status: non-GLP*

Aims of this study were to investigate the effects of stevia on blood pressure and cortisol, because previous studies had produced conflicting results concerning both these parameters in humans. Participants were healthy male and female volunteers who were non-smokers, with no history of cardiovascular disease, hypertension or diabetes mellitus. The test article for this study was described as stevia powder, 100% purity. The placebo was sugar. The participants were randomly assigned to two groups with eight volunteers in each group. The first group was allocated to take stevia for seven days while the second group took sugar, with specific instructions to avoid additional sugar in their diet. The intended dose of stevia was 0.6 g/day. At the end of the seven days there was a three-day washout period before a second seven-day intervention with the sweeteners provided to the groups reversed. Prior to the interventions, on the seventh day of the first intervention, and on the seventh day of the second intervention, participants were asked to provide 24-hour urine collection and three saliva samples (morning, afternoon and evening) for determination of urinary and saliva cortisol. Before the first intervention participants had blood pressure, weight, and height measured, and BMI calculated. Blood pressure and weight were also measured in the washout period between the interventions and again at the end of the second intervention, and BMI was calculated. Stevia was associated with a small but statistically significant increase in blood pressure, with mean systolic blood pressure increasing 4.4% (p < 0.001) and mean diastolic blood pressure increasing 6.9% (p < 0.01). Stevia consumption was also associated with statistically significant increases in mean free cortisol in urine (approximately 37% increase) and in urinary free cortisol/cortisone ratio (53% increase). Slight reductions in bodyweight and BMI were observed in association with stevia but were not statistically significant. The authors suggested that the increase in blood pressure was secondary to the increase in cortisol and cortisol/cortisone ratio. It is suggested that stevia may inhibit 11β-hydroxysteroid dehydrogenase 2, which converts cortisol to cortisone. An increase in circulating cortisol would lead to increased reabsorption of sodium and water by the renal nephrons, leading in turn to increased blood pressure.

A limitation of this study is that the dosage of stevia was likely to be variable, because of the different body weights of the participants. The range of body weights is not stated but the standard deviations around the mean body weights are large, at ±19.8 kg for men and ±13.4 kg for women. Other limitations of this study are the small number of participants and the short durations of the interventions. Because of these limitations, FSANZ considers this study to be preliminary and not sufficiently robust to be useful for safety assessment of stevia or steviol glycosides.

## 4.4 Assessments by other regulatory agencies

There have been no new assessments by other regulatory agencies since FSANZ finalized A1157. FSANZ has previously reviewed the assessments by JECFA and by Health Canada. In contrast to the JECFA and Health Canada assessments, EFSA has declined to expand the definition of steviol glycosides to include all individual steviol glycosides, because they consider that there are uncertainties relating to the rate and extent of metabolism of individual steviol glycosides to steviol (EFSA 2018).

## 4.5 Hazard assessment discussion and conclusion

Previous assessments of steviol glycosides by FSANZ and JECFA have confirmed that all steviol glycosides undergo the same metabolic pathway to steviol, which is then glucuronidated and excreted in the urine. A group ADI, expressed as steviol, is therefore appropriate to all steviol glycosides.

No new evidence has been identified that would justify a decrease in the ADI of 0 to 4 mg/kg bw, expressed as steviol, for steviol glycosides established by JECFA at their 69th meeting and confirmed at their 82nd meeting in 2016. The ADI of 0 to 4 mg, when expressed as steviol, is therefore appropriate for enzymatically-produced rebaudioside D.

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