

**29 November 2019**

**[103-19]**

**Supporting document 1**

Risk and technical assessment – Application A1183

Enzymatic production of rebaudioside E from stevia leaf extract

# Executive summary

Blue California has applied to amend the Australia New Zealand Food Standards Code (the Code) to seek approval for a new specification for the steviol glycoside rebaudioside E, produced by an enzymatic conversion method using an enzyme processing aid derived from a genetically modified strain of *Pichia pastoris (P. pastoris)*. The starting material is purified stevia leaf extract.

The enzymatic conversion process produces a highly purified preparation containing no less than 85% rebaudioside E and no less than 95% total steviol glycosides on a dry weight basis. The enzymatic conversion process makes use of an enzyme processing aid (designated as UGT-A) that has previously been assessed and approved by FSANZ under A1157 (FSANZ 2018) and is listed in Schedule 18 for the production of rebaudiosides M and D. UGT-A contains two plant enzymes (uridine diphosphate (UDP)-glucosyltransferase and a sucrose synthase) expressed as a fusion protein.

Steviol glycosides are currently permitted by the Code to be used in certain foods as food additives up to specified maximum permitted levels. They are used as an intense sweetener or flavour enhancer. Substances used as food additives must comply with any relevant identity and purity specifications listed in Schedule 3 – Identity and Purity. Schedule 3 of the Code contains a specification for steviol glycosides prepared from the leaves of *Stevia rebaudiana* Bertoni, which includes rebaudiosides M and D produced from stevia leaf extract, using enzymatic conversion (S3—35).

An acceptable daily intake (ADI) of 0-4 mg/kg bodyweight for steviol glycosides, expressed as steviol, was established by FSANZ in 2008 and the the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2009, and confirmed at their 82nd meeting in 2016. This ADI is appropriate for rebaudioside E produced using enzymes from genetically modified *P. pastoris* as it is chemically the same as rebaudioside E extracted traditionally from *Stevia 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.

FSANZ is aware that a number of research papers have reported on possible links between consumption of intense sweeteners and unwanted metabolic effects resulting in weight gain, but considers that the current evidence does not support a causal relationship. FSANZ will continue to monitor the emerging scientific literature in this area.

Blue California is not requesting a change to the foods permitted to contain steviol glycosides as a food additive nor do they propose to increase the maximum permitted levels of steviol glycosides in foods. FSANZ has previously conducted a dietary exposure assessment using the current permissions to use steviol glycosides as a food additive and therefore no dietary exposure assessment was necessary for this Application.

In conclusion, FSANZ’s risk assessment has not identified any safety concerns associated with Blue California’s high purity rebaudioside E preparation (≥85% rebaudioside E; ≥95% total steviol glycosides) produced from purified stevia leaf extract.

Table of contents

[Executive summary i](#_Toc25564300)

[1 Introduction 2](#_Toc25564301)

[**1.1** **Objectives of the assessment** 2](#_Toc25564302)

[2 Food technology assessment 3](#_Toc25564303)

[2.1 Assessment of the rebaudioside E preparation 3](#_Toc25564304)

[2.1.1 Identity of the rebaudioside E and the rebaudioside E preparation 3](#_Toc25564305)

[2.1.2 Physical and chemical properties of the rebaudioside E preparation 4](#_Toc25564306)

[2.1.3 Technological purpose of the food additive 4](#_Toc25564307)

[2.1.4 Technological justification 5](#_Toc25564308)

[2.1.5 Manufacturing process 5](#_Toc25564309)

[2.1.6 Specification for identity and purity 5](#_Toc25564310)

[2.1.7 Analytical method for detection 6](#_Toc25564311)

[2.1.8 Product stability 6](#_Toc25564312)

[2.2 Assessment of the enzymes used 6](#_Toc25564313)

[2.2.1 Identity of the enzymes and manufacturing process 6](#_Toc25564314)

[2.2.2 Specifics of the enzymatic reaction 7](#_Toc25564315)

[2.2.3 Specification for identity and purity for the enzymes 7](#_Toc25564316)

[2.3 Food technology conclusion 7](#_Toc25564317)

[3. Risk Assessment 8](#_Toc25564318)

[**3.1** **Previous FSANZ assessments** 8](#_Toc25564319)

[3.1.1 Previous FSANZ safety assessment of the UGT-A processing aid 8](#_Toc25564320)

[3.1.2 Previous FSANZ hazard assessments of steviol glycosides 8](#_Toc25564321)

[**3.2** **Characterisation of rebaudioside E** 8](#_Toc25564322)

[3.3.1 Toxicity 9](#_Toc25564323)

[**3.4**  **Assessments by other regulatory agencies** 13](#_Toc25564324)

[**3.5**  **Risk assessment discussion and conclusion** 13](#_Toc25564325)

[4 References 15](#_Toc25564326)

# 1 Introduction

Blue California has applied to Food Standards Australia New Zealand (FSANZ) to amend the Australia New Zealand Food Standards Code (the Code) to include a new production method in Schedule 3 of the Code for rebaudioside E, a steviol glycoside. The traditional method to produce steviol glycosides uses hot water extraction of the *Stevia rebaudiana* Bertoni (stevia) leaf, followed by purification and recrystallisation using methanol or ethanol. Blue California’s rebaudioside E preparation adds an additional step to the traditional method whereby the initial extract is modified by an enzymatic conversion process using an enzyme processing aid derived from a genetically modified (GM) strain of *Pichia pastoris (P. pastoris).* This produces a highly purified preparation containing no less than 85% rebaudioside E and no less than 95% total steviol glycosides on a dry weight basis. This preparation is designated as high purity rebaudioside E (≥85% rebaudioside E; ≥95% total steviol glycosides).

The enzyme processing aid, designated UGT-A, contains two plant enzymes expressed as a fusion protein from a GM strain of *P. pastoris*. The plant enzymes include an uridine diphosphate (UDP)-glucosyltransferase and a sucrose synthase. UGT-A[[1]](#footnote-2) has previously been assessed and approved by FSANZ under A1157, and is listed in Schedule 18 for the production of rebaudioside M and rebaudioside D (FSANZ 2018 and FSANZ 2019a, respectively).

Schedule 3 of the Code contains specifications for steviol glycosides in S3—35, for which Blue California’s rebaudioside E preparation 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 laying down specifications for food additives). Specifications for steviol glycosides in these primary sources also do not include the enzymatic conversion process. As such, Blue California’s rebaudioside E preparation is not consistent with specifications in Schedule 3. Under the Code, steviol glycosides must meet an assay value of not less than 95% total steviol glycoside content, on a dry weight basis, however the individual steviol glycosides can be in any combination and ratio.

Steviol glycosides are currently permitted by the Code to be added to certain foods as a food additive up to specified maximum permitted levels. Blue California is not requesting a change to the foods permitted to contain steviol glycosides as a food additive nor do they propose to increase the maximum permitted levels of steviol glycosides in foods.

FSANZ has previously conducted a dietary exposure assessment using the current permissions to use steviol glycosides as a food additive and therefore no dietary exposure assessment was necessary for this Application.

**1.1 Objectives of the assessment**

The objectives of this risk and technical assessment for the enzymatic conversion of purified stevia leaf extract to produce a high purity preparation of rebaudioside E (containing no less than 85% rebaudioside E and no less than 95% total steviol glycosides) were to:

* confirm the technological purpose of Blue California’s rebaudioside E preparation, the justification for its use and assess the manufacturing method, including use of a specific enzyme processing aid
* determine whether Blue California’s rebaudioside E preparation meets the current identity and purity requirements of the steviol glycoside specifications listed in Schedule 3, aside from the production method
* confirm that the enzyme processing aid (UGT-A fusion protein), used to produce Blue California’s rebaudioside E preparation is the same as that evaluated under A1157, and therefore that there are no public health and safety concerns arising from the use of the enzyme processing aid
* determine whether the proposed production method produces an equivalent rebaudioside E, chemically and metabolically, to that obtained by the traditional extraction method from the *Stevia rebaudiana* Bertoni leaf
* determine whether the current acceptable daily intake (ADI) is appropriate for Blue California’s rebaudioside E preparation, by assessing any recent toxicological studies and other data published subsequent to FSANZ’s most recent assessment of steviol glycosides.

# 2 Food technology assessment

This assessment covers both the method of production for the high purity rebaudioside E preparation (≥85% rebaudioside E; ≥ 95% total steviol glycosides), and the enzyme processing aid used in the production of rebaudioside E.

## Assessment of the rebaudioside E preparation

### 2.1.1 Identity of the rebaudioside E and the rebaudioside E preparation

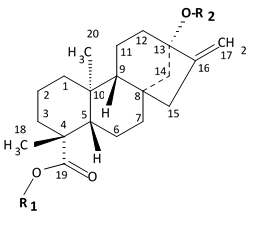
Steviol glycosides are a group of compounds naturally occurring in the *Stevia rebaudiana* Bertoni plant. Rebaudioside E is a minor steviol glycoside that is present in the leaves of *S. rebaudiana* Bertoni at less than 0.2%. Blue California’s method allows enrichment of rebaudioside E resulting in a high purity preparation containing ≥85% rebaudioside E in a mixture of ≥ 95% total steviol glycosides, on a dry weight basis.

To confirm the identity of the rebaudioside E preparation, HPLC was performed. [[2]](#footnote-3) The data presented showed a major peak that had the same retention time as purified rebaudioside E. There were several minor peaks that corresponded to a range of steviol glycosides found in standard leaf extracts from stevia. These data indicate that the rebaudioside E produced by enzymatic conversion is chemically the same as the plant-extracted form and that the rebaudioside E is present in a mixture containing other well-characterised steviol glycosides.

In addition, HPLC analysis data contained in Appendix H of the application (commercial in-confidence) demonstrates that the approximately ten percent content of other steviol glycosides contained in the high purity rebaudioside E preparation complies with the specifications in Schedule 3. These are major and/or minor steviol glycosides as defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA 2017).

***Structural formula:***

All steviol glycosides share the same steviol backbone structure (Figure 1) but have different sugar moieties attached, as conjugated glycosides. R1 and R2 can be one or more sugar moieties, including but not limited to glucose, rhamnose, xylose, fructose, galactose and deoxyglucose, which can be attached in various combinations, quantity and orientation (JECFA 2017). Rebaudioside E is an ent-kaurane diterpene glycoside with a steviol backbone conjugated to four glucose units (two on each of the R1 and R2 positions).



**Figure 1** Chemical backbone structure for steviol glycosides

The chemical information for rebaudioside E is provided in Table 1 below.

*Table 1* Chemical information for rebaudioside E

|  |  |
| --- | --- |
|  | Rebaudioside E |
| Chemical name | 13-[(*O*-β-D-glucopyranosyl-(1,2)-*O*-β-D-glucopyranosyl)-oxy]kaur-16-en-18-oic acid (4’)-*O*-β-D-glucopyranosyl-deoxy-(1,2)-*O*-β-D-glucopyranosyl ester |
| Chemical formula | C44H70O23 |
| Molecular weight | 967 |
| CAS number | 63279-14-1 |

### 2.1.2 Physical and chemical properties of the rebaudioside E preparation

According to certificates of analysis provided by Blue California (see Table B.6.1-1 of Application), their high purity rebaudioside E preparation is a white powder. The preparation has a pH of 4.5 to 7.0 (1% solution) and is soluble in water. Table B.6.1-1 of the application provides information on the chemical properties of the preparation.

### 2.1.3 Technological purpose of the food additive

Blue California states that the technological purpose of its high purity rebaudioside E preparation (≥85% rebaudioside E; ≥ 95% total steviol glycosides) is that of an intense sweetener which would replace sugar in food in reduced energy or no added sugar products. They also note it may be used as a flavour enhancer.

Steviol glycosides are currently permitted as a food additive at maximum permitted levels in a variety of food classes and at Good Manufacturing Practice (GMP) level for table top sweeteners in Schedule 15. Blue California has not requested any amendments to these maximum permitted levels; rather, their application relates to the method of production for their high purity rebaudioside E preparation (≥85% rebaudioside E; ≥ 95% total steviol glycosides).

### 2.1.4 Technological justification

Steviol glycosides are present in the leaves of *S. rebaudiana* Bertoni. Those present at higher levels are called major glycosides (such as rebaudioside A and stevioside). As noted in section 2.1.1 above, rebaudioside E is a minor glycoside, as it is present at much lower levels in the leaf. New technologies such as enzymatic conversion allow product developers to produce glycosides with more preferential sensory characteristics with a taste profile more reflective of sucrose, for example by producing a steviol glycoside preparation with much higher levels of minor glycosides. Blue California is one such product developer, using enzymes expressed as a fusion protein such as UGT-A. The enzymes facilitate the transfer of glucose to purified stevia leaf extract.

The enzymatic conversion method used by Blue California is technologically justified in that it yields a higher amount of rebaudioside E, compared to the low levels in the stevia leaf. Blue California claims its high purity rebaudioside E preparation has preferential taste characteristics compared to preparations containing major individual steviol glycosides alone.

### 2.1.5 Manufacturing process

Blue California states in its application that the rebaudioside E is enzymatically converted from a purified stevia leaf extract (≥95% total steviol glycosides). The stevia leaf extract is produced from stevia leaves using the traditional hot water extraction process, consistent with the process already defined for recognised steviol glycosides (JECFA 2017). The production process results in a food additive preparation that contains at least 95% total steviol glycosides, with rebaudioside E representing at least 85% of the finished product.

UGT-A, containing the two enzymes uridine diphosphate (UDP)-glucosyltransferase and sucrose synthase, is used as a processing aid to catalyse the conversion of rebaudioside E to generate the higher purity rebaudioside E preparation (≥85% rebaudioside E; ≥ 95% total steviol glycoside) via a series of purification and isolation steps. The purification process following the enzymatic conversion is consistent with that already defined for recognised steviol glycosides (JECFA 2017).

All materials and processing aids utilised in the manufacture of Blue California’s rebaudioside E are food-grade and comply with relevant Food Chemical Codex or other internationally-recognised standards. A detailed description of the manufacturing process, including a flow chart and details of the raw materials, processing aids and equipment used in the production process is provided in section B.5 of the application.

### 2.1.6 Specification for identity and purity

Blue California has established product specifications, specifically identity and purity, for the rebaudioside E preparation. The application contains comprehensive product specification information in Tables B.6.1-1 and B.6.2.1-1, based on five non‐consecutive batches of Blue California’s rebaudioside E preparation. The product specifications are consistent with the specifications in Schedule 3 for steviol glycosides in S3—35, with the exception of the method of production for rebaudioside E, and sweetness equivalency (see below). The product specifications also meet the assay and impurity specifications in the FAO JECFA Monograph 20 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. Blue California’s rebaudioside E preparation meets these additional specifications for cadmium and mercury.

The sweetness equivalency to sucrose of Blue California’s rebaudioside E preparation was reported in the application to be 137 times sweeter than sucrose upon evaluation by a sensory panel. The applicant has since provided additional information, which shows that the sweetness equivalency is ‘approximately 179 times sweeter than sucrose’. This is less than that in S3—35(4), which describes the sweetness for steviol glycosides as ‘approximately 200 to 300 times sweeter than sucrose’.

As noted in section 2.1.1, the applicant has provided evidence that the approximately ten percent content of other steviol glycosides contained in the high purity rebaudioside E preparation complies with the specifications in S3—35.

### 2.1.7 Analytical method for detection

The steviol glycoside purity of the rebaudioside E preparation can be measured using the JECFA HPLC method for steviol glycosides (JECFA 2017).

### 2.1.8 Product stability

JECFA have previously concluded that steviol glycosides are sufficiently thermally and hydrolytically stable for use in foods, including acidic beverages, under normal conditions of processing and storage (JECFA 2007).

Blue California provided results of a 6‐month accelerated stability study conducted on 5 representative batches of their rebaudioside E preparation, when stored at 40±2°C at a relative humidity of 75±5%. Blue California’s rebaudioside E preparation was observed to be stable over the course of the accelerated stability study, based on appearance, moisture content, and percent rebaudioside E content measured by HPLC compared to baseline. These results are shown in Table B.1.3-1 of the application.

## Assessment of the enzymes used

### 2.2.1 Identity of the enzymes and manufacturing process

The first stage of the manufacturing process involves the preparation of the processing aid UGT-A by fermentation. The processing aid is produced by a GM strain of *P. pastoris* expressing two enzymes in a single polypeptide (fusion protein). The two enzymes are a UDP‐glucosyltransferase and a sucrose synthase. UGT-A has previously been assessed and approved under A1157 and is listed in Schedule 18 of the Code.

Information on the two enzymes (that together form the fusion protein UGT-A) used to produce rebaudioside E is provided below.

**UDP‐glucosyltransferase enzyme**

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

*Common:* Glucosyltransferase

*EC Number:* Not yet fully classified by the IUBMB[[3]](#footnote-4)

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

*CAS Number:*9033-07-2

**Sucrose synthase enzyme**

*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

### 2.2.2 Specifics of the enzymatic reaction

The information regarding the specifics of the enzyme reaction was provided in section 2.4 of the Supporting documents under applications A1157 and A1172 (FSANZ 2018, FSANZ 2019a).

### 2.2.3 Specification for identity and purity for the enzymes

There are international specifications for enzyme preparations used in the production of food. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its Compendium of Food Additive Specifications (JECFA 2006) and in the Food Chemicals Codex (FCC 2018). These specifications are included in S3—2 of the Code and enzymes used as a processing aid must meet these specifications. The applicant states that the processing aids comply with the relevant FCC or other internationally-recognised standards.

## Food technology conclusion

Steviol glycosides are already permitted for use in certain foods as a food additive and are used as an intense sweetener or flavour enhancer. The technological purpose of the applicant’s rebaudioside E preparation matches that of currently permitted steviol glycosides. However the current specifications for identity and purity for steviol glycosides produced from enzymatic conversion in the Code (S3—35) does not include rebaudioside E produced by enzymatic conversion.

The food technology assessment concludes that Blue California’s rebaudioside E preparation (≥85% rebaudioside E; ≥ 95% total steviol glycosides, on a dry weight basis) produced by enzymatic conversion meets the purity specification in S3—35, i.e. containing no less than 95% total steviol glycosides on a dry weight basis. Rebaudioside E produced by enzymatic conversion is chemically the same as the rebaudioside produced by hot water extraction of the stevia leaf.

Blue California demonstrated that its particular method of production of rebaudioside E (as the primary component in its rebaudioside E preparation) produces a consistent product that conforms to the specifications in Schedule 3. Aside from the production method and sweetness equivalency, the applicant’s preparation meets the relevant purity and identity specification in the Code.

# 3. Risk Assessment

**3.1 Previous FSANZ assessments**

### 3.1.1 Previous FSANZ safety assessment of the UGT-A processing aid

The enzymatic process used to convert stevia leaf extract to rebaudioside E uses the processing aid UGT-A produced from a GM *P. pastoris* strain. UGT-A and the source microorganism have previously been assessed by FSANZ under A1157 (FSANZ 2018). 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. 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.

### 3.1.2 Previous FSANZ hazard assessments of steviol glycosides

FSANZ established an ADI for steviol glycosides of 0-4 mg/kg bw/day steviol in 2008 under application A540 (FSANZ 2008). The ADI was derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a two-year rat study.

The FSANZ ADI is consistent with the ADI established by JECFA at the 69th meeting held in 2008, and published in 2009. JECFA re-assessed steviol glycosides at the 82nd meeting in 2016 and confirmed the existing ADI. The assessments confirmed that steviol glycosides share a metabolic pathway to steviol. The ADI, expressed as steviol, is therefore appropriate for all steviol glycosides.

FSANZ updated the hazard assessment for steviol glycosides as a part of applications A1037, A1108, A1132, A1157, A1172 and A1176 (FSANZ 2011, FSANZ 2015, FSANZ 2017, FSANZ 2018, FSANZ 2019a, FSANZ 2019b). These assessments did not identify a need to change the ADI.

**3.2 Characterisation of rebaudioside E**

Rebaudioside E produced using a processing aid from *P. pastoris* is chemically the same as rebaudioside E extracted directly from leaves of *Stevia rebaudiana* Bertoni. The rebaudioside E preparation comprises ≥85% rebaudioside E and ≥95% total steviol glycosides.

Certificates of analysis for three separate batches showed that protein was not detectable in the rebaudioside E preparation, supporting the conclusion that enzymes and other proteins used in production are effectively removed and do not pose an allergenic hazard.

**3.3 Hazard assessment - toxicological data**

### 3.3.1 Toxicity

The Applicant submitted a number of studies investigating reproductive parameters and a number of special animal studies not specifically related to safety of rebaudioside E.

FSANZ also conducted a literature search in PubMed and EBSCO using the search terms ’steviol’ or ‘rebaudioside’ and ‘toxic-‘, ‘safety’ or ‘hazard’.

#### 3.3.1.1 Reproductive and Developmental Toxicity in Laboratory Animals

*Effect of* Stevia rebaudiana *extract on sexual dysfunction in diabetic male rats (Ghaheri* et al. *2018). Non-GLP*

The test article for this study was an extract from *Stevia rebaudiana* leaves, of unspecified composition and concentration. Dried leaves were treated with warm water, followed by ethanol extraction, filtration and concentration. The test subjects were healthy adult Wistar rats, maintained at 22°C with a 12 h light/dark cycle, with free access to water and standard rat feed. Prior to the study, all male rats were assessed for normal sexual activity with receptive female rats and only males exhibiting normal libido and mounting behaviour were selected for the study. Similarly, only females exhibiting normal sexual receptivity following hormonal induction of oestrus were selected for the study. Diabetes was induced in male rats by intraperitoneal administration of streptozotocin, and confirmed by measurement of blood glucose. A group of seven male rats without diabetes served as a negative control group, while diabetic male rats were distributed to four groups of seven rats each. One of the diabetic groups served as the positive control group. Both control groups were dosed with distilled water, while the other diabetic groups were gavaged daily with 5, 50 or 100 mg/kg bw of the Stevia extract in aqueous solution, at a dose volume of 2 mL/day for 28 days. Sexual behaviour of males with receptive females was observed by remote camera for 30 min on treatment days 0, 14 and 28, and quantified in terms of latency and frequency. At the end of the treatment phase, male rats were anesthetised, blood was collected for measurement of serum testosterone, rats were killed and the right testis and epididymis of each male was collected, weighed, preserved and processed for histopathology.

Administration of stevia extract did not have a consistent effect on blood glucose measured at 28 days. Males in the 5 mg/kg bw and 100 mg/kg bw/day groups had lower group mean blood glucose than the positive (diabetic) controls, but the males in the 50 mg/kg bw/day group did not. All groups of diabetic rats had higher group mean blood glucose than the normal controls. No significant differences in group mean testis weight or epididymis weight were discovered between any groups. There was no consistent dose-response effect of stevia extract on mating parameters of treated groups when compared to either control group. All diabetic groups had lower group mean values for number of Leydig cells per field than the negative controls, and the groups treated with stevia extract had greater group mean values for Leydig cell numbers than the untreated positive control group, but a dose-response relationship between dose of stevia extract and Leydig cell numbers was not apparent. All diabetic rats had lower group mean values for serum testosterone than the negative control group. Diabetic rats treated with stevia extract had higher group mean values for serum testosterone than the positive control group, but a dose-response relationship between dose of stevia extract and serum testosterone was not apparent. The authors concluded that stevia extract is protective against destruction of Leydig cells in diabetes.

Although no adverse effects of treatment were observed, this study is not informative to hazard assessment of steviol glycosides because the test article was not analysed to determine its composition.

*Effects of saccharin and rebaudioside A on ovarian function in aged mice (Ngekure* et al. *2019). Regulatory status: Non-GLP*

Thirty-six 12 month old female ICR mice were used as test subjects in this study. Mice were maintained under standard laboratory housing conditions although it is not stated whether they were individually housed or group-housed. Mouse chow was provided *ad libitum*. Mice were randomly allocated to three groups, 12 mice/group. The control group was provided with tap water to drink. The second group was provided with water containing 7.5 mM sodium saccharin, and the third group was provided with water containing 2.5 mM rebaudioside A. Treatment duration was 30 days. Mice were weighed daily, and water consumption was measured daily. The estrous cycles of 6 mice/group were assessed during the final 14 days of the study by daily collection and assessment of vaginal smears. At the end of the treatment period mice were anesthetised with CO2 for blood sample collection, and then killed by cervical dislocation. Ovaries and uteri were collected and fixed for histopathology and immunohistochemistry. Blood serum was analysed for total cholesterol, triglycerides, glucose and progesterone.

Water consumption was comparable between the three groups through almost all of the treatment period, although water consumption of saccharin-treated mice was significantly higher than that of controls on Day 30. Treatment had no effect on body weight. Mice in the control group had a significantly higher percentage of normal cycling days than mice administered sweeteners, although the definition of normal versus abnormal cycling is not clear. There was no difference in cycling between saccharin-treated mice and rebaudioside A-treated mice. Mice treated with sweeteners showed a significant increase in the group mean number of corpora lutea on the ovaries, compared to control mice. Saccharin-treated mice exhibited a greater number of apoptotic cells in the corpora lutea, compared to control mice. Ovarian cells of mice administered sweeteners exhibited stronger immunostaining for apoptosis-inducing factor (AIF) than those of control mice. This effect was most marked in the saccharin-treated mice. Mice treated with sweeteners had significantly higher group mean serum glucose than control mice, but there was no significant difference in this parameter between the two sweetener-treated groups. Group mean total cholesterol was significantly higher in rebaudioside A-treated mice than in controls, but this effect was not observed in saccharin-treated mice. Treatment had no effect on serum triglycerides. Treatment with rebaudioside A had no apparent effect on serum progesterone, but treatment with saccharin was associated with a significant increase in group mean progesterone in those mice killed in dioestrus, compared to controls.

The authors of the study concluded that both saccharin and rebaudioside A had deleterious effects on the ovaries of aged mice. FSANZ considers that this finding is not likely to be relevant to human beings. A 2.5 mM solution of rebaudioside A contains approximately 2.43 g/L (molecular mass of rebaudioside A is 967.01 g/mole). Data on water consumption are not clearly presented but tend to indicate mean water consumption around 65 mL/d/mouse in the rebaudioside A group, which would deliver 0.16 g/day. The daily group mean bodyweight of the rebaudioside A mice was 42.5 g over the course of the study and they were therefore consuming 3.7 g/kg bw rebaudioside A, a dose not relevant to human exposure levels.

*Effects of aspartame, rebaudioside A, and prebiotic oligofructose on fertility and reproduction in obese rats. Cho* et al. *(2018). Regulatory status: Non-GLP*

Test subjects for this study were female Sprague-Dawley rats that were 10 weeks old at the start of the experiment. Rats were individually housed under standard laboratory environmental conditions. Obesity was induced in 71 rats using a high-fat, high-sucrose (HFHS) diet. There were five groups: A lean control group of 24 rats fed a standard diet; an obese control group of 27 rats; an obese + aspartame group of 14 rats; an obese + rebaudioside group of 15 rats; and an obese + oligofructose (OFS) group of 15 rats. Aspartame (5-7 mg/kg bw/day) and rebaudioside A (2-3 mg/kg bw/day) were administered in drinking water from 2 weeks prior to mating, while OFS was administered in the diet at 10% w/w from time of mating. Rats were weighed weekly and the amount of sweetener added to water adjusted according to bodyweight. Reproductive parameters included number of days mated, percentage of rats that conceived (fertility index), percentage of successful pregnancies (pregnancy index); percentage of successful deliveries (delivery index), number of liveborn pups, number of stillbirths, and pup survival.

The fertility index and pregnancy index of lean controls were both 100% but obese controls had an 85.7% fertility index and the pregnancy index was only 60.7%. Obesity was also associated with fewer pups born alive and more pups born dead.

The fertility index of obese rats treated with aspartame was 80%, not significantly different to that of obese controls, but the fertility index of obese rats treated with rebaudioside A was significantly lower at 53.3%. There were no other significant differences in reproductive parameters between obese controls and obese rats treated with sweeteners. The pregnancy and delivery indices of the obese + rebaudioside A group were both 100%.

Treatment of obese rats with OFS resulted in a pregnancy index of 91.7%, significantly better than that of obese controls, but had no effect on other reproductive parameters.

Obese rats treated with sweeteners showed a higher group mean bodyweight at day 20 of gestation during pregnancy than obese controls. Obese rats treated with aspartame, but not those treated with rebaudioside A, also had higher group mean food consumption at day 20 than obese controls.

The authors concluded that obesity has a detrimental effect on reproductive parameters in female Sprague-Dawley rats and that consumption of rebaudioside A may impair the ability to conceive in obese rats. However they acknowledged that the results are preliminary and further investigation is required to confirm their findings. FSANZ considers the relevance of this study to human health to be uncertain. The study is small and preliminary. Only one dose of rebaudioside A was administered, so a dose-response relationship has not been established; and the results are not consistent with a multigeneration rat study reviewed by JECFA (2009) that did not reveal adverse effects of rebaudioside A at ≤4066 mg/kg bw/day.

#### 3.3.1.2 Other Studies in Laboratory Animals

*Study of the effects of chronic intake of commercial sweeteners on feeding behaviour and signalling pathways related to control of appetite in BALB/c mice (Barrios-Correa* et al*. 2018). Regulatory status: Non-GLP*

BALB/c mice were raised and maintained under standard laboratory husbandry conditions with *ad libitum* access to food and water. When mice were 8 weeks old, they were assigned to four study groups (9/sex/group). Mice were group-housed with others of the same sex and treatment group, 3/cage. The control article/vehicle was purified water, supplied for drinking, and measured and replenished daily. Sucrose was provided to the second group at 10% concentration in 100 mL purified water/day. The test articles, Splenda® sucralose and Svetia® steviol glycosides, for the third and fourth group respectively, were administered as one commercial 1 g packet per day in purified water, equal to 0.012 g sucralose and 0.025 g steviol glycoside. Treatment was continued daily for 6 weeks. Food and water intake were measured daily, and mice were individually weighed once a week. At the end of the six-week treatment period, mice were anesthetised and adiposity was measured using a tetrapolar spectroscopy bioimpedance system. Mice were killed by sodium pentobarbital overdose at the end of the treatment period. Brains from six mice/sex/group were used for protein extraction and western blot analysis, while the remaining brains of three mice/sex/group were used for immunofluorescence staining and confocal microscopy. Expression of proteins involved in the JAK2/STAT3 signalling pathway, which regulates appetite and body composition, was investigated.

When compared to the group mean values for sex-matched controls, mice treated with sucrose showed significant lower group mean values for food consumption (47 - 62% of control for males; 55 - 81% for females) and significantly higher water intake (195 - 252% of control for males; 197 - 263% for females) in every week of over the treatment period. When food consumption was calculated as energy intake, that of males treated with sucrose was lower than that of negative control males throughout the study (68 – 93%), but that of females was comparable in all weeks except Week 6, when it was significantly lower (78%). In the steviol glycoside-treated group, males showed significantly lower group mean values for water intake (71 - 82%) in every week of the treatment period, and significantly lower group mean food consumption in Weeks 2, 4 and 6 (74, 82 and 83% respectively). Females treated with steviol glycosides also showed significantly lower group mean water intake than that of female negative controls in Weeks 2 – 4 (76 - 81% of controls) and significantly lower group mean values for food consumption in study Weeks 2, 4 and 6 (79 – 91% of control values). When food consumption was converted to energy intake, group mean values for males treated with steviol glycoside were significantly lower than those of controls in Weeks 2, 4, 5 and 6 (71 – 83% of control), but values for females were only lower than those of controls in Week 4 (91% of control). Group mean values for food consumption in male mice treated with sucralose were significantly lower than those for male controls only in Weeks 1 and 2 (79 and 80% respectively) and their group mean water intake was lower only in Weeks 1 and 4 (86 and 84% respectively). Group mean food consumption of females treated with sucralose was significantly lower than those of female controls only in Week 6 (84%) and group mean water intake was also lower than that of female controls only in Week 6 (88%). When food consumption was converted to energy intake, group mean values for males in the sucralose-treated group were significantly lower than that of control males in Weeks 1 to 4 inclusive (67 – 88%) while those for females were significantly lower than those of female controls only in Week 6 (84%).

Male mice in the groups treated with sucrose or sucralose had significantly higher values for group mean body weights in Week 6 than the control group (112 and 111% respectively) but that of the steviol glycoside group was not significantly different to that of male controls. In females, the only group that had a group mean bodyweight significantly different to that of controls in Week 6 was the steviol glycoside group, which had a lower group mean bodyweight (91% of control). Weight gain was also assessed, because mice had different body weights at the start of the study. Group mean weight gain of the males treated with steviol glycosides was 63% that of male controls over the six weeks of treatment, whereas group mean weight gain of males treated with sucrose or sucralose was not significantly different to that of controls. In the females, group mean weight gain of mice treated with steviol glycosides was lower than that of female controls, but the difference was not statistically significant. Group mean weight gain of sucrose-treated females was significantly greater than that of controls (133%). Group mean body fat mass was not affected in any treated group of males, relative to male controls, but group mean adiposity was significantly increased in female mice treated with sucrose, when compared to female controls (147% of control). Expression of phosphorylated forms of some proteins in the JAK2/STAT3 pathway was altered by treatment with steviol glycoside. Immunofluorescence assays showed that female mice, but not male mice, treated with steviol glycoside showed increased expression of ObRb, the long isoform of the leptin receptor, relative to sex-matched controls. ObRb is able to activate the JAK2/STAT3 pathway.

The authors concluded that intake of steviol glycosides for 6 weeks downregulates feeding behaviour and total energy intake in mice, and that this effect may be mediated through effects on the JAK2/STAT3 pathway.

The authors of the study did not calculate the dose of steviol glycoside consumed by the mice, but from group mean bodyweight data and group mean water consumption data, it can be calculated that group mean SG consumption of males averaged 47.7 mg/kg bw/day (range 42.8 to 52.1 mg/kg bw/day) and that of females averaged 48.5 mg/kg bw/day (range 41.5 to 57.9 mg/kg bw/day). No adverse effects of steviol glycoside consumption were observed, which is not surprising since the administered dose is well below the rat NOEL on which the ADI was set.

A number of other non-GLP studies in laboratory animals were submitted by the applicant or located by literature search. Many of these are not useful for risk assessment of steviol glycosides because the test article was not adequately characterised and therefore the dose administered to the animals cannot be determined. These included the studies by Ahmad *et al*. (2018a) who presented evidence that an aqueous extract has beneficial effects on hyperlipidaemia in the rat; the investigation by El-Mesallamy *et al.* (2018) of effects of *Stevia rebaudiana* extracts on skeletal muscle in Type 1 diabetic rats; and the investigation by Ramos-Tovar *et al.* (2018) of the effects of *Stevia rebaudiana* tea on hepatic cirrhosis in the rat.

Studies of modulating effects of steviol glycosides on diabetes-associated lesions in rats following induction of diabetes mellitus by administration of streptozotocin (Rotimi *et al.* 2018; Gholizadeh *et al*. 2019) were not considered to be relevant to the risk assessment of steviol glycosides as sweeteners, although FSANZ notes that there were no adverse effects of steviol glycoside consumption reported in the rats. A study by Han et al. (2018) found that stevioside supplementation improved food intake and digestibility of dietary fibre in goats, but the relevance of this finding in ruminants to human beings is uncertain.

#### 3.3.1.3 Studies in Humans

New studies of steviol glycosides in human beings submitted by the applicant, or located by literature search, were not considered to be relevant to risk assessment of rebaudioside E as a sweetener.

The composition and/or concentration of a Stevia preparation was not adequately described in the study by Ahmad *et al*. (2018) investigating the effects of incorporating Stevia in cookies on postprandial glycaemia, appetite, palatability and gastrointestinal comfort, or in the study by Chupeerach *et al*. (2018) investigating the effects of stevia in coconut jelly on blood glucose, insulin and C-peptide responses.

A study by Rizwan *et al*. (2018) found evidence that co-administration of stevioside with conventional antihypertensive treatment may delay or prevent the progression of chronic kidney disease, but this was not considered to be relevant for the general population. FSANZ notes that no adverse effects of stevioside were reported by the patients studied by Rizwan *et al* (2018).

**3.4 Assessments by other regulatory agencies**

There have been no new assessments by other regulatory agencies since FSANZ reviewed A1176 in 2019 (FSANZ, 2019b). FSANZ has previously reviewed the assessments of JECFA, Health Canada and the European Food Safety Authority (EFSA).

**3.5 Risk assessment discussion and conclusion**

UGT-A and the source microorganism have previously been assessed by FSANZ. The assessment concluded 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. 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 allergenicity concern associated with the enzyme. Furthermore, no protein was detectable in Blue California’s rebaudioside E preparation indicating that it is effectively removed during the production process.

An ADI of 0-4 mg/kg bodyweight for steviol glycosides, expressed as steviol, was established by FSANZ in 2008 and JECFA in 2009, and confirmed at their 82nd meeting in 2016. This ADI is appropriate for rebaudioside E produced using enzymes from GM *P. pastoris* as it is chemically the same as rebaudioside E extracted traditionally from *Stevia 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.

Three studies investigated the effects of steviol glycoside exposure on reproductive parameters in rodents. Ngekure et al. (2019) concluded that rebaudioside A had deleterious effects on the ovaries of aged mice, but the dose administered was not relevant to human exposure scenarios. Although a small and preliminary study by Cho et al. (2018), suggested that rebaudioside A may have adverse effects on reproduction in obese rats at low doses, the findings are inconsistent with a multigeneration rat study using much higher doses reviewed by JECFA (2009). Barrios-Correa et al. (2018) found that repeat-dose intake of steviol glycosides, at a dose below the NOEL for rats on which the human ADI is based, downregulates feeding behaviour and total energy intake in mice, although not to an extent considered to be adverse. A number of other rodent studies lacked sufficient characterisation of the test article to be of value. Two studies of the possible therapeutic effects of steviol glycosides in diabetic rats, and one study of steviol glycosides in goats, were not considered to be relevant to risk assessment of the use of steviol glycosides as sweeteners by the general human population. Likewise, two studies in human volunteers were not considered to be informative because the test article was insufficiently characterised, and a study of possible beneficial effects in patients with chronic kidney disease did not identify any adverse effects.

Further to the assessment presented above, FSANZ is aware that a few research papers have reported on possible links between consumption of intense sweeteners and unwanted metabolic effects resulting in weight gain, but considers that the current weight of evidence does not support a causal relationship. FSANZ will continue to monitor the emerging scientific literature in this area.

Blue California is not requesting a change to the foods permitted to contain steviol glycosides as a food additive nor do they propose to increase the maximum permitted levels of steviol glycosides in foods. FSANZ has previously conducted a dietary exposure assessment using the current permissions to use steviol glycosides as a food additive and therefore no dietary exposure assessment was necessary for this application.

In conclusion, no new evidence of adverse effects of steviol glycosides has been identified that would justify a change in the ADI of 0 to 4 mg/kg bw, expressed as steviol, for steviol glycosides established by FSANZ in 2008 and 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 the rebaudioside E produced by enzymatic conversion that is the subject of this application.

# 4 References

Ahmad U, Ahmad RS, Arshad MS, Mushtaq Z, Hussain SM ad Hameed A (2018). Antihyperlipidemic efficacy of aqueous extract of *Stevia rebaudiana* Bertoni in albino rats. Lipids in Health and Disease 17: 175 <https://do.or/10.1186/s12944-018-0810-9>

Ahmad J, Khan I, Johnson SK, Alam I and ud Din, Z (2018). Effect of incorporating Stevia and Moringa in cookies on postprandial glycemia, appetite, palatability, and gastrointestinal well-being. Journal of the American College of Nutrition 37:133-139

Barrios-Correa AA, Estrada JA, Martel C, Olivier M, López-Santiago R and Contreras I (2018). Chronic Intake of Commercial Sweeteners Induces Changes in Feeding Behavior and Signaling Pathways Related to the Control of Appetite in BALB/c Mice. Biomedical Research International <https://doi.org/10.1155/2018/3628121>

Cho NA, Klancic T, Nettleton JE, Paul HA and Reimer RA (2018). Impact of food ingredients (aspartame, stevia, prebiotic oligofructose) on fertility and reproductive outcomes in obese rats. Obesity 26(110); 1692-1695

Chupeerach C, Yothakulsiri C, Chamchan R, Suttisansanee U, Sranacharoenpong K, Tungtrongchitr A and On-Nom N (2018). The effect of coconut jelly with Stevia as a natural sweetener on blood glucose, insulin and C-Peptide responses in twelve healthy subjects. Recent Patents on Food, Nutrition and Agriculture 9(2):127-133

El-Mesallamy AMD, Mahmoud SA, Elazab KM, Hussein SAM and Hussein AM (2018). Attenuation of metabolic dysfunctions in the skeletal muscles of Type 1 diabetic rats by Stevia rebaudiana extracts, via AMPK upregulation and antioxidant activities. Acta Scientiarum Polonorum Technologia Alimentaria 17: 289-297

FAO (2016). Steviol glycosides. In: *82nd JECFA - Chemical and Technical Assessment (CTA)* [82nd meeting held June 7-16, 2016]. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives Meeting (JECFA). Available at: <http://www.fao.org/3/a-br566e.pdf> .

FCC (2018). Enzyme preparations. In: Food Chemicals Codex, 11th edition. Rockville (MD): United States Pharmacopeial Convention, pp. 413-417.

FSANZ (2008) Application A540 [Steviol glycosides as Intense Sweeteners](http://www.foodstandards.gov.au/code/applications/Pages/applicationa540stevi3096.aspx). Food Standards Australia New Zealand, Canberra.

FSANZ (2011) Application A1037 [Steviol glycosides Increase in permitted levels](http://www.foodstandards.gov.au/code/applications/Pages/applicationa1037stev4605.aspx). Food Standards Australia New Zealand, Canberra.

FSANZ (2015) Application A1108 [Rebaudioside M as a steviol glycoside intense sweetener](http://www.foodstandards.gov.au/code/applications/Pages/A1108-RebaudiosideM-SteviolGlycosideIntenseSweetener.aspx). Food Standards Australia New Zealand, Canberra.

FSANZ (2017) Application A1132 [Broaden definition of Steviol Glycosides (Intense sweetener)](http://www.foodstandards.gov.au/code/applications/Pages/A1132Definition-of-Steviol-Glycosides.aspx). Food Standards Australia New Zealand, Canberra.

FSANZ (2018) Application A1157 [Enzymatic production of rebaudioside M](http://www.foodstandards.gov.au/code/applications/Pages/A1157–EnzymaticproductionofRebaudiosideM.aspx). Food Standards Australia New Zealand, Canberra.

FSANZ (2019a) Application A1172 [Enzymatic production of rebaudioside D](http://www.foodstandards.gov.au/code/applications/Pages/A1172EnzymaticproductionofRebaudiosideD.aspx). Food Standards Australia New Zealand, Canberra.

FSANZ 2019b) Application A1176 [Enzymatic production of steviol glycosides](http://www.foodstandards.gov.au/code/applications/Pages/A1176.aspx). Food Standards Australia New Zealand, Canberra.

Ghaheri M, Miraghaee S, Babaei A, Mohammadi B, Kahrizi D, Saivosh Haghighi ZM and Bahrami G (2018). Effect of *Stevia rebaudiana* Bertoni extract on sexual dysfunction in Streptozotocin-induced diabetic male rats. Cellular and Molecular Biology 64(2): 6-10

Gholizadeh F, Dastghaib S, Koohpeyma F, Bayat E and Mokarram P (2019) The protective effect of Stevia rebaudiana Bertoni on serum hormone levels, key steroidogenesis enzymes, and testicular damage in testes of diabetic rats. Acta Histochemica 121(7):833-840. doi: 10.1016/j.acthis.2019.08.001

Han X, Chen C, Zhang X, Wei Y, Tang S, Wang J, Tan Z and Xu L. Effects of dietary stevioside supplementation on feed intake, digestion, ruminal fermentation, and blood metabolites of goats. Animals 9, 32 doi:10:3990/ani9020032

JECFA (2006). General specifications and considerations for enzyme preparation used in food processing [prepared by the Committee at its sixty-seventh meeting (2006)]. In: *Combined Compendium of Food Additive Specifications [Online Edition]. General Specifications for Enzymes Analytical Methods, Volume 4: Analytical Methods, Test Procedures and Laboratory Solutions Used by and Referenced in the Food Specifications*. 1st to 67th JECFA Meetings, 1956-2006. (FAO JECFA Monographs 1). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA), xxi-xxv. Available at: <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/>. Accessed 13 September 2019

JECFA (2007). 3.1.9 Steviol glycosides. [*Evaluation of Certain Food Additives and Contaminants*](http://whqlibdoc.who.int/publications/2007/9789241209472_eng.pdf). Sixty eighth Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 19‐28, 2007, Geneva, Switz. (WHO Technical Report Series, no 947). Geneva, Switz.: World Health Organization (WHO), pp. 50‐54, 206, 215. Accessed 13 September 2019

JECFA (2009). 3.1.8 Steviol glycosides. Evaluation of Certain Food Additives. Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 17-26, 2008, Rome, Italy. (WHO Technical Report Series 952. WHO, p 52. Available at <http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID+272> Accessed October 2019

JECFA (2017). Steviol glycosides from Stevia *rebaudiana Bertoni* [New specifications prepared at the 84th JECFA, 2017), Superseding tentative specifications prepared at the 82nd JECFA (2016)]. In: *Compendium of Food Additive Specifications*. 84th Meeting, Rome, 6‐15 June 2017 (FAO JECFA Monographs 20). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) /Geneva, Switz.: World Health Organization (WHO), pp. 50‐69. Available at: <http://www.fao.org/documents/card/en/c/4b06cdda-3e70-4c80-b7e5-56034601836b/> Accessed 13 September 2019

Ngekure MXK, Jiang J, Enayatullah H, Ennab W, Mustafa S, Rodeni S, Wei Q and Shi F (2019). Sweet taste receptor agonists alter ovarian functions and ovarian cycles in aged mice. Reproductive Biology doi:10.1016/j.repbio.2019.07.007

Ramos-Tovar E, Flores-Beltrán RE, Galindo-Gómez S, Vera-Aguilar E, Diaz-Ruiz A, Montes S, Camacho J, Tsutumi V and Muriel P (2018). *Stevia rebaudiana* tea prevents experimental cirrhosis via regulation of NF-KB, Nrf-2, transforming growth factor beta, Smad7, and hepatic stellate cell activation. Phytotherapy Research 32: 2568-2576

Rizwan F, Rashid HU, Yesmine S, Monjur F and Chatterjee TK (2018). Preliminary analysis of the effect of Stevia (*Stevia rebaudiana*) in patients with chronic kidney disease (Stage I to stage III). Contemporary Clinical Trials Communications 12: 17-25

Rotimi SO, Rotimi OA, Adelani IB, Onuzulu C, Obi P and Okungbaye R (2018). Stevioside modulates oxidative damage in the liverand kidney of high fat/low streptozocin diabetic rats. Heliyon 4 e00640 doi: 10.1016/j.heliyon.2018.e00640

1. UGT is an abbreviation of UDP-glucosyltransferase [↑](#footnote-ref-2)
2. This information was provided by Blue California as an additional appendix, after the application was accepted by FSANZ. [↑](#footnote-ref-3)
3. An EC number for UDP-glucosyltransferase was incorrectly included for the draft variations prepared under applications A1157 and A1172. This will be corrected by FSANZ in a Code Maintenance Proposal. [↑](#footnote-ref-4)