

---

**Safety Assessment: Outcome of  
Assessment of the Modification  
of use of Steviol Glycosides (E  
960) Produced by *Yarrowia  
lipolytica***

**Reference number RP1140**

---

FOOD STANDARDS AGENCY (FSA) AND FOOD  
STANDARDS SCOTLAND (FSS)

# Regulated Product Dossier Assessment

**Assessment finalised: 20/06/2023**

## Summary

An application was submitted to the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in June 2021 from Avansya V.O.F. (Avansya) (“the Applicant”) for the changes of the existing production method of steviol glycosides (E 960) to amend the existing specifications for steviol glycosides to allow for inclusion of fungal fermentation of simple sugars (dextrose) by *Yarrowia lipolytica* as an alternative method to manufacture high purity steviol glycosides.

To support the FSA and FSS in evaluating the dossier the Joint Expert Group on Additives, Enzymes and other regulated products (AEJEG) were asked to review the dossier and the supplementary information from the Applicant. The AEJEG concluded that the new method for the production of steviol glycosides using *Yarrowia lipolytica* was safe under the proposed conditions of use. The proposed uses and use levels for steviol glycosides produced using fungal fermentation of simple sugars (dextrose) by *Yarrowia lipolytica* remain the same as the already authorised food additive steviol glycosides (E 960).

The views of the AEJEG have been taken into account in this safety assessment which represents the opinion of the FSA and FSS on the method for the production of steviol glycosides using *Yarrowia lipolytica*. The Committee on Toxicity (COT) also reviewed the AEJEG safety assessment agreeing with the conclusions of the AEJEG.

## 1. Introduction

The FSA and FSS have undertaken a partial risk assessment for the production of steviol glycosides using fungal fermentation of simple sugars (dextrose) by *Yarrowia lipolytica* under the common authorisation procedure for food additives, food enzymes and food flavourings legislation, retained EU Regulation No 1331/2008. To support the risk assessment by FSA and FSS, the AEJEG provided advice to the FSA and FSS outlined in this opinion.

The dossier was evaluated on behalf of the FSA and the FSS by the AEJEG. In line with Article 3 of retained EU regulation 1331/2008 (REUL 1331/2008), the assessment has considered the aspects of the food additive and its modification of use. This, and the guidance put in place by EFSA for food additive applications, has formed the basis and structure for the assessment (EFSA, 2012).

With thanks to the members of the AEJEG during the course of the assessment who were: Dr Allain Bueno, Dr Claude Lambré, Dr Martin Rose, Dr Olwenn Martin and Professor Qasim Chaudhry.

Following the review by the AEJEG at their meeting in June 2022, further information was requested with regard to differing specifications for steviol glycosides and in order to address information gaps in the dossier and complete the risk assessment.

This document outlines the conclusions of the AEJEG assessment on the safety of the new manufacturing process to produce high purity steviol glycosides.

## 2. Assessment

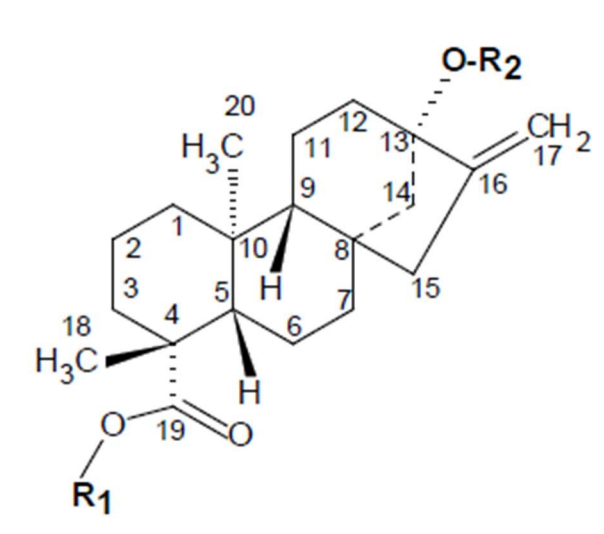
### 2.1 Identity and Characterisation of the additive

Current specifications for steviol glycosides, set out in retained EU Regulation No. 231/2012, stipulate that they are obtained from the leaves of the *Stevia rebaudiana* Bertoni plant and contain no less than 95 % of 11 named steviol glycosides. These include: stevioside; rebaudiosides A, B, C, D, E, F and M; steviolbioside; rubusoside; and dulcoside.

The Applicant proposed to amend the existing specifications for steviol glycosides to allow for inclusion of fungal fermentation of simple sugars (dextrose) by *Y. lipolytica* as an alternative method to manufacture high purity steviol glycosides. The purified mixture will mostly comprise rebaudioside M and may contain, in 'various concentrations,' the other glycosides naturally present in the leaves of the *S. rebaudiana* Bertoni plant (rebaudiosides A, B, C, D, E and F; stevioside; rubusoside; and or dulcoside A).

### 2.1.1 Identity of the Components

The Applicant summarised the Chemical Abstracts Service (CAS) Numbers, empirical formulae, molecular weights and R1 and R2 groups (Figure 1) for the individual steviol glycosides that may be present in the steviol glycoside mixture produced by *Y. lipolytica*, as well as the aglycone steviol, and explained that the distribution of the mixture components will vary depending on the production process and final product formulation.



**Figure 1.** Backbone structure for steviol glycosides

### 2.1.2 Description of Physical and Chemical Properties

The Applicant described steviol glycosides produced by *Y. lipolytica* as: 'a white to off-white powder with a characteristic sweet taste, consistent with the description of commercial steviol glycoside preparations in the most recent Chemical and Technical Assessment (CTA) published by the JECFA (FAO, 2016)'. They explained that they have the same backbone as steviol glycosides extracted from *S. rebaudiana* Bertoni leaf, and that the individual glycosides differ only in terms of the type and number of sugar moieties at positions R1 and R2. Therefore, the Applicant concluded that: 'the physiochemical properties of steviol glycosides produced by *Y. lipolytica* will be identical'.

### 2.1.3 Solubility

The Applicant stated that steviol glycosides produced by *Y. lipolytica* are 'slightly soluble in water,' commensurate with the current specification for steviol glycosides in retained EU Regulation No. 231/2012.

## 2.2 Manufacturing Process

### 2.2.1 Identity of Raw Materials, Processing Aids and Equipment

The Applicant stated that raw materials, processing aids and equipment used during manufacture of steviol glycosides with *Y. lipolytica* are of food-grade quality and comply with relevant Food Chemicals Codex (FCC) or other internationally recognised standards. The AEJEG had access to the full information on the raw materials, processing aids and equipment used in the manufacture of of steviol glycosides with *Y. lipolytica*.

### 2.2.2 Details of the Manufacturing Process

The Applicant has submitted detailed and concise descriptions and a schematic breakdown of steviol glycoside production using *Y. lipolytica*. A non-confidential summary has been included below:

Steviol glycosides produced by *Y. lipolytica* is a purified steviol glycoside mixture that is produced via fermentation of simple sugars using a *Y. lipolytica* production strain that has been engineered to produce steviol glycosides. Steviol glycosides produced by *Y. lipolytica* are manufactured in accordance with current Good Manufacturing Practice. The *Y. lipolytica* production strain is added to the fermentation medium and is allowed to produce steviol glycosides under aerobic conditions. The fermentation is stopped via heat treatment that kills the yeast cells, and the biomass is separated from the steviol glycosides by microfiltration. The steviol glycosides are purified in accordance with the methodologies outlined in the CTA published by Food and Agriculture Organization of the United Nations/JECFA for steviol glycosides (FAO, 2016) that includes the use of filtration aids, purification resins, and crystallisation. The final product is primarily comprised of rebaudioside M and may contain a mixture of the following additional glycosides in various concentrations, which are naturally present in the leaves of the *S. rebaudiana* Bertoni plant: rebaudiosides A, B, C, D, E, F, stevioside, steviolbioside, rubusoside, and/or dulcoside A, such that the total steviol glycoside content is no less than 95%.

The *Y. lipolytica* production organism is a species of non-pathogenic and non-toxicogenic yeast that contains no known pathogenicity-related genes, proteins, toxins, or allergens. The European Food Safety Authority (EFSA) has granted Qualified Presumption of Safety (QPS) status for *Y. lipolytica* and therefore has deemed it safe to derive genetically modified strain lineages to use in the production of food additives and enzymes with the qualification “QPS applies for production purposes only” (EFSA BIOHAZ Panel, 2020).

The AEJEG noted that the metabolic pathway in the modified *Y. lipolytica* strain was constructed from genes derived from different and unspecified sources. Although the Applicant had provided high-performance liquid chromatography (HPLC) data confirming similar retention times for rebaudiosides M and D from *S. rebaudiana* Bertoni and *Y. lipolytica*, The AEJEG further discussed whether the Applicant

provided sufficient information to establish whether steviol glycosides from both sources differed in terms of isomeric structure (which might result in differences in their toxicological profiles).

However, the AEJEG noted JECFA's (2020) conclusion in their Compendium of Food Additive Specifications, that:

'No safety issues [existed] for steviol glycosides produced by any one of these methods [including fermentation] resulting in products with  $\geq 95\%$  steviol glycosides as per existing specifications... The Committee recognized that steviol glycosides could be produced via a new method or the modification or combination of the methods currently described in the annexes of the specifications monograph. If the final product [met] the current specification of  $\geq 95\%$  steviol glycosides, the Committee [would] evaluate possible impurities from the method of manufacture' (JECFA, 2020, Annex 2).

Considering this, the AEJEG agreed that the genetic modification of *Y. lipolytica* for steviol glycoside production was likely to be of low safety concern and focused on evaluating application RP 1140 on the basis of impurities.

### 2.2.3 Presence of Impurities

As mentioned above, Steviol glycosides produced by *Y. lipolytica* are subject to several purification steps to produce a 'high purity' product 'containing no less than 95 % steviol glycosides'. The Applicant has submitted in support of this application, batch analysis data which 'demonstrate that the purity is consistent with the purity specification parameters established for steviol glycosides E 960, namely total ash <1 %, loss on drying <6 %, methanol <200 mg/kg, ethanol <5000 mg/kg, arsenic <1 mg/kg and lead <1 mg/kg'.

The AEJEG noted that the Applicant had used PCR testing of steviol glycosides produced by *Y. lipolytica* to confirm that no DNA from the production organism was detectable. It was also noted that the Applicant had performed batch testing for

different impurities, all of which were found to be within acceptable parameters, based on the current specifications for steviol glycosides laid down by retained EU Regulation No. 231/2012, as amended. However, the AEJEG had originally queried if the Applicant had carried out any testing for impurities other than those stipulated in Regulation No. 231/2012, as amended (such as kaurenoic acid). The Applicant confirmed that they had carried out testing using high-performance liquid chromatography (HPLC) on five non-consecutive lots of steviol glycosides produced by *Y. lipolytica*. The data presented indicated that kaurenoic acid level had not been detected in any samples, which the AEJEG deemed acceptable.

The AEJEG noted that the specifications require a loss on drying amount of 'not more than 6 %' but that the identity of the substances responsible for this loss had not been stated. The AEJEG noted that this loss may represent unspecified impurities (besides water) and requested that the Applicant clarified these points. Upon further information received by the Applicant, the AEJEG was satisfied with the Applicant's clarification that the substances accounting for the loss of up to 6 % detailed in the technical dossier was attributed to water only.

Members noted that in the proposed specifications for steviol glycosides produced by *Y. lipolytica*, the Applicant had stated a residual solvent level of 'not more than 200 mg/kg methanol.' This was higher than current maximum residue limit for methanol (when used as an extraction solvent) set in the 2013 England SI No.2210. The Applicant clarified that methanol is not used in the production of steviol glycosides and the methanol specification parameter and corresponding limit were included in the proposed specifications solely for alignment with the current specifications for steviol glycosides in the UK. Overall, the AEJEG was satisfied with the information provided with regards to the presence of impurities in the current product, however questioned the need for inclusion of methanol in the proposed specifications, due to the fact that the solvent is not utilised in the manufacturing of the current product.

### **2.3 Specification**



### 2.3.1 Existing Specifications for Steviol Glycosides

The Applicant has provided the current specifications for steviol glycosides in Retained EU Regulation No. 231/2012, which all commercial preparations must comply with (Table 1).

**Table 1.** Existing specifications for steviol glycosides in the United Kingdom

Parameter	Specification																																							
Definition	<p>The manufacturing process comprises two main phases: the first involving water extraction of the leaves of the <i>Stevia rebaudiana</i> Bertoni plant and preliminary purification of the extract by employing ion exchange chromatography to yield a steviol glycoside primary extract, and the second involving recrystallisation of the steviol glycosides from methanol or aqueous ethanol resulting in a final product containing not less than 95 % of the below identified 11 related steviol glycosides, in any combination and ratio.</p> <p>The additive may contain residues of ion-exchange resins used in the manufacturing process. Several other related steviol glycosides that may be generated as a result of the production process, but do not occur naturally in the <i>Stevia rebaudiana</i> plant have been identified in small amounts (0,10 to 0,37 % w/w).</p>																																							
Chemical name	<p>Steviolbioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid</p> <p>Ruboside: 13-β-D-glucopyranosyloxykaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Dulcoside A: 13-[(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Rebaudioside B: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid</p> <p>Rebaudioside C: 13-[(2-O-α-L-rhamnopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Rebaudioside D: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester</p> <p>Rebaudioside E: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester</p> <p>Rebaudioside F: 13-[(2-O-β-D-xylofuranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Rebaudioside M: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester</p>																																							
Molecular formula	<table border="1"> <thead> <tr> <th>Trivial name</th> <th>Formula</th> <th>Conversion factor</th> </tr> </thead> <tbody> <tr> <td>Steviol</td> <td>C<sub>20</sub> H<sub>30</sub> O<sub>3</sub></td> <td>1,00</td> </tr> <tr> <td>Steviolbioside</td> <td>C<sub>32</sub> H<sub>50</sub> O<sub>13</sub></td> <td>0,50</td> </tr> <tr> <td>Ruboside</td> <td>C<sub>32</sub> H<sub>50</sub> O<sub>13</sub></td> <td>0,50</td> </tr> <tr> <td>Dulcoside A</td> <td>C<sub>38</sub> H<sub>60</sub> O<sub>17</sub></td> <td>0,40</td> </tr> <tr> <td>Stevioside</td> <td>C<sub>38</sub> H<sub>60</sub> O<sub>18</sub></td> <td>0,40</td> </tr> <tr> <td>Rebaudioside A</td> <td>C<sub>44</sub> H<sub>70</sub> O<sub>23</sub></td> <td>0,33</td> </tr> <tr> <td>Rebaudioside B</td> <td>C<sub>38</sub> H<sub>60</sub> O<sub>18</sub></td> <td>0,40</td> </tr> <tr> <td>Rebaudioside C</td> <td>C<sub>44</sub> H<sub>70</sub> O<sub>22</sub></td> <td>0,34</td> </tr> <tr> <td>Rebaudioside D</td> <td>C<sub>50</sub> H<sub>80</sub> O<sub>28</sub></td> <td>0,29</td> </tr> <tr> <td>Rebaudioside E</td> <td>C<sub>44</sub> H<sub>70</sub> O<sub>23</sub></td> <td>0,33</td> </tr> <tr> <td>Rebaudioside F</td> <td>C<sub>43</sub> H<sub>68</sub> O<sub>22</sub></td> <td>0,34</td> </tr> <tr> <td>Rebaudioside M</td> <td>C<sub>56</sub> H<sub>90</sub> O<sub>33</sub></td> <td>0,25</td> </tr> </tbody> </table>	Trivial name	Formula	Conversion factor	Steviol	C <sub>20</sub> H <sub>30</sub> O <sub>3</sub>	1,00	Steviolbioside	C <sub>32</sub> H <sub>50</sub> O <sub>13</sub>	0,50	Ruboside	C <sub>32</sub> H <sub>50</sub> O <sub>13</sub>	0,50	Dulcoside A	C <sub>38</sub> H <sub>60</sub> O <sub>17</sub>	0,40	Stevioside	C <sub>38</sub> H <sub>60</sub> O <sub>18</sub>	0,40	Rebaudioside A	C <sub>44</sub> H <sub>70</sub> O <sub>23</sub>	0,33	Rebaudioside B	C <sub>38</sub> H <sub>60</sub> O <sub>18</sub>	0,40	Rebaudioside C	C <sub>44</sub> H <sub>70</sub> O <sub>22</sub>	0,34	Rebaudioside D	C <sub>50</sub> H <sub>80</sub> O <sub>28</sub>	0,29	Rebaudioside E	C <sub>44</sub> H <sub>70</sub> O <sub>23</sub>	0,33	Rebaudioside F	C <sub>43</sub> H <sub>68</sub> O <sub>22</sub>	0,34	Rebaudioside M	C <sub>56</sub> H <sub>90</sub> O <sub>33</sub>	0,25
Trivial name	Formula	Conversion factor																																						
Steviol	C <sub>20</sub> H <sub>30</sub> O <sub>3</sub>	1,00																																						
Steviolbioside	C <sub>32</sub> H <sub>50</sub> O <sub>13</sub>	0,50																																						
Ruboside	C <sub>32</sub> H <sub>50</sub> O <sub>13</sub>	0,50																																						
Dulcoside A	C <sub>38</sub> H <sub>60</sub> O <sub>17</sub>	0,40																																						
Stevioside	C <sub>38</sub> H <sub>60</sub> O <sub>18</sub>	0,40																																						
Rebaudioside A	C <sub>44</sub> H <sub>70</sub> O <sub>23</sub>	0,33																																						
Rebaudioside B	C <sub>38</sub> H <sub>60</sub> O <sub>18</sub>	0,40																																						
Rebaudioside C	C <sub>44</sub> H <sub>70</sub> O <sub>22</sub>	0,34																																						
Rebaudioside D	C <sub>50</sub> H <sub>80</sub> O <sub>28</sub>	0,29																																						
Rebaudioside E	C <sub>44</sub> H <sub>70</sub> O <sub>23</sub>	0,33																																						
Rebaudioside F	C <sub>43</sub> H <sub>68</sub> O <sub>22</sub>	0,34																																						
Rebaudioside M	C <sub>56</sub> H <sub>90</sub> O <sub>33</sub>	0,25																																						

**Table 1 cont.** Existing specifications for steviol glycosides in the United Kingdom

Parameter	Specification		
Molecular weight and CAS No.	Trivial name	CAS No.	Molecular weight (g/mol)
	Steviol		318,46
	Steviolbioside	41093-60-1	642,73
	Rubusoside	64849-39-4	642,73
	Dulcoside A	64432-06-0	788,87
	Stevioside	57817-89-7	804,88
	Rebaudioside A	58543-16-1	967,01
	Rebaudioside B	58543-17-2	804,88
	Rebaudioside C	63550-99-2	951,02
	Rebaudioside D	63279-13-0	1 129,15
	Rebaudioside E	63279-14-1	967,01
	Rebaudioside F	438045-89-7	936,99
	Rebaudioside M	1220616-44-3	1 291,30
Assay	Not less than 95 % steviolbioside, rubusoside, dulcoside A, stevioside, rebaudiosides A, B, C, D, E, F and M on the dried basis, in any combination and ratio.		
Description	White to light yellow powder, approximately between 200 and 350 times sweeter than sucrose (at 5 % sucrose equivalency).		
<b>Identification</b>			
Solubility	Freely soluble to slightly soluble in water		
pH	Between 4,5 and 7,0 (1 in 100 solution)		
<b>Purity</b>			
Total ash	Not more than 1 %		
Loss on drying	Not more than 6 % (105 °C, 2h)		
Residual solvents	Not more than 200 mg/kg methanol Not more than 5 000 mg/kg ethanol		
Arsenic	Not more than 1 mg/kg		
Lead	Not more than 1 mg/kg		

CAS = Chemical Abstracts Service

### 2.3.2 Proposed Specifications for Steviol Glycosides Produced by *Yarrowia lipolytica*

The Applicant has submitted proposed specifications for steviol glycosides produced by *Y. lipolytica* (Table 2). The Applicant highlighted that they are high-purity glycosides produced by fermentation of simple sugars by *Y. lipolytica* that meet the same assay requirements as UK steviol glycosides (E960) of ‘not less than 95 % steviolbioside, rubusoside, dulcoside A, stevioside, rebaudiosides A, B, C, D, E, F, and M, on the dried basis, in any combination and ratio’. The Applicant also stated that they also meet the description, identification (solubility and pH), and purity (ash, loss on drying, residual methanol and ethanol, arsenic and lead) stipulations.

Although purified in the same manner as steviol glycosides E 960, the Applicant noted that those produced by *Y. lipolytica* are not extracted from the leaves of the *S. rebaudiana* Bertoni plant, meaning they do not meet the definition in the current specification. The Applicant therefore proposed to extend the definition in the current specification.

**Table 2.** Proposed specifications for E 960 steviol glycosides in the United Kingdom

Parameter	Specification
Definition	<p><b>Extraction:</b> The manufacturing process comprises two main phases: the first involving water extraction of the leaves of the <i>Stevia rebaudiana</i> Bertoni plant and preliminary purification of the extract by employing ion exchange chromatography to yield a steviol glycoside primary extract, and the second involving recrystallisation of the steviol glycosides from methanol or aqueous ethanol resulting in a final product containing not less than 95 % of the below identified 11 related steviol glycosides, in any combination and ratio. The additive may contain residues of ion-exchange resins used in the manufacturing process. Several other related steviol glycosides that may be generated as a result of the production process, but do not occur naturally in the <i>Stevia rebaudiana</i> plant have been identified in small amounts (0,10 to 0,37 % w/w).</p> <p><b>Fermentation:</b> The manufacturing process comprises two main phases: the first involving fermentation of a non-toxicogenic non-pathogenic strain of <i>Yarrowia lipolytica</i> (VRM) that has been genetically modified with heterologous genes to overexpress steviol glycosides, followed by heat treatment and removal of the biomass by solid-liquid separation; and the second involving purification by employing ion exchange chromatography and then recrystallisation of the steviol glycosides from methanol or aqueous ethanol resulting in a final product containing not less than 95 % of the below identified 11 related steviol glycosides, in any combination and ratio.</p>
Chemical name	<p>Steviolbioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid</p> <p>Rubusoside: 13-β-D-glucopyranosyloxykaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Dulcoside A: 13-[(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Rebaudioside B: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid</p> <p>Rebaudioside C: 13-[(2-O-α-L-rhamnopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester</p> <p>Rebaudioside D: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester</p> <p>Rebaudioside E: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester</p>

**Table 2 cont.** Proposed specifications for E 960 steviol glycosides in the United Kingdom

Parameter	Specification		
	Rebaudioside F: 13[[2-O-β-D-xylofuranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester		
	Rebaudioside M: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester		
Molecular formula	Trivial name	Formula	Conversion factor
	Steviol	C <sub>20</sub> H <sub>30</sub> O <sub>3</sub>	1,00
	Steviolbioside	C <sub>32</sub> H <sub>50</sub> O <sub>13</sub>	0,50
	Rubusoside	C <sub>32</sub> H <sub>50</sub> O <sub>13</sub>	0,50
	Dulcoside A	C <sub>38</sub> H <sub>60</sub> O <sub>17</sub>	0,40
	Stevioside	C <sub>38</sub> H <sub>60</sub> O <sub>18</sub>	0,40
	Rebaudioside A	C <sub>44</sub> H <sub>70</sub> O <sub>23</sub>	0,33
	Rebaudioside B	C <sub>38</sub> H <sub>60</sub> O <sub>18</sub>	0,40
	Rebaudioside C	C <sub>44</sub> H <sub>70</sub> O <sub>22</sub>	0,34
	Rebaudioside D	C <sub>50</sub> H <sub>80</sub> O <sub>28</sub>	0,29
	Rebaudioside E	C <sub>44</sub> H <sub>70</sub> O <sub>23</sub>	0,33
	Rebaudioside F	C <sub>43</sub> H <sub>68</sub> O <sub>22</sub>	0,34
	Rebaudioside M	C <sub>56</sub> H <sub>90</sub> O <sub>33</sub>	0,25
Molecular weight and CAS No.	Trivial name	CAS No.	Molecular weight (g/mol)
	Steviol		318,46
	Steviolbioside	41093-60-1	642,73
	Rubusoside	64849-39-4	642,73
	Dulcoside A	64432-06-0	788,87
	Stevioside	57817-89-7	804,88
	Rebaudioside A	58543-16-1	967,01
	Rebaudioside B	58543-17-2	804,88
	Rebaudioside C	63550-99-2	951,02
	Rebaudioside D	63279-13-0	1 129,15
	Rebaudioside E	63279-14-1	967,01
	Rebaudioside F	438045-89-7	936,99
	Rebaudioside M	1220616-44-3	1 291,30
Assay	Not less than 95 % steviolbioside, rubusoside, dulcoside A, stevioside, rebaudiosides A, B, C, D, E, F and M on the dried basis, in any combination and ratio.		
Description	White to light yellow powder, approximately between 200 and 350 times sweeter than sucrose (at 5 % sucrose equivalency).		
Identification			
Solubility	Freely soluble to slightly soluble in water		
pH	Between 4,5 and 7,0 (1 in 100 solution)		
Purity			
Total ash	Not more than 1 %		
Loss on drying	Not more than 6 % (105 °C, 2h)		
Residual solvents	Not more than 200 mg/kg methanol Not more than 5 000 mg/kg ethanol		
Arsenic	Not more than 1 mg/kg		
Lead	Not more than 1 mg/kg		

CAS = Chemical Abstracts Service

## 2.4 Analytical Data for Steviol Glycosides Produced by *Yarrowia lipolytica*

### 2.4.1 Composition

The steviol glycoside distribution of 5 non-consecutive commercial lots of steviol glycosides produced by *Y. lipolytica* was determined using a similar HPLC method to the method described in the JECFA specifications for “Steviol Glycosides from *Stevia rebaudiana* Bertoni” (JECFA, 2017a).

The results of the analyses are presented in Table 3. The applicant demonstrated that the mixture consists mainly of rebaudiosides M (85.4-96.3 %) and D (3.1-10.7 %), with traces of rebaudiosides A (0.86-0.92 %) and B (0.14-0.68 %), and a total steviol glycoside content of 97.2-100.2 %. The AEJEG was satisfied with the information provided regarding the composition of the product.

**Table 3.** Steviol Glycoside Composition of Steviol Glycosides Produced by *Yarrowia lipolytica*. Numbers indicate manufacturing lot number.

<b>Steviol Glycosides (% dry weight)</b>	<b>200124-B1</b>	<b>200221-B1</b>	<b>200226-B1</b>	<b>200302-B1</b>	<b>200306-B1</b>
Rebaudioside A	0.589	0.447	0.428	0.640	0.650
Rebaudioside B	0.683	0.421	0.142	0.671	0.504
Rebaudioside D	9.440	3.103	3.404	10.744	8.752
Rebaudioside M	86.519	96.262	95.690	85.436	89.530
<b>Total steviol glycosides</b>	<b>97.231</b>	<b>100.232</b>	<b>99.664</b>	<b>97.491</b>	<b>99.435</b>

### 2.5 Stability of the Substance and Reaction and Fate in Foods

The Applicant cites several published studies (Chang and Cook, 1983; Kroyer et al. 1999) and regulatory agencies which have considered the stability of steviol glycosides.

In particular, the Applicant cites the conclusions drawn by the JECFA (2007a), which considered that based on the results of studies submitted for review, steviol glycosides were thermally and hydrolytically stable for use in foods and acidic beverages under normal storage and processing conditions. Those with purities of 90-94 % were considered stable for at least 180 days when stored at 24°C in acidic solutions (pH 2-4). However, their stability appeared to be pH and temperature-dependent, as increasing the temperature from 80-100°C caused the rate of steviol glycoside decomposition to accelerate over eight hours from 4-10 % at pH 4 and from 8-40 % at pH 3.

#### 2.5.1 Stability of Steviol Glycosides Produced by *Y. lipolytica*

Stability trials have been conducted by the Applicant on 3 non-consecutive lots of steviol glycosides produced by *Y. lipolytica*.

The first trial was a conventional shelf-life stability study over 3-6 months at 25°C and 60 % relative humidity. The second was an accelerated shelf-life stability study over 1-9 months at 40°C and 75 % relative humidity. Both looked at steviol glycoside content and loss on drying, based on analysis of duplicate samples. The Applicant has noted that continued time point testing in each case is still underway.

It was concluded that the data from both studies: 'support the stability of steviol glycosides produced by *Y. lipolytica* under conventional shelf-life stability conditions for up to 9 months.'

## 2.6 Proposed Use and Anticipated Intake

The Applicant stated that: 'the intent of the current application is to modify the existing specifications in the UK for steviol glycosides (E 960) to include fermentation as an acceptable method of manufacture...proposed uses for steviol glycosides produced by *Y. lipolytica*...will adhere to the existing use levels for steviol glycosides (E 960)'.

According to the Applicant: 'the expected intakes [of steviol glycosides produced by *Y. lipolytica*] would be similar to the intakes from other steviol glycosides that are currently on the market in the UK...Based on the foregoing, a separate intake assessment for steviol glycosides produced by *Y. lipolytica* was not performed for the purposes of this amendment application'.

Following consideration of the provided information, the AEJEG concluded that there would be no significant change in exposure to steviosides as a result of this application, due to the fact that the steviosides produced using the process in the current application will be subject to the existing conditions of use for steviol glycosides.

The information provided was sufficient and does not raise any further areas for evaluation.

## **2.7 Biological and Toxicological Data**

The Applicant noted that the safety of steviol glycosides has been extensively evaluated and is supported by conclusions from several scientific bodies and regulatory agencies, including the European Commission's SCF (1985, 1999), EFSA (2010, 2012, 2015, 2018, 2019, 2020), JECFA (1999, 2006, 2007, 2009, 2010, 2016, 2017a, 2017b, 2019, 2020), FSANZ (2008, 2015, 2017, 2020a, 202b), Health Canada (2012a, 2012b, 2016, 2017, 2019, 2020a, 2020b) and the US FDA (2000, 2008, 2016a, 2016b, 2018, 2020a, 2020b)'. The Applicant noted that while much of the data examining the safety of steviol glycosides were conducted on steviosides or high purity rebaudioside A, 'several studies in the public domain conducted with stevia extracts have demonstrated the shared metabolic fate of all steviol glycosides.

It was highlighted that in its safety review following the proposal to amend the steviol glycoside (E 960) specifications to include all 60 steviol glycosides, the EFSA FAF Panel (2020) used additional unpublished data submitted to predict that each would share the same metabolic fate. Therefore, the Panel considered previous read-across data sufficient to assess the safety of all 60 steviol glycosides. EFSA concluded that based on the data submitted, there was no change to the previous



safety-related conclusions drawn and that the proposed amendment would not pose a safety concern, provided that the assay value was not less than 95 % steviol glycosides. However, the Applicant acknowledged that: 'the Panel recognised that the mixtures tested in the previous genotoxicity studies may not have sufficiently represented mixtures from the proposed new specifications.

The Applicant added that: 'since steviol glycosides produced by a *Y. lipolytica* production strain are identical and therefore chemically equivalent to those extracted from *S. rebaudiana* Bertoni, the extensive safety database that exists for steviol glycosides extracted from the plant may be applied to establish the safety of steviol glycosides produced by *Y. lipolytica*'. The Applicant has also provided details of new studies on the safety of steviol glycosides which has emerged since the 2015 and 2020 EFSA opinions in support of the application.

Considering the studies presented in the following sections, the AEJEG agreed that the Applicant had provided sufficient justification with regards to the use of read-across to support the safety of steviol glycosides produced via the proposed manufacturing method.

#### 2.7.1 Microbial Degradation of Steviol Glycosides

The Applicant has submitted a summary of a number of *in vivo*, *ex vivo* and *in vitro* as well as their own studies conducted in support of this application. In their own study, human faecal homogenates pooled from 2 healthy male and 2 healthy female volunteers were mixed with steviol glycosides produced by *Y. lipolytica* to a concentration of 0.2 mg/ml and incubated anaerobically for 4-48 hours at  $37 \pm 5^{\circ}\text{C}$  in triplicate. Rebaudioside A from *S. rebaudiana* Bertoni and steviol were also incubated under the same conditions and used as a chemical comparison and stability control, respectively. Liquid Chromatography - Mass Spectrometry (LC-MS) was used to measure steviol formation in each sample.

According to the Applicant, 'near complete deglycosylation of steviol glycosides produced by *Y. lipolytica* occurred within an incubation period of 24 to 48 hours,' with steviol glycosides from *S. rebaudiana* Bertoni having a 'similar rate and overall

degree of hydrolysis'. They concluded: 'these data demonstrate that steviol glycosides produced by *Y. lipolytica*, comprised of primarily rebaudiosides M and D, in the presence of human faecal homogenates is metabolised nearly completely to steviol within 48 hours and confirms that these glycosides share the same metabolic fate as steviol glycosides, such as rebaudioside A, from *S. rebaudiana* Bertoni'.

#### 2.7.2 Absorption, Distribution, Metabolism and Elimination

Following hydrolysis to steviol, the applicant explained that the aglycone is systemically absorbed via the portal vein and distributed to various organs and tissues, including the liver (where further metabolism occurs), spleen, adrenal glands, fat and blood. Several *in vivo* studies in rats are cited to support this, including Nakayama et al. (1986), Sung (2002), and Koyoma et al. (2003a), Wang et al. (2004), and a review by Roberts and Renwick (2008).

Following colonic absorption, the Applicant described that steviol is mainly conjugated with glucuronic acid to steviol glucuronide in the liver, citing Roberts and Renwick (2008) and an *in vivo* rat study by Nikiforov et al. (2013) in support of this. Several human studies detecting peak plasma concentrations of steviol glucuronide after 8 and 12 hours of ingestion for stevioside and rebaudioside A, respectively, were also cited to support this (Geuns and Pietta, 2004; Simonetti et al., 2004; Geuns et al., 2007; Wheeler et al., 2008).

The Applicant continues to describe that in rats, free and conjugated steviol, in addition to unhydrolysed glycosides, are excreted primarily via the faeces based on *in vivo* studies (usually within 48 hours), with smaller amounts of free and conjugated steviol appearing in urine (<3 %) (Wingard et al., 1980; Nakayama et al., 1986; Sung, 2002; Roberts and Renwick et al., 2008). Conversely, in humans, *in vivo* studies suggest that elimination of steviol glycosides primarily occurs via the urine as steviol glucuronide (along with small amounts of the unchanged glycoside or steviol) (Kraemer and Maurer, 1994; Geuns and Pietta, 2004; Simonetti et al., 2004; Geuns et al., 2006; Geuns et al., 2007; Wheeler et al., 2008). It is mentioned that several of these studies also showed that relatively larger amounts of steviol, including unabsorbed steviol released back from steviol glycosides in the colon or from small

amounts of steviol glucuronide secreted back into the gut via the bile, were eliminated in human faeces compared with urine (Geuns and Pietta, 2004; Simonetti et al., 2004; Geuns et al., 2007; Wheeler et al., 2008).

The Applicant attributes the difference in the elimination route of systematically absorbed steviol as steviol glucuronide between rats and humans (via bile and in the urine respectively) to the lower molecular weight threshold for biliary excretion in rats compared with humans, based on threshold values from Renwick (2008). However, they commented that: 'the difference is considered to be of no toxicological significance, due to the fact that the water-soluble phase II metabolites are rapidly cleared in both species.'

The Applicant concluded that: 'collectively the degradation and pharmacokinetic studies on steviol glycosides confirm [that]...steviol glycosides are rapidly hydrolysed to steviol, steviol is absorbed and conjugated with glucuronic acid, and steviol glucuronide is excreted primarily via the urine in humans. Steviol glycosides, whether produced by fermentation or extracted from the *S. rebaudiana* Bertoni plant, share this same metabolic fate...Considering the common pathway of metabolism, and the fact that systemically, exposure only occurs to steviol following consumption of steviol glycosides, the safety data and conclusions drawn for individual steviol glycosides from *S. rebaudiana* Bertoni, therefore, can be extended to include all steviol glycosides, including those derived from fermentation of yeast, such as steviol glycosides produced by *Y. lipolytica*'.

The AEJEG was satisfied that the information provided supported the conclusions of the Applicant.

## **2.8 Further Toxicological Information**

### **2.8.1 Subchronic Toxicity**

The Applicant has cited a number of *in vivo* studies in support of this application. These studies investigated the possible effects of rebaudioside A on the gut

microbiota in rats. (Nettleton et al., 2019); The effects of steviol glycosides on the functionality of adipocytes in conjunction with a normal or high-fat diet (Sánchez-Tapia et al., 2019); the impact of exposure to several sweeteners, including rebaudioside A, in mice for effects on endothelial progenitor cells, inflammation, and behaviour (Schiano et al., 2019) and a 90-day oral toxicity study (Rumelhard et al., 2016) to evaluate the safety of rebaudioside A (>95% purity) produced via fermentation, using a *Y. lipolytica* strain genetically engineered to express the *S. rebaudiana* metabolic pathway.

The Applicant concluded that the studies corroborate the safety of steviol glycosides and also noted that the study by Rumelhard et al., (2016) also confirmed that 'steviol glycosides produced by *Y. lipolytica* are no different in their safety profile than steviol glycosides extracted from *S. rebaudiana* Bertoni.

#### 2.8.2 Genotoxicity

The Applicant has cited a number of *in vitro* and *in vivo* studies in support of this application. These studies included an *in vitro* assessment of the effect of steviol on human lymphocytes obtained and cultured from a single healthy individual (Pasqualli et al., 2020), single dose pharmacokinetics in rats (Roberts et al., 2016) and the potential oxidative and genotoxic capabilities of steviol glycosides in mice (Yilmaz et al., 2020).

Rumelhard et al., (2016) reported that rebaudioside A (>95 % purity) produced via fermentation of a yeast strain (*Y. lipolytica*) genetically engineered to express the *S. rebaudiana* metabolic pathway was not mutagenic in the Ames reverse mutation assay when tested at concentrations of up to 5000 µg/plate in the presence or absence of metabolic activation. Additionally, fermentative rebaudioside A was not cytotoxic and did not induce micronuclei formation in cultured peripheral human lymphocytes when incubated for up to 3 hours with or without metabolic activation, or up to 24 hours without metabolic activation, at concentrations of up to 5000 µg/plate as part of an *in vitro* micronucleus assay.

The Applicant concluded that: 'these findings corroborate the previous conclusions by JECFA (2010) that steviol glycosides are not genotoxic'.

### 2.8.3 Chronic Toxicity and Carcinogenicity

The Applicant has not submitted any new information in support of this application and noted that the chronic toxicity and carcinogenicity of steviol glycosides was previously addressed in the safety evaluations by scientific bodies and regulatory and stated that 'no new data were identified in relation to this endpoint'.

### 2.8.4 Reproductive and Developmental Toxicity

The Applicant has submitted *in vivo* studies in support of this application. These studies covered the effects of stevia extract on testicular steroidogenesis, spermatogenesis, stereological characteristics, and reproductive function in diabetic rats. (Gholizadeh et al., 2019) and the effect of rebaudioside A on the expression of guinea pig uterine taste receptors, (Li et al., 2020).

### 2.8.5 Human studies

The Applicant has submitted a number of studies in support of this application. These studies included a double-blind, randomised, controlled clinical study, which investigated the effects of repeat-consumption of a snack containing stevia on the development of dental caries in children (Cocco et al., 2019); a parallel-arm randomised control trial, investigating the effects of low-calorie sweeteners on body weight, ingestive behaviour, and glucose tolerance in overweight or obese adults (Higgins and Mattes, 2019); a 3-arm, single-blinded randomised crossover trial, investigating the effect of stevia consumption on glucose levels, food consumption, and appetite compared to water and sugar (Farhat et al., 2019); a parallel-arm, double-blind, randomised, controlled trial on the glycaemic and lipid profile in adults with type 2 diabetes in response to daily consumption of stevia extract or sucralose in tea (Ajami et al., 2020) and a randomised, parallel, controlled, open-label study, evaluating the effect of daily stevia consumption on glycemia (Stamataki et al., 2020).

Overall, The AEJEG considered that the toxicological information presented from the literature was sufficient to evaluate the safety of steviol glycosides produced by fungal fermentation of simple sugars (dextrose) by *Y. lipolytica*.

## **2.9 Immunotoxicity, Hypersensitivity/Allergy and Food Intolerance**

The Applicant has submitted studies on the effects of sweetener consumption on nutrient and caloric intake, adipose mass, triglycerides, and cytokine concentrations in healthy male and female adults (Sánchez-Delgado et al., 2019) and a randomised, controlled study investigating the effects of stevia consumption for 6 or 12 weeks on glycaemia, cytokines, hormones, and gut-associated lymphoid tissue (GALT) in CD1 mice, (Rosales-Gómez et al. 2018) in support of this application.

The Applicant concludes that there are no safety concerns regarding allergenicity associated with exposure to steviol glycosides produced by fungal fermentation of simple sugars (dextrose) by *Y. lipolytica*.

The AEJEG was satisfied that the information provided supported the conclusions of the Applicant.

## **3. Discussion**

The Applicant concluded that:

‘Steviol glycosides, whether produced by fermentation or extracted from the *S. rebaudiana* plant, are metabolised and biologically handled in an identical manner following oral administration. This was demonstrated for steviol glycosides produced by *Y. lipolytica* based on the results of the *in vitro* microbial metabolism study... Because of this shared metabolic fate, the extensive safety database that exists for steviol glycosides extracted from *S. rebaudiana* Bertoni may be applied using a read across approach to establish the safety of highly purified steviol glycosides produced

by *Y. lipolytica*. Specifically, the safety of steviol glycosides has been extensively evaluated and is supported by conclusions from several scientific bodies and regulatory agencies, including the European Commission's SCF, EFSA, JECFA, FSANZ, Health Canada, and the U.S. FDA and an ADI of 4 mg/kg body weight for steviol glycosides, expressed as steviol equivalents has been established (FSANZ, 2008; EFSA ANS Panel, 2010; Health Canada, 2012a; JECFA, 2016). Furthermore, no toxicologically significant results were reported from recently published toxicity studies, covering endpoints including metabolism, pharmacokinetics, genotoxicity, subchronic toxicity, reproductive/developmental toxicity, allergenicity, and human studies, which confirms the safety of steviol glycosides as food ingredients.'

The AEJEG considered the application for a proposed change in the steviol glycoside specification to include a production method using fermentation of sugars (dextrose) by *Yarrowia lipolytica* as an alternative method to manufacture high purity steviol glycosides.

Following clarifications from the applicant, the AEJEG concluded that sufficient information had been provided to allow for an evaluation of the proposal for modification of the manufacturing specifications of steviol glycosides (E 960) to include the fermentation process and agreed that the ADI of 4 mg/kg body weight for steviol glycosides, expressed as steviol equivalents has been established (FSANZ, 2008; EFSA, 2010; Health Canada, 2012a; JECFA, 2016) and would be applicable to steviol glycosides produced with the proposed manufacturing method.

Overall, the AEJEG have agreed that the application for the modification of the manufacturing specifications of steviol glycosides (E 960) to include fermentation of sugars (dextrose) by *Yarrowia lipolytica* as an alternative method to manufacture high purity steviol glycosides would not pose a risk to health. However, they questioned the proposal for inclusion of methanol in the proposed specifications, due to the fact that the solvent is not utilised in the manufacturing of the current product.

## 4. Conclusions

The FSA and FSS agreed on the assessment undertaken by the AEJEG for the changes of the existing production method of steviol glycosides (E 960) to amend the existing specifications for steviol glycosides and allow inclusion of fungal fermentation of simple sugars (dextrose) by *Y. lipolytica* as an alternative method to manufacture high purity steviol glycosides, as requested by the Applicant.

The FSA and FFS therefore conclude that the modification of the manufacturing specifications would not pose a risk to health as described within this application, noting the AEJEG had questioned the presence of methanol within the specification, where methanol was not used within the production process.

These conclusions were based on the information in the food additive dossier plus the supplementary information and could not have been reached without the data claimed as proprietary by the Applicant.



## 5. References

Ajami M, Seyfi M, Abdollah Pouri Hosseini F, Naseri P, Velayati A, Mahmoudnia F, Zahedirad M and Hajifaraji M, 2020. Effects of stevia on glycemic and lipid profile of type 2 diabetic patients: a randomized controlled trial. *Avicenna Journal of Phytomedicine*, 10, 118-127. [Effects of stevia on glycemic and lipid profile of type 2 diabetic patients: A randomized controlled trial - PubMed \(nih.gov\)](#)

Cargill, 2019. GRAS Exemption Claim for Steviol Glycosides Produced by *Yarrowia lipolytica*. Submitted by Cargill Incorporated, Wayzata, MN to U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety, College Park, MD. GRN No. 882. Available online: [GRAS Notices \(fda.gov\)](#) (Feb. 2, 2020 - FDA response - no questions).

Chang SS and Cook JM, 1983. Stability studies of stevioside and rebaudioside A in carbonated beverages. *Journal of Agricultural and Food Chemistry*, 31, 409-412. [Stability studies of stevioside and rebaudioside A in carbonated beverages | Journal of Agricultural and Food Chemistry \(acs.org\)](#)

Cocco F, Cagetti MG, Livesu R, Camoni N, Pinna R, Lingstrom P and Campus G, 2019. Effect of a daily dose of snacks containing maltitol or Stevia rebaudiana as sweeteners in high caries risk schoolchildren. A double-blind RCT study. *Oral Health & Preventive Dentistry*, 17, 515-522. [Effect of a Daily Dose of Snacks Containing Maltitol or Stevia rebaudiana as Sweeteners in High Caries Risk Schoolchildren. A Double-blind RCT Study - PubMed \(nih.gov\)](#)

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food) (2010). Scientific opinion on safety of steviol glycosides for the proposed uses as a food additive. *EFSA Journal*, 78, 1537 [85 pp]. DOI:10.2903/j.efsa.2010.1537. [Safety of steviol glycosides as a food additive | EFSA \(europa.eu\)](#)

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to food), 2012. Guidance for submission for food additive evaluations. *EFSA Journal*,

10, 2760. [60 pp]. DOI:10.2903/j.efsa.2012.2760. [Guidance for submission for food additive evaluations | EFSA \(europa.eu\)](#)

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to food), (2015). Scientific opinion on the safety of the proposed amendment of the specifications for steviol glycosides (E 960) as a food additive. EFSA Journal, 13, 4316 [29 pp]. DOI:10.2903/j.efsa.2015.4316. [Scientific opinion on the safety of the proposed amendment of the specifications for steviol glycosides \(E 960\) as a food additive | EFSA \(europa.eu\)](#)

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2018. Scientific opinion on the safety in use of glucosylated steviol glycosides as a food additive in different food categories (Question no EFSA-Q-2017-00247, adopted: 21 February 201). EFSA Journal, 16, 5181 [11pp]. DOI:10.2903/j.efsa.2018.5181. [Safety in use of glucosylated steviol glycosides as a food additive in different food categories | EFSA \(europa.eu\)](#)

EFSA FAF Panel (Panel on Food Additives and Flavourings), 2019. Scientific Opinion on the safety of the proposed amendment of the specifications for steviol glycosides (E 960) as a food additive: Rebaudioside M produced via enzyme-catalysed bioconversion of purified stevia leaf extract. EFSA Journal, 17, 5867 [19pp.]. DOI:10.2903/j.efsa.2019.5867. [Safety of the proposed amendment of the specifications for steviol glycosides \(E 960\) as a food additive: Rebaudioside M produced via enzyme-catalysed bioconversion of purified stevia leaf extract | EFSA \(europa.eu\)](#)

EFSA FAF Panel (Panel on Food Additives and Flavourings), 2020. Scientific Opinion on the safety of a proposed amendment of the specifications for steviol glycosides (E 960) as a food additive: to expand the list of / steviol glycosides to all those identified in the leaves of Stevia Rebaudiana Bertoni. EFSA Journal, 18, 6106 [32pp.]. DOI:10.2903/j.efsa.2020.6106. [Safety of a proposed amendment of the specifications for steviol glycosides \(E 960\) as a food additive: to expand the list of steviol glycosides to all those identified in the leaves of Stevia Rebaudiana Bertoni | EFSA \(europa.eu\)](#)

FAO (Food and Agriculture Organization of the United Nations), 2016. Steviol glycosides. In: 82nd JECFA - Chemical and Technical Assessment (CTA) [82nd meeting held June 7-16, 2016]. Joint FAO/WHO Expert Committee on Food Additives Meeting (JECFA), Geneva. [STEVIOLE GLYCOSIDES 82nd JECFA - Chemical and Technical Assessment \(CTA\), 2016 \(fao.org\)](#)

Farhat G, Berset V and Moore L, 2019. Effects of stevia extract on postprandial glucose response, satiety and energy intake: a three-arm crossover trial. *Nutrients*, 11, 3036 [8pp]. DOI:10.3390/nu11123036. [Effects of Stevia Extract on Postprandial Glucose Response, Satiety and Energy Intake: A Three-Arm Crossover Trial - PubMed \(nih.gov\)](#)

FSANZ (Food Standards Australia New Zealand), 2008. Final assessment report: application A540 steviol glycosides as intense sweeteners. [Microsoft Word - FAR A540 Steviol glycosides.doc \(foodstandards.gov.au\)](#)

FSANZ (Food Standards Australia New Zealand), 2015. A1108 – Rebaudioside M as a Steviol Glycoside Intense Sweetener. (Application to Change Food Standards Code). [A1108 – Rebaudioside M as a Steviol Glycoside Intense Sweetener \(foodstandards.gov.au\)](#)

FSANZ (Foods Standards Australia New Zealand), 2017. A1132 – Broaden definition of steviol glycosides (intense sweetener) [approval report]. 20 February 2017. [A1132 – Broaden Definition of Steviol Glycosides \(Intense Sweetener\) \(foodstandards.gov.au\)](#)

FSANZ (Foods Standards Australia New Zealand), 2020a. Schedule 3: Identity and purity (F2020C00234). In: Australia New Zealand food standards code. [Australia New Zealand Food Standards Code – Schedule 3 – Identity and purity \(legislation.gov.au\)](#)

FSANZ (Foods Standards Australia New Zealand), 2020b. Schedule 15: Substances that may be used as food additives (F2020203). In: Australia New Zealand food

standards code. [Australia New Zealand Food Standards Code – Schedule 15 – Substances that may be used as food additives \(legislation.gov.au\)](#)

Geuns JMC, 2003. Stevioside. *Phytochemistry*, 64, 913-921. [Stevioside - PubMed \(nih.gov\)](#)

Geuns JMC, 2008. Analysis of steviol glycosides: validation of the methods. In: *Steviol glycosides: technical and pharmacological aspects: proceedings of the 2nd stevia symposium*. Euprint ed., Heverlee, Belgium, 59-78. [\(PDF\) Analysis of steviol glycosides: development of an internal standard and validation of the methods | Jan Geuns - Academia.edu](#)

Geuns JMC and Pietta, P, 2004. Stevioside metabolism by human volunteers. Report from Laboratory Functional Biology, Kuleuven, Leuven Belgium and ITB-CNR, from Segrate (MI), Italy. Submitted to WHO by Federal Ministry of Social Affairs, Public Health and the Environment, Belgium. (cited in: JECFA, 2006).(Unpublished)

Geuns JM, Augustijns P, Mols R, Buyse JG and Driessen B, 2003. Metabolism of stevioside in pigs and intestinal absorption characteristics of stevioside, rebaudioside A and steviol. *Food and Chemical Toxicology*, 41, 1599-1607. [Metabolism of stevioside in pigs and intestinal absorption characteristics of stevioside, rebaudioside A and steviol - ScienceDirect](#)

Geuns JMC, Buyse J, Vankeirsbilck A, Temme EHM, Compennolle F and Toppet S, 2006. Identification of steviol glucuronide in human urine. *Journal of Agricultural and Food Chemistry*, 54, 2794-2798. [Identification of Steviol Glucuronide in Human Urine | Journal of Agricultural and Food Chemistry \(acs.org\)](#)

Geuns JMC, Buyse J, Vankeirsbilck A and Temme EHM, 2007. Metabolism of stevioside by healthy subjects. *Experimental Biology and Medicine / Society for Experimental Biology and Medicine*, 232, 164-173. [Metabolism of stevioside by healthy subjects - PubMed \(nih.gov\)](#)

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, 833-840. [The protective effect of Stevia rebaudiana Bertoni on serum hormone levels, key steroidogenesis enzymes, and testicular damage in testes of diabetic rats - ScienceDirect](#)

Health Canada, 2012a. Consultation on Health Canada's proposal to allow the use of the food additive steviol glycosides as a table-top sweetener and as a sweetener in certain food categories. Health Canada, Bureau of Chemical Safety, Food Directorate, Ottawa, ON. [Consultation on Health Canada's Proposal to Allow the Use of the Food Additive Steviol Glycosides as a Table-Top Sweetener and as a Sweetener in Certain Food Categories - Canada.ca](#) (Date modified: 2012-11-30).

Health Canada, 2012b. Notice of modification to the lists of permitted food additives to enable the use of steviol glycosides as a table-top sweetener and as a sweetener in certain food categories. Health Canada, Bureau of Chemical Safety, Ottawa (ON). [Notice of Modification to the Lists of Permitted Food Additives to Enable the Use of Steviol Glycosides as a Table-Top Sweetener and as a Sweetener in Certain Food Categories - Canada.ca](#) (Date Modified: 2012-11-30).

Health Canada, 2016. Notice of modification to the list of permitted sweeteners to enable the use of rebaudioside M as a sweetener in various unstandardized foods. Health Canada, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Ottawa, ON. Reference Number: NOM/ADM-0065. [Notice of Modification to the List of Permitted Sweeteners to Enable the Use of Rebaudioside M as a Sweetener in Various Unstandardized Foods - Reference Number: NOM/ADM-0065 - Canada.ca](#) (Date Modified: 2016-01-15)

Health Canada, 2017. Notice of modification to the list of permitted sweeteners to enable the use of steviol glycosides from Stevia rebaudiana Bertoni as a sweetener. Health Canada, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Ottawa, ON. Reference Number: NOM/ADM-0102. [Notice of Modification to the List of Permitted Sweeteners to Enable the Use of Steviol](#)

[Glycosides from Stevia rebaudiana Bertoni as a Sweetener - Canada.ca](#) (Date Modified: 2017-08-29).

Health Canada, 2019. Notice of modification to the list of permitted sweeteners to enable the use of steviol glycosides from *Saccharomyces cerevisiae* CD15380 and CD15407 in a variety of foods. Health Canada, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Ottawa, ON. Reference Number: NOM/ADM-0132. [Notice of Modification to the List of Permitted Sweeteners to Enable the Use of Steviol Glycosides from \*Saccharomyces cerevisiae\* CD15380 and CD15407 in a Variety of Foods - Canada.ca](#) (Date Modified: 2019-05-14).

Health Canada, 2020a. Notice of modification to the list of permitted sweeteners to enable the use of steviol glycosides from *Saccharomyces cerevisiae* Y63348 in a variety of foods. Health Canada, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Ottawa, ON. Reference Number: NOM/ADM-0151. [Notice of Modification to the List of Permitted Sweeteners to Enable the Use of Steviol Glycosides from \*Saccharomyces cerevisiae\* Y63348 in a Variety of Foods - Canada.ca](#) (Date Modified: 2020-09-03).

Health Canada, 2020b. 9. List of permitted sweeteners (lists of permitted food additives). In: Lists of permitted food additives. [9. List of Permitted Sweeteners \(Lists of Permitted Food Additives\) - Canada.ca](#) (Date issued: 2020-09-01).

Higgins KA and Mattes RD, 2019. A randomized controlled trial contrasting the effects of 4 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. *The American Journal of Clinical Nutrition*, 109, 1288-1301. [A randomized controlled trial contrasting the effects of 4 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity - ScienceDirect](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Sweetening agent: Stevioside. In: Safety evaluation of certain food additives. 51st Report of JECFA, June 9-18, 1998, Geneva. World Health Organization (WHO) / International Programme on Chemical Safety (IPCS), Geneva. WHO Food Additives Series, No.

42, 119-143. [933. Sweetening agent: Stevioside \(WHO Food Additives Series 42\) \(inchem.org\)](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Steviol glycosides. In: Safety evaluation of certain food additives. Sixty-third report of JECFA, June 8-17, 2004, Geneva. World Health Organization (WHO) / International Programme on Chemical Safety (IPCS), Geneva. WHO Food Additives Series, No. 54, 117-144, 638. [Safety evaluation of certain food additives / prepared by the sixty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives \(JECFA\)](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2007a. Steviol glycosides. In: Chemical and Technical Assessment (CTA). [Sixty-eighth report of JECFA, June 17-26, 2008]. (Prepared by Harriet Wallin and revised by Paul M. Kuznesof PhD). Food and Agriculture Organization of the United Nations (FAO), Rome. [Microsoft Word - CTA Steviol glycosides - Final 2007.doc \(fao.org\)](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2007b. 3.1.9 Steviol glycosides. Steviol glycosides. In: Evaluation of certain food additives and contaminants. Sixty-eighth report of JECFA, June 19-28, 2007, Geneva. Food and Agriculture Organization of the United Nations (FAO), Rome / World Health Organization (WHO), Geneva. WHO Technical Report Series No. 947, 50-54, 78, 206, 215. [Evaluation of certain food additives and contaminants : sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2009. Steviol glycosides (addendum). In: Toxicological evaluation of certain food additives. Sixty-ninth report of JECFA, June 17-26-29, 2008, Rome. World Health Organization (WHO) / International Programme on Chemical Safety (IPCS), Geneva. WHO Food Additives Series, No. 60, 183-220, 618-619. [Safety evaluation of certain food additives \(who.int\)](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2010. Steviol glycosides [Prepared at the 73rd JECFA, 2010) and published in FAO JECFA Monographs 10, 2010)]. In: Combined compendium of food additive specifications

[online edition]. General specifications for enzymes analytical methods, volume 4. Food and Agriculture Organization of the United Nations (FAO), Rome / World Health Organization (WHO), Geneva. FAO JECFA Monographs No. 10. [Microsoft Word - additive-442-m10.doc \(fao.org\)](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2016. Rebaudioside A from multiple gene donors expressed in *Yarrowia lipolytica* [Prepared at the 82nd JECFA, 2016]. In: Combined compendium of food additive specifications. 82nd Meeting, June 7-16, Geneva. Food and Agriculture Organization of the United Nations (FAO), Rome / World Health Organization (WHO), Geneva. FAO JECFA Monographs No. 19, 91-96. [Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives \(JECFA\), 82nd meeting 2016. Rebaudioside a From Multiple Gene Donors Expressed in Yarrowia Lipolytica](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2017a. Steviol glycosides from *Stevia rebaudiana* Bertoni [New specifications prepared at the 84th JECFA, 2017), Superseding tentative specifications prepared at the 82nd JECFA (2016)]. In: Combined compendium of food additive specifications. 84th Meeting, June 6-15, Rome. Food and Agriculture Organization of the United Nations (FAO), Rome / World Health Organization (WHO), Geneva. FAO JECFA Monographs No. 20, 50-69. [bu297en.pdf \(fao.org\)](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2017b. Stevia glycosides (addendum). In: Safety evaluation of certain food additives. Eighty-second Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 7-16, 2016. Food and Agriculture Organization of the United Nations (FAO), Rome / World Health Organization (WHO), Geneva. WHO Food Additives Series, No. 73, 181-218, 492. [9789241660730-eng.pdf \(who.int\)](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2019). 2.2.8. Framework for developing specifications for steviol glycosides by method of production. In: Evaluation of certain food additives, June 4-13, 2019, Rome. World Health Organization (WHO) / Food and Agriculture Organization of the United



Nations (FAO). WHO Technical Report Series, No. 1020, 10-11, 103.

[9789241210294-eng.pdf \(who.int\)](#)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2020. (Framework for) Steviol glycosides (tentative). In: Combined compendium of food additive specifications. 87th Meeting of JECFA, June 4-13, 2019, Rome, Italy. Food and Agriculture Organization of the United Nations (FAO), Rome / World Health Organization (WHO), Geneva. FAO JECFA Monographs 23, 62-85. [Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives \(JECFA\), 87th Meeting June 2019](#)

Koyama E, Sakai N, Ohori Y, Kitazawa K, Izawa O, Kakegawa K, Fujino A and Ui A, 2003a. Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglycone, steviol, in rats and humans. Food and Chemical Toxicology, 41, 875-883. [Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglycone, steviol, in rats and humans - PubMed \(nih.gov\)](#)

Koyama E, Kitazawa K, Ohori Y, Izawa O, Kakegawa K, Fujino A and Ui M, 2003b. In vitro metabolism of the glycosidic sweeteners, stevia mixture and enzymatically modified stevia in human intestinal microflora. Food and Chemical Toxicology, 41, 359-374. [In vitro metabolism of the glycosidic sweeteners, stevia mixture and enzymatically modified stevia in human intestinal microflora - PubMed \(nih.gov\)](#)

Kraemer T and Maurer HH, 1994. On the metabolism of the sweetener stevioside in humans. European Journal of Pharmaceutical Sciences, 2, 103 [abstract FC12].

Kroyer GT, 1999. The low calorie sweetener stevioside: stability and interaction with food ingredients. Lebensmittel-Wissenschaft und -Technologie. Food & Science Technology. Science & Technologie Alimentaire, 32, 509-512. [FC12 on the metabolism of the sweetener stevioside in humans - ScienceDirect](#)

Li J, Shen T, Shi F and Fu Y, 2020. Influences of non-nutritive sweeteners on ovarian and uterine expression of T1R2 and T1R3 in peripubertal female guinea pigs. Animal Science Journal, 91, e13348 [16pp]. DOI:10.1111/asj.13348. [Influences](#)

[of non-nutritive sweeteners on ovarian and uterine expression of T1R2 and T1R3 in peripubertal female guinea pigs - PubMed \(nih.gov\)](#)

Nakayama K, Kasahara D and Yamamoto F, 1986. Absorption, distribution, metabolism and excretion of stevioside in rats. Shokuhin Eiseigaku Zasshi. Journal of the Food Hygienic Society of Japan, 27, 1-8. [Absorption, Distribution, Metabolism and Excretion of Stevioside in Rats \(jst.go.jp\)](#)

Nettleton JE, Klancic T, Schick A, Choo AC, Shearer J, Borgland SL, Chleilat F, Mayengbam S and Reimer RA, 2019. Low-dose stevia (Rebaudioside A) consumption perturbs gut microbiota and the mesolimbic dopamine reward system. Nutrients, 11, 1248 [17pp, plus supplementary data]. DOI:10.3390/nu11061248. [Low-Dose Stevia \(Rebaudioside A\) Consumption Perturbs Gut Microbiota and the Mesolimbic Dopamine Reward System - PubMed \(nih.gov\)](#)

PureCircle Stevia Institute, 2020. Where in the world is stevia approved? A map of stevia regulatory approvals. PureCircle Stevia Institute, formerly Global Stevia Institute (GSI). [PCSI Map Infographic - A Map of Stevia Regulatory Approvals \(purecirclestevia institute.com\)](#)

Nikiforov AI, Rihner MO, Eapen AK and Thomas JA, 2013. Metabolism and toxicity studies supporting the safety of rebaudioside D. International Journal of Toxicology, 32, 261-273. [Metabolism and Toxicity Studies Supporting the Safety of Rebaudioside D - Andrey I. Nikiforov, Marisa O. Rihner, Alex K. Eapen, Jennifer A. Thomas, 2013 \(sagepub.com\)](#)

Pasqualli T, Chaves PEE, da Veiga Pereira L, Serpa ÉA, Souza de Oliveira LF and Machado MM, 2020. Steviol, the active principle of the stevia sweetener, causes a reduction of the cells of the immunological system even consumed in low concentrations. Immunopharmacology and Immunotoxicology, 42, 504-508. [Steviol, the active principle of the stevia sweetener, causes a reduction of the cells of the immunological system even consumed in low concentrations: Immunopharmacology and Immunotoxicology: Vol 42, No 5 \(tandfonline.com\)](#)

Renwick AG, 2008. Toxicokinetics [section on elimination: excretion via the gut]. In: Principles and Methods of Toxicology, 5th edition. Ed Hayes W. Taylor and Francis/CRC Press, Philadelphia, 179-230 [see 188].

Renwick AG and Tarka SM, 2008. Microbial hydrolysis of steviol glycosides. Food and Chemical Toxicology, 46(7, Suppl.), S70-S74. [Microbial hydrolysis of steviol glycosides - PubMed \(nih.gov\)](#)

Roberts A and Renwick AG, 2008. Comparative toxicokinetics and metabolism of rebaudioside A, stevioside and steviol in rats. Food and Chemical Toxicology, 46(7, Suppl.), S31-S39. [Comparative toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol in rats - ScienceDirect](#)

Roberts A, Lynch B, Rogerson R, Renwick A, Kern H, Coffee M, Cuellar-Kingston N, Eapen A, Crincoli C, Pugh G, Bhusari S, Purkayashtha S and Carakostas M, 2016. Chemical-specific adjustment factors (inter-species toxicokinetics) to establish the ADI for steviol glycosides. Regulatory Toxicology and Pharmacology: RTP, 79, 91-102. [Chemical-specific adjustment factors \(inter-species toxicokinetics\) to establish the ADI for steviol glycosides - ScienceDirect](#)

Rosales-Gómez CA, Martínez-Carrillo BE, Reséndiz-Albor AA, Ramírez-Durán N, Valdés-Ramos R, Mondragón-Velásquez T and Escoto-Herrera JA, 2018. Chronic consumption of sweeteners and its effect on glycaemia, cytokines, hormones, and lymphocytes of GALT in CD1 mice. BioMed Research International, 2018, Article ID 1345282 [15pp]. DOI:10.1155/2018/1345282 [Chronic Consumption of Sweeteners and Its Effect on Glycaemia, Cytokines, Hormones, and Lymphocytes of GALT in CD1 Mice \(hindawi.com\)](#)

Rumelhard M, Hosako H, Eurlings IMJ, Westerink WMA, Staska LM, van de Wiel JAG and La Marta J, 2016. Safety evaluation of rebaudioside A produced by fermentation. Food and Chemical Toxicology, 89, 73-84. [Safety evaluation of rebaudioside A produced by fermentation - ScienceDirect](#)

Sánchez-Delgado M, Estrada JA, Paredes-Cervantes V, Kaufer-Horwitz M and Contreras I, 2019. Changes in nutrient and calorie intake, adipose mass, triglycerides and TNF- $\alpha$  concentrations after non-caloric sweetener intake: pilot study. International Journal for Vitamin and Nutrition Research, [online ahead of print - Oct. 28, 2019, plus supplementary table]. DOI:10.1024/0300-9831/a000611. [Changes in nutrient and calorie intake, adipose mass, triglycerides and TNF- \$\alpha\$  concentrations after non-caloric sweetener intake: A pilot study | International Journal for Vitamin and Nutrition Research \(hogrefe.com\)](https://doi.org/10.1024/0300-9831/a000611)

Sánchez-Tapia M, Martínez-Medina J, Tovar AR and Torres N, 2019. Natural and artificial sweeteners and high fat diet modify differential taste receptors, insulin, and TLR4-mediated inflammatory pathways in adipose tissues of rats. Nutrients 11, 880 [15pp, plus supplementary table]. DOI:10.3390/nu11040880. [Natural and Artificial Sweeteners and High Fat Diet Modify Differential Taste Receptors, Insulin, and TLR4-Mediated Inflammatory Pathways in Adipose Tissues of Rats - PubMed \(nih.gov\)](https://doi.org/10.3390/nu11040880)

SCF (Scientific Committee on Food), 1985. Reports of the Scientific Committee for Food concerning sweeteners (opinion expressed by the SCF on 14 September 1984) [Stevioside]. In: Food science and techniques. Reports of the Scientific Committee for Food, 16th series. [Reports of the Scientific Committee for Food \(Twenty-fifth series\) \(pitt.edu\)](https://www.fao.org/3/a/06202e.htm)

SCF (Scientific Committee on Food), 1999. Opinion on stevioside as a sweetener (adopted on 17/6/99). CS/ADD/EDUL/167 Final. Available online: [Health and Consumer Protection - Opinion of the SCF on Stevioside as sweetener \(adopted on 17/06/99\) \(europa.eu\)](https://ec.europa.eu/food/safety/biotechnology/stevia/stevia_en)

Schiano C, Grimaldi V, Boccella S, Iannotta M, Zullo A, Luongo L, Mancini FP, Maione S and Napoli C, 2019. Sweeteners modulate bioactivity of endothelial progenitor cells but not induce detrimental effects both on inflammation and behavioural changes. International Journal of Food Sciences and Nutrition, 70, 725-737 [plus supplementary figures] [Sweeteners modulate bioactivity of endothelial progenitor cells but not induce detrimental effects both on inflammation and](https://doi.org/10.1080/09637454.2019.1644444)

[behavioural changes: International Journal of Food Sciences and Nutrition: Vol 70, No 6 \(tandfonline.com\)](#)

Simonetti P, Gardana C, Bramati L and Pietta PG, 2004. Bioavailability of stevioside from *Stevia rebaudiana* in humans: preliminary report. In: Geuns JMC, Buyse J, editors. Safety of stevioside: proceedings of the first symposium sponsored by KULeuven, Apr. 16, 2004, Leuven, Belgium. Euprint ed., Heverlee, Belgium, 51-62.

Stamataki NS, Crooks B, Ahmed A and McLaughlin JT, 2020. Effects of the daily consumption of stevia on glucose homeostasis, body weight, and energy intake: a randomised open-label 12-week trial in healthy adults. *Nutrients*, 12, 3049 [16pp, plus supplementary figure]. DOI:10.3390/nu12103049 [Nutrients | Free Full-Text | Effects of the Daily Consumption of Stevia on Glucose Homeostasis, Body Weight, and Energy Intake: A Randomised Open-Label 12-Week Trial in Healthy Adults \(mdpi.com\)](#)

Sung LH, 2002. Report on pharmacokinetic (PK) studies of T100 sunstevia 95% stevioside in rats. From: Sunlabel Pte Ltd., Singapore. Submitted to WHO by the Ministry of Health and Welfare, Japan. (cited in: JECFA, 2006).(Unpublished)

Toyoda K, Matsui H, Shoda T, Uneyama C, Takada K and Takahashi M (1997). Assessment of the carcinogenicity of stevioside in F344 rats. *Food and Chemical Toxicology*, 35, 597-603. DOI: 10.1016/s0278-6915(97)00023-9 [Assessment of the carcinogenicity of stevioside in F344 rats - ScienceDirect](#)

U.S. FDA (U.S. Food and Drug Administration), 2000. Toxicological principles for the safety assessment of food ingredients: Redbook 2000 [updated to July 2007]. [Guidance for Industry and Other Stakeholders: Redbook 2000 | FDA](#)

U.S. FDA (U.S. Food and Drug Administration), 2008. Agency response letter GRAS Notice No. GRN 253 [Rebaudioside A purified from *Stevia Rebaudiana* (Bertoni) Bertoni, Cargill, Inc., Wayzata, MN]. <https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=253> (Dec.17, 2008).

U.S. FDA (U.S. Food and Drug Administration), 2016a. Agency response letter GRAS Notice No. GRN 626 [Steviol glycosides produced in *Saccharomyces cerevisiae*, Cargill, Inc., Wayzata, MN].

[GRAS Notices \(fda.gov\)](#) (May 27, 2016 - FDA response - no questions).

U.S. FDA (U.S. Food and Drug Administration), 2016b. Agency response letter GRAS Notice No. GRN 632 [Rebaudioside A from *Yarrowia lipolytica*, DSM Nutritional Products, LLC, Parsippany, NJ].

<https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=632> (Jun. 24, 2016 - FDA response - no questions).

U.S. FDA (U.S. Food and Drug Administration), 2018. Agency response letter GRAS Notice No. GRN 759

[Steviol glycosides consisting primarily of rebaudioside M produced in *Yarrowia lipolytica*, DSM Food Specialties, Delft, The Netherlands / DSM Nutritional Products North America, Parsippany, NJ].

[GRAS Notices \(fda.gov\)](#) (May 25, 2018 - FDA response - no questions).

U.S. FDA (U.S. Food and Drug Administration), 2020a. Agency response letter GRAS Notice No. GRN 882

[Rebaudioside M, Cargill, Inc., Wayzata, MN].

[GRAS Notices \(fda.gov\)](#) (Feb. 2, 2020 - FDA response - no questions).

U.S. FDA (U.S. Food and Drug Administration), 2020b. GRAS notices [inventory].

[GRAS Notices \(fda.gov\)](#) (Page Last Updated: 01/11/2021).

Wang LZ, Goh BC, Fan L and Lee HS, 2004. Sensitive high-performance liquid chromatography/mass spectrometry method for determination of steviol in rat plasma. *Rapid Communications in Mass Spectrometry*, 18, 83-86. [Sensitive high-performance liquid chromatography/mass spectrometry method for determination of steviol in rat plasma - PubMed \(nih.gov\)](#)

Wheeler A, Boileau AC, Winkler PC, Compton JC, Prakash I, Jiang X and Mandarino DA, 2008. Pharmacokinetics of rebaudioside A and stevioside after single oral doses in healthy men. Food and Chemical Toxicology, 46(7, Suppl.), S54-S60.

[Pharmacokinetics of rebaudioside A and stevioside after single oral doses in healthy men - ScienceDirect](#)

Wingard RE, Brown JP, Enderlin FE, Dale JA, Hale RL and Seitz CT, 1980.

Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. Experientia, 36, 519-520.

[Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A | SpringerLink](#)

Yılmaz SG, Uçar A and Yılmaz S, 2020. Do steviol glycosides affect the oxidative and genotoxicity parameters in BALB/c mice? Drug and Chemical Toxicology, [online ahead of print - Jan. 21, 2020]. [Do steviol glycosides affect the oxidative and genotoxicity parameters in BALB/c mice?: Drug and Chemical Toxicology: Vol 45, No 1 \(tandfonline.com\)](#)

## 6. Abbreviations

ADI	Acceptable Daily Intake
ADME	Absorption, distribution, metabolism and excretion
AEJEG	Joint Expert Group on Additives, Enzymes and other Regulated Products
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstracts Service
CTA	Chemical and Technical Assessment
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority

EU	European Union
FAF	EFSA Panel on Food Additives and Flavourings
FAO	Food and Agriculture Organisation
FCC	Food Chemicals Codex
FDA	United States Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	EFSA Panel on Genetically Modified Organism
GMP	Good manufacturing practices
GRAS	Generally recognized as safe
JECFA	Joint FAO/WHO Expert Committee on Food Additives
HPLC	High-performance liquid chromatography
LC-MS	Liquid chromatography–mass spectrometry
NOAEL	No Observed Adverse Effect Level
QPS	Qualified Presumption of Safety (QPS)
PCR	Polymerase chain reaction
SCF	European Commission’s Scientific Committee on Food
SG	Steviol Glycoside
WHO	World Health Organization

© Crown copyright 2023

This publication (not including logos) is licensed under the terms of the Open Government Licence v3.0 except where otherwise stated. Where we have identified any third party copyright information you will need to obtain permission from the copyright holders concerned.

For more information and to view this licence:

- visit [the National Archives website](#)
- email [psi@nationalarchives.gov.uk](mailto:psi@nationalarchives.gov.uk)



- write to: Information Policy Team, The National Archives, Kew, London, TW9 4DU

For enquiries about this publication, [contact the Food Standards Agency](#).



Follow us on Twitter:

[@foodgov](https://twitter.com/foodgov)



Find us on Facebook:

[facebook.com/FoodStandardsAgency](https://facebook.com/FoodStandardsAgency)