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SCIENTIFIC OPINION

Scientific opinion on the extension of the authorisation of use of the food additive steviol glycosides (E 960a–d) and the modification of the acceptable daily intake (ADI) for steviol

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Abstract

The EFSA Panel on Food Additive and Flavourings (FAF Panel) evaluated the safety of proposed changes to the currently permitted uses of the food additive steviol glycosides (E 960a–d) and of a proposed modification of the current acceptable daily intake (ADI) from 4 mg/kg body weight (bw) per day to 6 or 16 mg/kg bw per day, expressed as steviol equivalents. Currently, steviol glycosides (E 960a–d) are authorised in the EU in 32 different food categories (FCs). An extension of use was proposed for four new uses within FC 7.2 'Fine bakery wares'. In addition, an increase of the maximum permitted levels (MPLs) for FC 14.1.3 'Fruit nectars' and for three uses within FC 14.1.4 'Flavoured drinks' was requested. Consequently, the Panel updated the exposure estimates using the protocol for assessing exposure to sweeteners, developed to consider the specificities related to consumers' exposure to this functional class of food additives. Considering the proposed extension of use and increase of the MPLs, together with the currently authorised uses (at the MPLs) of E 960a–d, the highest 95th percentiles of exposure are 4.1 and 6.9 mg/kg bw per day for infants and toddlers, respectively. Based on the currently available absorption, distribution, metabolism and excretion (ADME) dataset for steviol glycosides (E 960a–d), the Panel concluded that that there is insufficient justification to increase the current ADI of 4 mg/kg bw per day, expressed as steviol equivalents. With respect to the proposed extension of use and increase of the MPLs, the Panel concluded that the calculated, conservative, dietary exposure would result in an increased exceedance of the ADI for toddlers at the 95th percentile.

KEYWORDS

ADI, E 960a–d, extension of use, food additive, steviol glycosides

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Panel on Food Additives and Flavourings (FAF Panel) was asked to provide a scientific opinion on the safety of proposed changes to the currently permitted uses of the food additive steviol glycosides (E 960a–d) and of the proposed modification of the current acceptable daily intake (ADI).

The safety of steviol glycosides (E 960a–d) as a food additive was evaluated by EFSA in 2010 and an ADI of 4 mg/kg body weight (bw) per day, expressed as steviol equivalents, was established, based on the application of a 100-fold uncertainty factor (UF) to the no observed adverse effect level (NOAEL) from a 2-year carcinogenicity study in rat.

Currently, steviol glycosides (E 960a–d) is authorised as a food additive in the EU in 32 different food categories (FCs) (representing 42 uses) with maximum permitted levels (MPLs) ranging from 20 to 3300 mg steviol equivalents/kg. In addition, they are permitted at *quantum satis* (QS) in table-top sweeteners. The latest regulatory maximum level exposure assessment scenario of steviol glycosides was performed by EFSA in 2015. This scenario was based on food consumption data available at the time in the EFSA Comprehensive Database and the standard methodology used for estimating the regulatory MPL exposure assessment scenario applicable to the general population.

The applicant proposed four new uses of steviol glycosides (E 960a–d) as sweetener within the already authorised FC 7.2 'Fine bakery wares'. Additionally, an increase of the MPLs was proposed for three uses within the already authorised FCs 14.1.4 'Flavoured drinks' and 14.1.3 'Fruit nectars as defined by Directive 2001/112/EC'. In all food categories for which an extension of use was proposed, the use is for foods that are energy reduced or with no added sugar.

A new methodology for assessing the dietary exposure has been developed and implemented by the Panel in the context of the re-evaluation of already authorised sweeteners under Regulation (EU) No 257/2010. The Panel considered that this new methodology would be applicable also to the present assessment and was therefore used to update the exposure to steviol glycosides (E 960a–d) via the currently permitted uses and taking into account the proposed extension of use and increase in MPLs.

Currently, for the regulatory maximum level exposure assessment scenario, the mean exposure to steviol glycosides (E 960a–d), expressed as steviol equivalents, ranged from 0.02 mg/kg bw per day in the elderly to 1.3 mg/kg bw per day in toddlers. The 95th percentile of exposure to steviol glycosides, expressed as steviol equivalents, ranged from 0.2 mg/kg bw per day in toddlers, adolescents and the elderly, to 4.8 mg/kg bw per day in toddlers. Considering the proposed extension of use and increase in the MPLs, together with the currently authorised uses (at the MPLs), the mean exposure to steviol glycosides (E 960a–d), expressed as steviol equivalents, ranged from 0.02 mg/kg bw per day in the elderly to 1.8 mg/kg bw per day in toddlers. The corresponding 95th percentile of exposure ranged from 0.2 mg/kg bw per day in toddlers and adolescents to 6.9 mg/kg bw per day in toddlers. In both cases, the exceeding exposure estimates represent one country per population group. However, based on the uncertainty analysis, the Panel considered the exposure assessment to be an overestimation.

In addition to the proposed extension of use and increase of the MPLs, the applicant also requested a modification of the current ADI for steviol glycosides (E 960a–d). The Panel noted that for addressing this request no new biological and toxicological data were provided, whereas studies already considered in several previous opinions by the ANS and FAF Panel were submitted (EFSA ANS Panel, 2010, 2015a, 2015b, 2018; EFSA FAF Panel, 2019, 2020, 2021, 2022a, 2022b, 2023). According to the applicant, the current ADI for steviol glycosides (E 960a–d) could be increased from the currently established 4 mg kg/bw per day (expressed as steviol equivalents) by applying a chemical-specific adjustment factor(s) (CSAF) to account for toxicokinetic difference between rat and humans. According to the applicant, the default toxicokinetic component of 4 can be modified to 1, based on C_{max} values, or to 2.8, based on area under the curve (AUC) values, resulting in an ADI of 16 or 6 mg/bw per day, expressed as steviol equivalents, respectively. The proposals were based on the results of the study carried out in rats and human from Roberts et al. (2016).

Taking into account the currently available dataset on ADME of steviol glycosides (E 960a–d), the Panel considered that data are not sufficiently robust to support the use of a CSAF as proposed by the applicant in place of the standard default factor applied to the derivation of the current ADI for steviol glycosides (E 960a–d).

The Panel concluded that there is insufficient justification to increase the current ADI for steviol glycosides (E960a–d) of 4 mg/kg bw per day (expressed as steviol equivalents).

With respect to the proposed extension of use and increase in the MPLs, the Panel concluded that the calculated, conservative, dietary exposure would result in an increased exceedance of the ADI for toddlers at the 95th percentile.

1 | INTRODUCTION

The present scientific opinion deals with the evaluation of proposed changes to the currently permitted uses of the food additive steviol glycosides (E 960a–d). In addition, the applicant is proposing a modification of the current acceptable daily intake (ADI) for E 960a-d, which is also considered in the current opinion.

1.1 | Background and Terms of Reference as provided by the European Commission

1.1.1 | Background

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008[1] on food additives. Only food additives that are included in the Union list, in particular in Annex II to that regulation, may be placed on the market and used in food under the conditions of use specified therein. Moreover, food additives shall comply with the specifications as referred to in Article 14 of that Regulation and laid down in Commission Regulation (EU) No 231/2012.[2] An application has been introduced for the extension of the authorisation of use of the food additive steviol glycosides in certain food categories of Annex II to Regulation (EC) No 1333/2008 and the modification of the ADI.

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority (EFSA) to perform a safety assessment to provide a scientific opinion on the safety of the proposed use of the food additive steviol glycosides (E 960a-d) and the assessment of possible confidentiality requests in accordance with Regulation (EC) No 1331/2008[3] establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

1.1.3 | Interpretation of the Terms of Reference

For the present opinion, in conjunction with the assessment of the proposed changes to the currently permitted uses of E 960a–d, the Panel has also evaluated the data provided by the applicant in support of its request of the modification of the ADI for E 960a–d, as referred to in Section 1.1.1, Background.

1.2 | Information on existing evaluations and authorisations

According to Annex II to Regulation (EC) No 1333/2008, the following food additives are authorised for use in the EU, listed under the group of steviol glycosides (E 960a–d): steviol glycosides from stevia (E 960a); enzymatically produced steviol glycosides (E 960c) and glucosylated steviol glycosides (E 960d). These food additives have combined maximum permitted levels (MPLs) for use in foods, expressed as steviol equivalents and are listed in the functional group of sweeteners.

Steviol glycosides from stevia (E 960a) was the first steviol glycosides to be authorised as a food additive in the EU. The food additive is obtained by water extraction of the leaves of the *Stevia rebaudiana* Bertoni plant. According to the specifications defined in Commission Regulation (EU) No 231/2012, it is described as: '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 safety of steviol glycosides from stevia (E 960a) as a food additive was evaluated by EFSA in 2010 and an acceptable daily intake (ADI) of 4 mg/kg body weight (bw) per day, expressed as steviol equivalents, was established, based on application of a 100-fold uncertainty factor (UF) to the no observed adverse effect level (NOAEL) from a 2-year carcinogenicity study in rat (EFSA ANS Panel, 2010). Following the EFSA assessment in 2015 (EFSA ANS Panel, 2015a), rebaudioside D and M were included in the specifications for steviol glycosides (E 960a). The latest exposure assessment to steviol glycosides (E 960a) was carried out by the EFSA ANS Panel in 2015 (EFSA ANS Panel, 2015b).

In 2020, the FAF Panel evaluated an application to amend the existing EU specifications for steviol glycosides to allow for the inclusion of 60 steviol glycosides identified in *S. rebaudiana* Bertoni leaves, including both 'major' and 'minor' steviol glycosides, that may comprise the assay value of not less than 95% total steviol glycosides. The Panel concluded that the overall metabolic fate of these steviol glycosides is the same, and therefore, it would be acceptable to use a read-across

[1]Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

[2]Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

[3]Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1.

approach for the safety assessment of the 60 steviol glycosides and the ADI of 4 mg/kg bw per day would apply to all those steviol glycosides. However, the Panel noted at that time that the proposed change from 11 to 60 specified steviol glycosides, while maintaining an assay value of not less than 95% as proposed by the applicant, would allow less pure preparations of the food additive into the market. According to the proposed change in specifications, there would remain a small but not insignificant fraction of the additive that was undefined and therefore could not be evaluated by the Panel. Therefore, while inclusion of the 60 steviol glycosides in the specifications for steviol glycoside (E 960) would not be of safety concern, the FAF Panel could not conclude on the safety of the proposed amendment to the specifications of steviol glycosides (E 960) as a food additive if the purity assay value of not less than 95% for the total content of steviol glycosides was maintained (EFSA FAF Panel, 2020).

In July 2021, a new entry for 'enzymatically produced steviol glycosides (E 960c)' was added to Annex II to Regulation (EC) No 1333/2008.[4] This amendment to the Regulation was based on the conclusions from the opinion on the safety of a proposed amendment of the specifications of the food additive steviol glycosides (E 960) concerning rebaudioside M produced by enzyme modification of steviol glycosides, using UDP-glucosyl transferase and sucrose synthase enzymes produced by the genetically modified yeasts *Komagaetella phaffii* UGT-A and *K. phaffii* UGT-B (EFSA FAF Panel, 2019). Regulation (EU) No 231/2012 was also amended accordingly, with the inclusion of a new entry for 'E 960c(i) Rebaudioside M produced via enzyme modification of steviol glycosides from stevia'.

In October 2022, Regulation (EU) No 231/2012 was further amended,[5] with the inclusion of the following new entries: 'E 960c(ii) Rebaudioside M produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts', 'E 960c(iii) Rebaudioside D produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts' and 'E960c(iv) rebaudioside AM produced via enzymatic conversion of highly purified stevioside stevia leaf extracts'. This amendment to the Regulation was based on evaluations by the FAF Panel (EFSA FAF Panel, 2020, 2021).

In March 2023, both Regulation (EC) No 1333/2008 and Regulation (EU) No 231/2012 were again amended[6] introducing the entry 'glucosylated steviol glycosides' (E 960d), based on the evaluation completed by the Panel (EFSA FAF Panel, 2022a). With this latest amendment introduced in the legislation, also the definition of the group of food additives named 'steviol glycosides' was changed to (E 960a – 960d).

In addition to the already authorised uses, the FAF Panel completed in 2022 the safety evaluation of an additional proposed amendment to the specifications of the food additive steviol glycosides (E 960). The application related to rebaudioside D produced by enzymatic bioconversion of purified *S. rebaudiana* Bertoni leaf extract, using UDP-glucosyltransferase (UGT) and sucrose synthase produced by a genetically modified strain of the yeast *K. phaffii* (EFSA FAF Panel, 2022b).

In December 2023, the Panel issued a further opinion on the safety of an additional proposed amendment to the specifications of the food additive steviol glycosides (E 960a–d) concerning steviol glycosides composed predominantly of rebaudioside M, manufactured by a new process by fermentation of simple sugars using a genetically modified strain of *Yarrowia lipolytica* (named *Y. lipolytica* VRM) (EFSA FAF Panel, 2023).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an ADI for steviol glycosides of 0–4 mg/kg bw per day, expressed as steviol (JECFA, 2008, 2009). In 2017, JECFA issued new specifications for 'Steviol Glycosides from *S. rebaudiana* Bertoni' that consist of a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties (glucose, rhamnose, xylose, fructose and deoxyglucose) in any of the orientations occurring in the leaves of *S. rebaudiana* Bertoni, provided that the total percentage of steviol glycosides is not less than 95% (JECFA, 2017). These specifications were superseded in 2019 at the 87th JECFA meeting by new tentative JECFA specifications adopted jointly with a framework approach based on the different methods of production applied to the manufacturing of steviol glycosides, i.e. water extraction, fermentation, enzymatic modification and glucosylation (JECFA, 2020). The framework adopted in 2019 was subsequently revised by JECFA at its 91st meeting in February 2021 (JECFA, 2021) and the tentative specifications prepared at its 87th meeting was replaced. Specifications for steviol glycosides manufactured using four different methods have been established, including specifications for 'Steviol Glycosides from Fermentation' (JECFA, 2021).

2 | DATA AND METHODOLOGIES

2.1 | Data

The present evaluation is based on the data submitted in the application dossier (Documentation provided to EFSA No. 1).

[4]Commission Regulation (EU) 2021/1156 of 13 July 2021 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards steviol glycosides (E 960) and rebaudioside M produced via enzyme modification of steviol glycosides from stevia. OJ L 249, 14.7.2021, p. 87–98.

[5]Commission Regulation (EU) 2022/1922 of 10 October 2022 amending the Annex to Regulation (EU) No 231/2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards specifications for rebaudiosides M, D and AM produced via enzymatic conversion of purified stevia leaf extracts and the specifications for rebaudioside M produced via enzyme modification of steviol glycosides from stevia (E 960c(i) OJ L 264, 11.10.2022, p. 1–7.

[6]Commission Regulation (EU) 2023/447 of 1 March 2023 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards the use of glucosylated steviol glycosides as sweetener. OJ L 65, 2.3.2023, p. 16–27.

In accordance with Art. 38 of the Commission Regulation (EC) No 178/2002[7] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation and of the Decision of the EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[8] the non-confidential version of the dossier is published on Open.EFSA.[9]

According to Article 32c(2) of Regulation (EC) No 178/2002[10] and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 16 April to 7 May 2024.[11] Comments were received and published.[12] None of the comments submitted was deemed relevant to the scope of the public consultation and were not considered further.

2.2 | Methodologies

This opinion was formulated following the principles described in the EFSA Guidance of the Scientific Committee on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidance documents from the EFSA Scientific Committee.

The current 'Guidance for submission for food additive evaluation' (EFSA ANS Panel, 2012) has been followed by FAF Panel for evaluating this application.

For this opinion, a regulatory maximum level exposure assessment scenario based on the current MPLs from the Regulation (EC) No 1333/2008) was performed and a regulatory maximum level exposure assessment scenario including also the proposed extension of use and increase in MPLs. The main purpose of this exposure scenario is to estimate the exposure to the additive that could theoretically result from the permitted uses and use levels (or the proposed uses and maximum use levels, in the case of new applications). This exposure is then compared with the health based guidance value (HBGV) of the additive. The Panel noted that this scenario considers that all foods within the permitted food categories contain the additive at the MPL or maximum use level in case of *quantum satis* (QS). This scenario will likely result in an overestimation of the actual dietary exposure, as it is unlikely that all foods that may contain the additive will indeed contain the additive and if so, that the additive is always present at the MPL or maximum use level.

In addressing the current mandate, by analogy with the approach applied to the re-evaluation programme of already permitted sweeteners, the Panel decided to update the dietary exposure estimates to steviol glycosides (E 960a–d) as published in EFSA ANS Panel (2015b). To do so, the protocol for assessing exposure to sweeteners as part of their safety assessment under the food additives re-evaluation programme (EFSA, 2020) was used since the methodological approach developed was considered suitable also to address the terms of reference of this new mandate related to the proposal for changes to the currently permitted uses of steviol glycosides (E 960a–d). This protocol has been developed to take into account the specificities related to consumers' exposure to sweeteners.[13]

3 | ASSESSMENT

3.1 | Technical data

3.1.1 | Identity of the food additive

Current EU specifications for individual steviol glycosides (E 960a–d) are listed in the Commission Regulation (EU) No 231/2012. The different entries (a, c, d) for E 960 (Table 1) qualifies different manufacturing processes – leaf extract of *S. rebaudiana* Bertoni plant (trivial name: stevia), enzymatic conversion of steviol glycosides preparations or glucosylation of steviol glycosides extracted from stevia – and consequently each preparation has a different content of the 11 approved steviol glycosides (i.e. steviol, steviolbioside, rubusoside, dulcoside A, stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F and rebaudioside M).

[7]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[8]Decision https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.

[9]The non-confidential version of the dossier, following EFSA's assessment of the applicant's confidentiality requests, is published on Open.EFSA and is available at the following link: https://open.efsa.europa.eu/dossier/FAD-2023-16355.

[10]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[11]https://connect.efsa.europa.eu/RM/s/consultations/publicconsultation2/a0lTk000000a5ij/pc0910.

[12]https://open.efsa.europa.eu/consultations/a0cTk0000012uDtIAI?status=Closed&search=steviol.

[13]The specificities related to consumers' exposure to sweeteners come from the fact that consumers can choose their foods based on label information concerning the presence of a sweetener in a consistent manner and these consumers represent the most relevant population for dietary exposure assessment of sweeteners.

TABLE 1 Description of steviol glycosides (E 960a–d) entries in the Commission Regulation (EU) No 231/2012.

E number	Food additive
E 960a	Steviol glycosides from stevia
E 960c(i)	Rebaudioside M produced via enzyme modification of steviol glycosides from stevia
E 960c(ii)	Rebaudioside M produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts
E 960c(iii)	Rebaudioside D produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts
E 960c(iv)	Rebaudioside AM produced via enzymatic conversion of highly purified stevioside stevia leaf extracts
E 960d	Glucosylated steviol glycosides

3.2 | Authorised uses and use levels

MPLs of use of steviol glycosides (E 960a–d) have been defined in Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives by establishing a Union list of food additives, as amended.

Currently, steviol glycosides (E 960a–d) is authorised as a food additive in the EU in 32 different food categories (FCs) (representing 42 uses) with MPLs ranging from 20 to 3300 mg steviol equivalents/kg or L. Additionally, they are permitted for use at QS in table-top sweeteners. Table 2 lists the FCs that are permitted to contain steviol glycosides (E 960a–d) and the corresponding MPLs as set out in Annex II to Regulation (EC) No 1333/2008, as amended.

TABLE 2 Authorised uses and use levels for steviol glycosides (E 960a–d) (expressed in mg steviol equivalents/kg or mg steviol equivalents/L).

Food category number	Food category	Restrictions or exceptions	MPL (mg/kg or mg/L)
1.4	Flavoured fermented milk products including heat-treated products	Only energy-reduced products or with no added sugar	100
3	Edible ices	Only energy-reduced products or with no added sugar	200
4.2.2	Fruit and vegetables in vinegar, oil or brine	Only sweet–sour preserves of fruit and vegetables	100
4.2.4.1	Fruit and vegetable preparations excluding compote	Only energy reduced	200
4.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EC	Only energy-reduced jams jellies and marmalades	200
4.2.5.2	Jam jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	Only energy-reduced jams jellies and marmalades	200
4.2.5.3	Other similar fruit or vegetable spreads	Only energy-reduced fruit or vegetable spreads and dried-fruit-based sandwich spreads, energy reduced or with no added sugar	200
5.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	Only energy reduced or with no added sugars	270
5.2	Other confectionery including breath-freshening microsweets	Only strongly flavoured freshening throat pastilles, energy reduced or with no added sugars	670
		Only breath-freshening microsweets, energy reduced or with no added sugars	2000
		Only confectionery with no added sugars only energy-reduced hard confectionery (candies and lollies) only energy-reduced soft confectionery (chewy candies, fruit gums and foam sugar products/marshmallows) only energy-reduced liquorice only energy-reduced nougat only energy-reduced marzipan	350
		Only cocoa, milk, dried fruit or fat based sandwich spreads, energy reduced or with no added sugar	330
		Only cocoa or dried fruit based, energy reduced or with no added sugar	270
5.3	Chewing gum	Only with no added sugar	3300
5.4	Decorations coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only cocoa or dried fruit based, energy reduced or with no added sugar	270
		Only confectionary with no added sugar	330

(Continues)

TABLE 2 (Continued)

Food category number	Food category	Restrictions or exceptions	MPL (mg/kg or mg/L)
6.3	Breakfast cereals	Only breakfast cereals with a fibre content of more than 15%, and containing at least 20% bran, energy reduced or with no added sugar	330
7.2	Fine bakery wares	Only essoblaten – wafer paper	330
9.2	Processed fish and fisheries products	Only sweet–sour preserves and semi preserves of fish and marinades of fish, crustaceans and molluscs	200
11.4.1	Table-top sweeteners in liquid form		QS
11.4.2	Table-top sweeteners in powder form		QS
11.4.3	Table-top sweeteners in tablets		QS
12.4	Mustard		120
12.5	Soups and broths	Only energy-reduced soups	40
12.6	Sauces	Only soy-bean sauce (fermented and non-fermented)	175
		Except soy-bean sauce (fermented and non-fermented)	120
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		330
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		270
14.1.3	Fruit nectars as defined by directive 2001/112 and vegetable nectars and similar products	Only energy reduced or with no added sugar	100
14.1.4	Flavoured drinks	Only energy reduced or with no added sugar	80
14.1.5.2	Other[a]	Only malt-based and chocolate/cappuccino flavoured drinks, energy reduced or with no added sugars	20
		Only flavoured instant coffee and instant cappuccino products, energy reduced or with no added sugars only coffee, tea and herbal infusion beverages, energy reduced or with no added sugars	30
		Only coffee, tea and herbal infusion beverages, energy reduced or with no added sugars	30
14.2.1	Beer and malt beverages	Only alcohol-free beer or with an alcohol content not exceeding 1,2% vol.; 'Bière de table/Tafelbier/Table beer (original wort content less than 6%) except for 'Obergäriges Einfachbier; beers with a minimum acidity of 30 milli-equivalents expressed as NaOH; Brown beers of the 'oud bruin type'	70
14.2.8	Other alcoholic drinks including mixtures of alcoholic beverages with non-alcoholic beverages and other alcoholic beverages based on distilled alcohol with alcoholic strength by volume less than 15%		150
15.1	Potato- cereal- flour- or starch-based snacks		20
15.2	Processed nuts		20
16	Desserts excluding products covered in category 1, 3 and 4	Only energy reduced or with no added sugar	100
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children	Only food supplements in chewable form	1800
			670
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children	Only food supplements in syrup form	1800
			200

Abbreviations: MPL, maximum permitted level; QS: *quantum satis*.

[a]MPL applies to the ready-to-drink products (e.g. canned) and their mixes and concentrates after preparation and ready for consumption.

3.2.1 | Proposed changes to the currently permitted uses

The applicant proposed four new uses of steviol glycosides (E 960a–d) as sweetener within the already authorised FC 7.2 'Fine bakery wares'. Additionally, an increase in the MPLs was proposed for three uses of steviol glycosides (E 960a–d)

within the already authorised FCs 14.1.4 'Flavoured drinks' and 14.1.3 'Fruit nectars as defined by Directive 2001/112/EC'. In all food categories for which an extension of use was proposed, the use is for foods that are energy reduced or with no added sugar. Table 3 summarises the proposed extension of use and the proposed increases in MPLs.

TABLE 3 Proposed extension of use and proposed increase in MPLs for steviol glycosides (E 960a–d) (expressed in mg steviol equivalents/kg or mg steviol equivalents/L) (Documentation provided to EFSA No. 1).

Food category number	Food category	Restrictions or exceptions	Proposed typical use levels (mg/kg or mg/L)	Proposed MPLs (mg/kg or mg/L)
7.2[a]	Fine bakery wares	Cereal bars energy reduced or with no added sugar	200	300
		Cookies/Biscuits energy reduced or with no added sugar	180	200
		Wafers/Cornet energy reduced or with no added sugar	240	300
		Cakes energy reduced or with no added sugar	220	240
14.1.3[b]	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Fruit nectars as defined by Directive 2001/112/EC, energy reduced or with no added sugar	100	125
14.1.4[b]	Flavoured drinks	Flavoured drinks energy reduced or with no added sugar	80	150
		Sport drinks energy reduced or with no added sugar	80	120
		Energy drinks energy reduced or with no added sugar	80	200

[a]Proposed new uses.
[b]Proposed increase in the MPLs.

3.3 | Exposure data

3.3.1 | Food consumption data used for the exposure assessment

EFSA comprehensive European food consumption database

To assess whether the proposed changes to the currently permitted uses (Table 3) pose a possible health concern, the potential chronic dietary exposure to steviol glycosides (E 960a–d) was calculated by the Panel using the authorised and proposed use levels, using food consumption data from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database). Since 2010, this database has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b). The version of the Comprehensive database taken into account in the exposure assessment was published in November 2023.[14] Data from EU Member States were considered for the estimations.

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons of the exposure estimates should be interpreted with caution. Depending on the food category and the level of detail used for the exposure calculations, uncertainties could be introduced owing to possible subjects' under-reporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database includes the currently best available food consumption data across Europe.

Food consumption data from infants, toddlers, children, adolescents, adults and the elderly were used in the exposure assessment. For the present assessment, food consumption data were available from 43 different dietary surveys carried out in 22 European countries (Table 4). Not all Member States provided consumption information for all population groups, and in some cases food consumption data from more than one consumption survey of one country was available. In most cases, when, for one country and population groups, different dietary surveys were available, the data from the most recent survey was used. However, when two national surveys from the same country gave a better coverage of the age range than using only the most recent one, both surveys were kept. For details on each survey, see Annex A, Table A.4.

[14]https://www.efsa.europa.eu/en/data-report/food-consumption-data.

TABLE 4 Population groups considered for the exposure estimates of steviol glycosides (E 960a–d).

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers[a]	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, the Netherlands, Portugal, Slovenia, Spain
Children[a]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, the Netherlands, Portugal, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
The elderly[b]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

[a]The term 'toddlers' in the Comprehensive Database (EFSA, 2011b) corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.[15]
[b]The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in Comprehensive Database (EFSA, 2011b).

Since 2018, all consumption records in the Comprehensive Database were codified according to the FoodEx2 classification system (EFSA, 2015). Nomenclature from the FoodEx2 classification system was linked to the food categorisation system of Annex II of Regulation (EC) No 1333/2008, part D, to perform the exposure assessments of food additives. In practice, the FoodEx2 food codes were matched to the food categories. For a detailed description of the methodology used to link these codes and the food categories, see section 5.2.1 of the protocol for assessing exposure to sweeteners (EFSA, 2020). In FoodEx2, facets are used to provide further information about different properties and aspects of foods recorded in the Comprehensive Database. These facets were used in the exposure assessment of steviol glycosides (E 960a–d) to further identify foods to be included in the assessment (e.g. sweetener-related facets for foods in relevant food categories, see details in Appendix D of EFSA, 2020).

The Panel noted that the applicant provided exposure assessments for the food categories in which use or use levels were proposed to be modified, using the Food Additive Intake Model 2.0 (FAIM) and the DietEx tool (Documentation provided to EFSA No. 1). Therefore, it was considered not appropriate and was not reported in the current opinion. The Panel estimated the exposure to steviol glycosides (E 960a–d) based on the methodology described in the protocol for the dietary exposure to sweeteners under re-evaluation (EFSA, 2020) (see Section 2.2).

Food categories considered for the exposure assessment of steviol glycosides (E 960a–d)

Dietary exposure was assessed for the population 'consumers only', following the protocol for assessing exposure to sweeteners (EFSA, 2020). Using this protocol means that facets[16] were used to identify eating events referring to foods reported to contain sweeteners (i.e. 'energy reduced or with no added sugar foods') and to foods related to the specific restrictions/exceptions defined in the legislation for the use of steviol glycosides (E 960a–d). However, as defined in the new protocol (EFSA, 2020), facets were not used to identify relevant eating events for foods belonging to five food categories:

- FC 11.4 'Table-top sweeteners',
- FC 05.3 'Chewing gum',
- Gum drops in FC 05.2 'Other confectionery including breath refreshening microsweets',
- Energy drinks in FC 14.1.4 'Flavoured drinks',
- Vitamin and mineral supplements in FC 17 'Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children'.

These five food categories are expected to be major contributors to the exposure to sweeteners according to the literature and cover a relatively high percentage of products labelled to contain at least one sweetener. Thus, all eating events belonging to these food categories were included in the dietary exposure assessment of steviol glycosides (E 960a–d).

[15]Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.

[16]Facets are collections of terms describing properties and aspects of foods from various perspectives (ingredient, process, packaging, …). They are used to add further details about the foods reported in the dietary surveys.

In the exposure assessment of steviol glycosides (E 960a–d) from the currently authorised uses, FC 7.2 'Fine bakery wares' was excluded, because the Panel considered the only authorised use 'only *essoblaten* – wafer paper' as niche and the inclusion of all wafers in the assessment was considered to result in large overestimation of the exposure to the sweetener.

For FC 11.4 'Table-top sweeteners', for which the current MPL is QS, a level of 12,000 mg/kg steviol glycosides (E 960a–d), expressed as steviol equivalents, retrieved from an exposure assessment of steviol glycosides by EFSA, performed in 2011 (EFSA, 2011a), was used for the exposure calculations.

An overview of the concentration levels of steviol glycosides (E 960a–d) used in the exposure assessment is provided in Annex A, Table A.1.

3.3.2 | Exposure estimates

For this opinion, the exposure was estimated using the regulatory maximum level exposure assessment scenario (see Section 2.2) using the current MPLs and using the current MPLs together with the proposed extension of use and proposed increases in the MPLs. Exposure was estimated by multiplying the (proposed) use level for the FC with the average daily consumption of foods belonging to that FC at individual level. Exposure estimates were summed across foods per individual and subsequently divided by the individual's body weight resulting in a distribution of daily individual average exposures per kg body weight. Based on these distributions, the mean and 95th percentiles of exposure were calculated per survey and per population group. Mean estimates based on dietary surveys/population groups with less than six consumers and 95th percetiles estimates with less than 60 consumers are not presented (EFSA, 2011a, 2011b).

In this evaluation, as stated in Section 5.2.3 in the protocol (EFSA, 2020), the dietary exposure was assessed for consumers only of at least one food category that could contain steviol glycosides (E 960a–d).

Dietary exposure to steviol glycosides (E 960a–d)

The summary of the dietary exposure (results per population group) to steviol glycosides (E 960a–d) is provided in Table 5. Detailed results per population group and survey are presented in Annex A, Table A.2.

TABLE 5 Summary of dietary exposure to steviol glycosides (E 960a–d) as a food additive in six population groups of consumers only (minimum-maximum across the dietary surveys in mg steviol equivalents/kg bw per day for the current uses and use levels and for the proposed extension of use and increase in the MPLs).

Estimated exposure (mg/kg bw per day)	Infants[a] (12 weeks-11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario - consumers only						
Mean	0.1–0.8	0.03–1.3	0.1–0.8	0.1–0.5	0.1–0.4	0.02–0.2
95th percentile	0.3–**4.1**	0.2–**4.8**	0.3–2.4	0.2–1.5	0.3–1.1	0.2–0.9
Regulatory maximum level exposure assessment scenario including the proposed extension of use and increase in MPLs – consumers only						
Mean	0.1–0.8	0.04–1.8	0.1–1.2	0.1–0.7	0.1–0.4	0.02–0.3
95th percentile	0.3–**4.1**	0.2–**6.9**	0.3–4.0	0.2–2.4	0.3–1.7	0.3–1.1

Abbreviation: MPLs, maximum permitted levels.

[a]The Panel noted that the highest mean and highest 95th percentile of exposure for infants were the same in both exposure assessments. These exposure estimates were from a survey in which no consumption was recorded for the food categories for which an extension of use was proposed, as well as increase in maximum permitted levels.

At the current MPLs, the lowest and highest estimates of mean exposure to steviol glycosides (E 960a–d), expressed as steviol equivalents, were 0.02 mg/kg bw per day in the elderly and 1.3 mg/kg bw per day in toddlers, respectively. The lowest and highest estimates of the 95th percentile of exposure to steviol glycosides, expressed as steviol equivalents, were 0.2 mg/kg bw per day in toddlers, adolescents and the elderly, and 4.8 mg/kg bw per day in toddlers, respectively.

Considering also the proposed extension of use and increase in MPLs (see Table 3), the lowest estimate of mean exposure to steviol glycosides (E 960a–d), expressed as steviol equivalents, was 0.02 mg/kg bw per day in the elderly and the highest 1.8 mg/kg bw per day in toddlers. The lowest and highest estimates of the 95th percentile of exposure to steviol glycosides (E 960a–d), expressed as steviol equivalents, were 0.2 mg/kg bw per day in toddlers and adolescents and 6.9 mg/kg bw per day in toddlers, respectively.

Main food categories contributing to exposure based on the exposure estimates following the sweetener protocol

At the current MPLs, the main food category contributing to the total mean exposure estimates for all population groups was FC 12.6 'Sauces'. FC 14.1.4 'Flavoured drinks' was also an important contributor for children, adolescents, adults and the elderly. Additionally, F 09.2 'Processed fish and fishery products including molluscs and crustaceans' contributed notably

to the exposure for adults and the elderly. Finally, FC 04.2.2 'Fruits and vegetables in vinegar, oil and brine' was also an important contributor to exposure for the elderly.

Considering also the proposed extension of use and increase in MPLs, the main food category contributing to the total mean exposure estimates for all population groups except infants was FC 12.6 'Sauces'. FC 14.1.4 'Flavoured drinks' was also an important contributor for children, adolescents, adults and the elderly. The contribution of FC 14.1.4 'Flavoured drinks' to total dietary exposure increased as a result of the proposed increase in MPLs. Additionally, for toddlers, adults and the elderly, FC 04.2.2 'Fruits and vegetables in vinegar, oil and brine' and FC 09.2 'Processed fish and fishery products including molluscs and crustaceans' contributed greatly to the exposure.

Detailed results by population group and survey are presented in Annex A, Table A.5.

Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties were considered and summarised in Table 6.

TABLE 6 Qualitative evaluation of influence of uncertainties on the dietary exposure assessment of steviol glycosides (E 960a–d).

Sources of uncertainties	Direction[a]
Consumption data	
Different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Underreporting of food descriptors (facets) concerning the presence or potential presence of sweeteners	–[b]
Use of the additive in table-top sweeteners added to home made products might not be captured for some surveys	
Use level of the additive in home made products may differ from industrial counterpart	+/–
Use of the additive in table-top sweeteners regardless of the type of the sweetener consumed	+
Methodology	
Methodology used to estimate high percentiles (95th) of long-term (chronic) exposure based on data from food consumption surveys covering only a few days	+
Concentration data	
Correspondence of (proposed) use levels to the food items in the EFSA Comprehensive Database: uncertainties to which types of food the levels refer	+/–
Use levels considered applicable to all foods within the entire food category, whereas most probably not all food belonging to a proposed food categories will contain steviol glycosides as a food additive	+
Food categories selected for the exposure assessment of the additive from the currently permitted uses and use levels: exclusion of essoblaten and wafer paper due to use in this food category being considered niche	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception ($n = 3$ for both MPL scenario and for MPL with extension of use scenario out of 32 food categories)	+
Regulatory maximum level exposure assessment scenario and regulatory maximum level exposure assessment scenario considering the currently authorised uses (MPL) and the extension of use: – exposure calculations based on the MPL according to Annex II to Regulation (EC) No 1333/2008 and proposed use levels	+

Abbreviation: MPLs, maximum permitted levels.

[a]+, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

[b]Direction of the uncertainty is based on the assumption that the underlying population of consumers does not change.

The Panel considered overall that the uncertainties identified resulted in an overestimation of the exposure to steviol glycosides (E 960a–d) in European countries available in the EFSA Comprehensive database in both the regulatory maximum level exposure assessment scenario and the regulatory maximum level exposure assessment scenarios considering the proposed extension of use and increase in MPLs.

Estimates of exposure using the methodology as in the previous steviol glycosides exposure assessment (EFSA ANS Panel, 2015b)

Additionally, the Panel carried out a dietary exposure assessment using the methodology considering the general population as performed in the previous exposure assessment for steviol glycosides (EFSA ANS Panel, 2015b) (Annex A, Table A.3 and A.4). The exposure estimates based on the current MPLs and on the current MPLs together with the proposed extension of use and increase in MPLs following this methodology were slightly lower than those obtained with the methodology described in the protocol for sweeteners (Table 4). At the current MPLs, the lowest and highest estimates of mean exposure to steviol glycosides (E 960a–d), expressed as steviol equivalents, were 0.0004 mg/kg bw per day in infants and 1.1 mg/kg bw per day in toddlers, respectively, while the corresponding 95th percentiles of exposure were 0 mg/kg bw per day in infants and 4.2 mg/kg bw per day in toddlers. Considering also the proposed extension of use and increase in MPLs, the lowest and highest estimates of mean exposure to steviol glycosides (E 960a–d), expressed as steviol equivalents, were

0.0004 mg/kg bw per day in infants and 1.6 mg/kg bw per day in toddlers, respectively, and those for the 95th percentiles of exposure were 0 mg/kg bw per day in infants and 6.6 mg/kg bw per day in toddlers, respectively. The Panel noted that the slightly lower estimates of exposure by considering also 'non-consumers of products with steviol glycosides (E 960a–d)' was due to the fact that non-consumers of the products containing steviol glycosides (E 960a–d) are not exposed to steviol glycosides, and therefore lower the mean and 95th percentile of exposure.

3.4 | Biological and toxicological data

In addition to the proposed changes to the currently permitted uses, the applicant requested an increase of the current ADI (4 mg steviol equivalents/kg bw per day) for steviol glycosides (E 960a–d) (Documentation provided to EFSA No. 1). This request is based on the proposal to modify the standard default factor by applying a Chemical-Specific Adjustment Factor (CSAF) to account for toxicokinetic difference between rats (species from which the NOAEL used for the derivation of the current ADI was established) and humans. According to the applicant, the default toxicokinetic component of 4 can be modified to 1, based on C_{max} values, or to 2.8, based on Area Under the Curve (AUC) values, resulting in an ADI of 16 or 6 mg steviol equivalents/bw per day, respectively. The proposals were based on the results of the study carried out in rats and human from Roberts et al. (2016), which are described in detail below.

Within the application dossier, scientific publications considered by the applicant relevant to the safety assessment of steviol glycosides were submitted (Documentation provided to EFSA No. 1).

The Panel noted that the majority of the references provided were studies already considered in previous evaluations of steviol glycosides (EFSA ANS Panel, 2010, 2015a, 2015b, 2018; EFSA FAF Panel, 2019, 2020, 2021, 2022a, 2023). Some additional publications were submitted within the current dossier, however these were not considered relevant by the Panel for the current assessment (e.g. purity of the substance not reported, investigation of beneficial effects).

3.4.1 | Absorption, distribution, metabolism and excretion (ADME)

Data on ADME of some of the steviol glycosides currently listed in the EU specifications were considered and summarised in previous EFSA opinions on this food additive (EFSA ANS Panel, 2010, 2015a, 2015b, 2018; EFSA FAF Panel, 2019, 2020, 2021, 2022a, 2022b, 2023). A detailed summary of all the studies previously considered is reported in Appendix A.

According to these opinions, stevioside and steviol glycosides are not hydrolysed by digestive enzymes of the upper gastrointestinal tract. After entering the colon, steviol glycosides are subject to degradation by the gut microbiota, resulting in the release of the aglycone steviol which is then absorbed. In rats and humans, absorbed steviol is glucuronidated; steviol glucuronide is then excreted in the urine and partly via bile into the faeces.

The microbial hydrolysis of different steviol glycosides preparations, in particular rebaudiosides A, B, C, D, E, F, M, steviolbioside and stevioside (with different purity levels or purity not specified) has been investigated in vitro with human faecal incubations (Purkayastha et al., 2014, 2015, 2016; Purkayastha & Kwok, 2020). The results demonstrate efficient deglycosylation of these steviol glycosides to the aglycone in the presence of colonic microbiota collected from adults or children.

In vitro metabolic studies in human faecal homogenate samples incubated with different steviol glycosides preparations have been previously assessed by the Panel for the evaluation of several proposed amendments to the specifications of the food additive steviol glycosides (E 960) (EFSA FAF Panel, 2019, 2020, 2021, 2022a, 2022b, 2023). In all these studies, nearly complete deglycosylation to the aglycone was shown to occur within the first 12 h of incubation.

Kinetic studies conducted in rats and humans provide evidence for the hydrolysis of steviol glycosides (stevioside, rebaudioside A) in the lower intestine (caecum and colon in rodents) to steviol which is then absorbed and further metabolised to glucuronides. The results of studies in rats with radiolabelled stevioside and rebaudioside A (Nakayama et al., 1986; Roberts & Renwick, 2008) or oral dosing of humans with unlabelled steviol glycosides (Simonetti et al., 2004; Geuns et al., 2006, 2007; Wheeler et al., 2008) were previously summarised by EFSA ANS Panel (2010).

In male and female rats, single oral doses of ^{14}C-compounds (5 mg/kg bw of rebaudioside A or 4.2 mg/kg bw stevioside) were hydrolised to steviol which was extensively and rapidly absorbed with plasma concentration – time profiles following similar patterns for both steviol glycosides (Roberts & Renwick, 2008). Elimination of radioactivity from plasma was essentially complete within 72 h, although the plasma kinetic data indicated a sex difference in C_{max} and AUC for total radioactivity. The predominant radioactive component in plasma was steviol, with far lower amounts of steviol glucuronides, the predominant form in bile. The vast majority (≥ 97%) of the radioactivity was eliminated in the faeces within 48 h whilst urinary excretion accounted for less than 2% of the administered dose. Overall, data on toxicokinetics and metabolism in rats indicated that stevioside and rebaudioside A were handled in a similar manner.

A study on kinetics in humans used the same doses as Roberts and Renwick (2008) of unlabelled steviol glycosides used in rats: Wheeler et al. (2008) conducted a double-blind, cross-over study with single oral doses of rebaudioside A (98.7% purity, 5 mg/kg bw) and stevioside (96.6% purity; 4.2 mg/kg bw) in eight healthy adult male volunteers, assessing the kinetics by analysis of steviol and steviol glucuronide in plasma collected between 0.5 and 72 h post administration. Free steviol was only found (≥ limit of quantification (LOQ) of 100 ng/mL) in one individual at one time-point, whilst steviol glucuronide appeared in plasma of all subjects after administration of rebaudioside A or stevioside, with median plasma peak times at 12 and 8 h post-dose, respectively. Two plasma peaks of steviol glucuronide occurred at 6–12 and 24 h post-dose.

This metabolite was eliminated from the plasma with similar half-life values of approximately 14 h for both compounds. After rebaudioside A dosing, a lower steviol glucuronide maximum plasma concentration (1472 ng/mL) was observed than after administration of stevioside (1886 ng/mL). Yet, there was no significant difference between the geometric mean AUC (0 – *t*) values for steviol glucuronide after administration of rebaudioside A (30.8 ng × h/mL) or of stevioside (34.1 ng × h/mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72 h collection period, accounting for 59% and 62% of the rebaudioside A and stevioside doses, respectively. No steviol glucuronide was detected in faeces. This kinetic analysis indicated that rebaudioside A and stevioside underwent similar metabolic and elimination pathways in humans with steviol glucuronide excreted primarily in the urine and steviol in the faeces.

Roberts et al. (2016) investigated single dose kinetics of stevioside in rats and humans with the aim to generate data in support of a chemical-specific inter-species toxicokinetic adjustment factor.

In the animal study, two groups of male and female Sprague–Dawley (Crl:CD(SD)) rats (72 animals/sex per dose group) were administered by gavage single oral doses of 40 or 1000 mg/kg bw of stevioside (≥ 95% purity) (equivalent to 16 or 396 mg steviol equivalents/kg bw). On the day of dosing, blood samples were collected from six animals/sex/time-point/group prior to dosing and at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 h after dose administration. Plasma samples were analysed for steviol and steviol glucuronide concentration using a validated liquid chromatography tandem mass spectrometry (LC–MS/MS) method. The lower limit of quantification (LLOQ) for steviol and steviol glucuronide was 20 and 4 ng/mL, respectively. After stevioside administration at 40 mg/kg bw, free steviol was detectable from 2 h post-dose and reached C_{max} between 4 and 6 h post-dose (76 ± 15 ng/mL in males and 87 ± 17 ng/mL in females). Steviol was no longer detectable at 36 h. At 1000 mg/kg bw, steviol was detected from 1 h post-dose reaching the C_{max} between 6 and 12 h post-dose followed by a strong decrease until 72 h post-dose. Steviol glucuronide was found at higher levels in plasma than steviol throughout the study. In the 40 mg/kg bw dose group, the toxicokinetic parameters for steviol and steviol glucuronide were similar for male and female rats, yet with higher C_{max} and AUC_{last} values in females. In the 1000 mg/kg bw dose group, the difference between sexes was more pronounced, C_{max} in female rats being three to five-fold higher than in males and AUC_{last} values, nearly three-fold higher in females than in male rats.

In an open-label, single dose trial in humans, 10 healthy adult male volunteers were provided 40 mg/kg bw of stevioside (equivalent to 16 mg steviol equivalents/kg bw) in an aqueous solution (Roberts et al., 2016). Blood samples were collected at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after dosing and analysed by the same LC–MS/MS method applied in the rat study. The data of one subject were excluded from the analysis. The plasma C_{max} of steviol (77 ± 17 ng/mL) was reached between 19 and 20 h post-dose. Free steviol was below the LOQ (20 ng/mL) at 48 h post-dose. Steviol glucuronide was present at far higher levels throughout the observation period.

The authors then compared the kinetic parameters derived for rats and humans after administration of the same stevioside dose: the C_{max} for steviol in humans occurred later than that in rats which is expected due to a longer transit time in the human gut. The AUC values for steviol in humans (1630–1680 ng × h/mL for $AUC_{0.75\text{-}72h}$) were approximately 2.8-fold higher than in rats dosed with 40 mg/kg bw stevioside (581 and 605 ng × h/mL AUC_{last} in male and female rats).

Roberts et al. (2016) considered justified the use of only male human subjects as the results of the in vitro metabolic studies in human faecal homogenates indicated that there were no gender or ethnicity-related differences with respect to the hydrolysis of various steviol glycosides to steviol (Purkayastha et al., 2014, 2015, 2016). However, the Panel noted that in the rat study by Roberts et al. (2016) and in an earlier study with radiolabelled steviol glycosides (Roberts & Renwick, 2008) some sex-specific differences in the kinetics were observed (see Appendix A, Table A.2).

In addition, the Panel noted that some, but not all, of the nine volunteers in the study had quantifiable steviol plasma levels and that the standard deviations were quite high. The Panel noted that the AUC (0–72 h) value for steviol glucuronide reported by Wheeler et al. (2008), once adjusted for dose, is 2.5-fold higher than the value reported by Roberts et al. (2016), which may indicate non-linearity in kinetics. Furthermore, plasma-concentration time curves (figure 3a,b in Wheeler et al. (2008) and figure 6 in Roberts et al. (2016)) show considerable inter-individual variability.

Overall, considering the above points, particularly because of the low number of only nine male volunteers with considerable inter-individual variability in the human study by Roberts et al. (2016), the Panel considered that the available data are not sufficiently robust to support the use of a CSAF as proposed by the applicant in place of the standard default factor applied to the derivation of the current ADI for steviol glycosides (E 960a–d).

4 | DISCUSSION

The present opinion deals with the evaluation of the proposed changes to the currently permitted uses of the already authorised food additive steviol glycosides (E 960a–d). The applicant is also proposing a revision of the current ADI for E 960a–d. The Panel noted that no new biological and toxicological data were provided in support of this request, but that the applicant referred to studies already considered in several previous opinions by the ANS and FAF Panel (EFSA ANS Panel, 2010, 2015a, 2015b, 2018; EFSA FAF Panel, 2019, 2020, 2021, 2022a, 2022b, 2023).

Currently, the food additive steviol glycosides (E 960a–d) is authorised in the EU as sweetener for use in 32 different food categories (representing 42 uses) with MPLs ranging from 20 to 3300 mg steviol equivalents/kg. The use in FC 11.4 'Table-top sweeteners' in their various forms is authorised at QS (see Section 3.2, Table 3). The applicant requested an extension of use for steviol glycosides (E 960a–d) for four new uses within the already authorised FC 7.2 'Fine bakery wares'. Additionally, an increase in the MPLs was proposed for three uses within the already authorised FCs 14.1.4 'Flavoured drinks' and 14.1.3

'Fruit nectars as defined by Directive 2001/112/EC'. In all food categories for which an extension of use or increase in MPLs was requested, the uses are restricted to foods that are energy reduced or with no added sugar.

The latest regulatory maximum level exposure assessment scenario to steviol glycosides was performed by EFSA in 2015 (EFSA ANS Panel, 2015b) based on food consumption data available at that time in the EFSA Comprehensive Database and the standard methodology for assessing the exposure in the general population. A new methodology for performing dietary exposure assessments of sweeteners has been developed and implemented by the Panel within the context of the re-evaluation of already authorised sweeteners under Regulation (EU) No 257/2010 (EFSA, 2020). The Panel considered this new methodology also applicable to the present assessment of steviol glycosides (a sweetener) and applied it to update the exposure assessment of steviol glycosides (E 960a–d) at the currently MPLs, and when taking into account the proposed extension of use and increase in MPLs (see Section 2.2).

In the previous EFSA opinion (EFSA ANS Panel, 2015b), exposure estimates were lower than the dietary exposure calculated in the current opinion with the 'sweeteners' methodology considering only the currently authorised uses. However, the 95th percentile of exposure of 4.3 mg steviol equivalents/kg bw per day for toddlers reported in 2015 already slightly exceeded the ADI of 4 mg steviol equivalents/kg bw per day.

In the current opinion, the 95th percentiles of exposure exceeding the current ADI, resulting from the currently authorised uses, are 4.1 and 4.8 mg/kg bw per day for infants and toddlers, respectively. Considering also the proposed extension of use and increase in MPLs, these exposure estimates are 4.1 and 6.9 mg/kg bw per day for infants and toddlers, respectively, which would lead to a greater exceedance of the ADI in toddlers compared to the currently authorised uses. In both cases, these two exposure estimates were based on data from one country per population. The increase of the highest 95th percentile of exposure for toddlers was entirely attributable to the consumption of energy reduced or no added sugar flavoured drinks for which increased MPLs were proposed.

In this opinion, the exposure was estimated considering all permitted and proposed food categories to contain steviol glycosides (E 960a–d) at the MPL (or proposed maximum level). The Panel noted that this regulatory scenario resulted in an overestimation of the actual dietary exposure (see Section 3.3.2, Uncertainty analysis).

When considering the proposed changes to the currently permitted uses, the impact on the dietary intake of steviol glycosides (E 960a–d) appears to be small and the Panel noted that overall, the exposure estimates remain at or below the current ADI of 4 mg steviol equivalents/kg bw per day for all population groups, with the exception of infants and toddlers at the maximum range of the 95th percentile of exposure (i.e. 4.1 and 6.9 mg steviol equivalents/kg bw per day, respectively) (see also Section 3.3.2, Table 5).

According to the applicant, the current ADI for steviol glycosides (E 960a–d) could be increased from the currently established 4 to 6 or 16 mg mg/kg bw per day, expressed as steviol equivalents. This request was based on the proposal to modify the standard default factor by applying a CSAF to account for toxicokinetic difference between rats and humans. Based on the results of the studies from Roberts et al. (2016), the applicant is of the opinion that the default toxicokinetic component of 4 can be modified to 1, based on C_{max} values, or to 2.8, based on AUC values, resulting in an ADI of 16 or 6 mg steviol equivalents/bw per day, respectively.

Considering the currently available dataset on ADME of steviol glycosides (E 960a–d) (see Section 3.4.1 and also Appendix A), the Panel considered that data are not sufficiently robust to support the use of a CSAF as proposed by the applicant in place of the standard default factor applied to the derivation of the current ADI for steviol glycosides (E 960a–d).

5 | CONCLUSIONS

The Panel concluded that there is insufficient justification to increase the current ADI for steviol glycosides (E960a–d) of 4 mg/kg bw per day (expressed as steviol equivalents).

With respect to the proposed extension of use and increase in the MPLs, the Panel concluded that the calculated, conservative, dietary exposure would result in an increased exceedance of the ADI for toddlers at the 95th percentile.

6 | DOCUMENTATION AS PROVIDED TO EFSA

1. Application to modify the conditions of use of the already authorised food additive steviol glycosides (E 960). Technical Dossier. January 2024. Submitted by International Stevia Council (ISC).[9]

ABBREVIATIONS

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AMP	adenosine 5′-phosphoric acid
ANS Panel	Panel on Food Additives and Nutrient Sources added to Food
AUC	area under the curve
bw	body weight
C_{max}	Maximum serum concentration

CEP Panel	Panel on Food Contact Materials, Enzymes and Processing Aids
CMP	cytidine 5′-monophosphoric acid
CSAF(s)	chemical-specific adjustment factor(s)
CONTAM Panel	Panel on Contaminants in the Food Chain
ESI-MS	electrospray ionisation mass spectrometry
FAF Panel	Panel on Food Additives and Flavourings
FAIM	Food Additives Intake Model
FAO/WHO	Food and Agriculture Organization/World Health Organization
FC	Food Categories
FCS	Food Categorization System
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
GC/MS	gas chromatography–mass spectrometry
GMO	genetically modified organisms
GRAS	generally recognised as safe
HPLC	high performance liquid chromatography
HPLC–MS/MS	high performance liquid chromatography–mass spectrometry
HPLC-UV	high performance liquid chromatography-ultraviolet
IR	infrared
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC/APCI-MS	liquid chromatography/atmospheric pressure chemical ionisation mass spectrometry
LC/ESI/MS	liquid chromatography-electrospray ionisation–mass spectrometry
LC/MS	liquid chromatography–mass spectrometry
LC/MS/MS	liquid chromatography–mass spectrometry–mass spectrometry
LC-UV	liquid chromatography-ultraviolet
LLOQ	lower limit of quantification
LOQ	limit of quantification
LSC	liquid scintillation
MPLs	maximum permitted levels
MS	mass spectroscopy
NIH	United States National Institutes of Health
NMR	nuclear magnetic resonance
No	number
NOAEL	no observed adverse effect level
OD	optical density
qPCR	quantitative polymerase chain reaction
RP-HPLC	reversed-phase high-performance liquid chromatography
Reb	rebaudioside
RSD	relative standard deviation
SOP	standard operating procedure
TLC	thin-layer chromatography
UF	uncertainty factor
UGT	UDP glucosyltransferase
UV	ultraviolet

REQUESTOR

European Commission

QUESTION NUMBER

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Tabulated summary of studies on ADME

TABLE A.1 In vitro studies.

Reference	Test system	Substance/purity	Test conditions	Sample analysed	Analysis	Results	Conclusions by the study authors
Wingard Jr et al. (1980) (see Table A.2 for the in vivo study)	Microbial cell suspension from Sprague Dawley Rats	Stevioside (2.5 mg/mL); Rebaudioside A (3.0 mg/mL); Steviol (0.2 mg/mL) Purity not specified	2–6 days incubation	Anaerobic incubation of cecal bacterial cells	HPLC	Stevioside was completely transformed into steviol within 2 days Rebaudioside A was completely transformed into steviol after 6 days	Both stevioside and rebaudioside A can be degraded to steviol by microbiota in the mammalian lower bowel with a degradation rate ranging between 0.4 and 0.8 mg converted/h/g cecal contents
Compadre et al. (1988)	Forward mutation assays, using Salmonella typhimurium strain TM677	Steviol	Assays both in the presence and absence of the rat S9 metabolic activator	Steviol hepatic metabolism: Steviol was incubated under conditions known to mediate the mutagenic response, including the presence of the S-9 fraction derived from the livers of Aroclor 1254-pretreated rats	GC/MS	Very low conversion (about 0.3% after 2 h) of steviol into oxidative metabolites. The major pathway of mammalian metabolism of steviol was allylic oxidation, to produce 15α-hydroxysteviol	15-Oxosteviol, a product of oxidation of the major steviol metabolite, 15alpha-hydroxysteviol, was found to be a direct-acting mutagen. Although it was not detected among the products of steviol metabolism, it is possible that 15-oxosteviol is, in fact, formed during incubation, and, due to its high reactivity, it could be trapped by a component of the mixture, as for example a cysteine residue in the S-9 proteins

(Continues)

TABLE A.1 (Continued)

Reference	Test system	Substance/purity	Test conditions	Sample analysed	Analysis	Results	Conclusions by the study authors
Hutapea et al. (1997)	Incubation with digestive enzymes or intestinal microflora from humans Wistar rats Swiss albino mice Syrian golden hamsters	Stevioside (1 mg/mL or 2.5 mg/mL) Purity not specified	1 and 2 h incubation for human saliva and gastric secretion 5 or 30 min incubation for human salivary α -amylase or pancreatic α-amylase, respectively 1 or 2 h incubation for human pepsin or pancreatin, respectively 1 or 2 h incubation for rodents intestinal brush border membrane (BBM) enzymes 2 or 4 days incubation for intestinal microflora (rodents and human)	Reaction media from incubations with digestive enzymes Anaerobial cecal bacterial suspension of mice, rats and hamsters Anaerobic faecal bacterial suspensions of human subjects	HPLC	None of the tested enzymes (salivary α -amylase, pancreatic α-amylase, saliva, pepsin, gastric secretion, pancreatin and intestinal brush border membrane enzymes) of rodents and humans were able to digest stevioside. Caecal microflora of rodents as well as of humans was able to metabolise stevioside to steviol. Instead, stevioside was digested by intestinal microflora of all rodent species used and of humans. A transient formation of steviol-16,17α-epoxide was observed in mice caecal contents and human faeces	These results suggested that steviol is the major metabolite produced by caecal microflora from various animal species and humans
Koyama, Ohori, et al. (2003)	Human faecal homogenates	Stevia mixture and enzymatically modified stevia 0.2 or 10 mg/mL stevia mixture, rebaudioside A, stevioside, α-monoglucosylrebaudioside A or α-monoglucosylstevioside or 0.08 or 0.2 mg/mL steviol Highest purity commercially available or of HPLC grade	24 h incubation	Faecal homogenate samples	LC/ESI/MS and LC/MS/MS	Stevioside is hydrolysed into steviol and similarly monoglucosylstevioside is first α-deglucosilate and then hydrolysed to steviol Rebaudioside A and α-monoglucosylrebaudioside A are eventually hydrolysed to steviol mainly via stevioside No degradation of steviol was found after 24 h incubation period and no other peaks were found for steviol of stevia mixture and enzymatically modified stevia	Steviol is confirmed as the final metabolite from stevia mixture and enzymatically modified stevia after human intestinal metabolism

TABLE A.1 (Continued)

Reference	Test system	Substance/purity	Test conditions	Sample analysed	Analysis	Results	Conclusions by the study authors
Koyama, Sakai, et al. (2003) (see Table A.2 for the in vivo study)	Rat everted gastroinstestinal sac method absorption) and rat or human liver microsomes (metabolism)	Stevia mixture and steviol, 50 μM or 1 mM		Hepatic metabolism of steviol	LC-ESI-MS and HPLC-UV	No absorption of stevia mixture was observed, but significant absorption of steviol. The intrinsic clearance of steviol in human liver microsomes was 4-times lower than that found in rat liver microsomes All metabolites required a NADPH generating system, and were estimated to be hydroxy or dihydroxy metabolites, suggesting that cytochrome P450 may be involved in steviol oxidation in both human and rat liver microsomes	No major species differences in steviol metabolites between rats and humans. Absorption from the human intestine can be predicted to occur in an analogous manner to that from the rat intestine The Panel notes that the authors concluded that extrapolation of toxicity data on steviol glycosides from rats to humans would therefore be valid
Gardana et al. (2003)	Human faecal homogenates	Stevia extract containing either 85% w/w stevioside or 90% w/w rebaudioside A and pure steviolbioside	Stevioside or rebaudioside A 40 mg Up to 24/48 h incubation under anaerobic conditions	Faecal homogenates or isolated bacterial strains from faecal materials	HPLC	Stevioside was completely degraded into steviol in approximately 10 h Rebaudioside A was completely degraded into steviol in approximately 24 h Steviol remained unchanged for 72 h incubation period	Human faecal microbiota completely hydrolysed stevioside and rebaudioside A to the common aglycon steviol in 10 and 24 h respectively. Steviol was not further degraded
Geuns, Malheiros, et al. (2003) (see Table A.2 for the in vivo study)	Caco-2 cells for transport studies	Stevioside (purity > 96%), Reb A, – 1 mM, steviol - 30, 100, 300 and 1000 μM), benzoic acid – 1.5 and 10 mM	Absorptive transport and secretory transport	Intestinal transport characteristics of stevioside, Reb A and steviol	HPLC or UV spectrophotometry	In comparison to steviol (apparent permeability (P_{app}) value of 31.9 × 10−6 cm/s), the transport of stevioside and Reb A through the Caco-2 monolayers was very low (P_{app} values of 0.16 × 10−6 and 0.11 × 10−6 cm/s, respectively). The P_{app} value for the absorptive transport of steviol was ~ 7 times higher than that for secretory transport of steviol, suggesting a carrier-mediated transport There was a 33% inhibition of steviol transport by benzoic acid	In this intestinal model, the P_{app} value for steviol is 200 to 300-times higher than that for stevioside or rebaudioside A The discrepancy between the relatively high absorptive transport of steviol and the lack of steviol in the blood may be explained by the fact that in the Caco-2 study, steviol is applied as a solution facilitating the uptake, whereas in the colon steviol probably is adsorbed to the compounds present in the colon of which the contents is being concentrated by withdrawal of water

(Continues)

TABLE A.1 (Continued)

Reference	Test system	Substance/purity	Test conditions	Sample analysed	Analysis	Results	Conclusions by the study authors
Nikiforov et al. (2013) (see Table A.2 for the in vivo study)	Rat caecal contents, rat liver microsomes, and simulated gastric and intestinal fluids	Rebaudioside D (93.5% purity with the remaining 6.5% comprising predominantly steviol glycosides) and Rebaudioside A (98.9% purity): 0.2 mmol/L		Simulated GI fluids with or without enzymes, rat liver microsomes with or wihout NADPH and rat cecal contents	LC/MS/MS or LC/UV absorption spectroscopy	Incubation with simulated gastric and intestinal fluids (with/without addition of pepsin or pancreatin, respectively) resulted in only minimal degradation of the test compounds (but no formation of steviol) No decrease in the concentration of Reb A or Reb D in rat liver microsomes with/without NADPH. A low concentration of steviolbioside was detected in microsomal incubations with Reb A and Reb D w/o NADPH In the presence of cecal contents, Reb A and Reb D concentrations were decreased extensively by microflora hydrolysis, corresponding with increasing concentrations of stevioside and/or steviol	While Reb D and Reb A were not susceptible to substantial degradation by the simulated GI fluids or liver enzymes, incubation in the cecal contents matrix did result in extensive degradation; therefore, the cecum is expected to be the primary site of hydrolysis in both Reb A and Reb D
Purkayastha et al. (2014)	Human faecal homogenates from 6 female and 6 male volunteers	Rebaudioside B (96.5% purity), rebaudioside D (96.46% purity), rebaudioside M (97.33% purity), steviolbioside (96.81% purity) and stevioside (97.37% purity)	0.2 or 2 mg/mL of each rebaudioside for 24 or 48 h incubation period; anaerobic conditions; Pooled homogenates ($n = 3$ donors)	Human faecal homogenate	LC/MS	In vitro metabolism of rebaudioside A, B and D are comparable. They were degraded by gut microbioma to steviol within 24 h but with the majority metabolised at 8 h. Rebaudioside M was tested at 0.2 mg/mL and showed a slower rate of metabolism after 8 h incubation. Rebaudioside M was fully degraded at 16 h	Rebaudioside A, B, D and M also defined as parent steviol glycosides. No significant differences have been identified between rebaudioside A and other rebaudiosides except for the slower rate of degradation of rebaudioside D and M compared to rebaudioside A, which could be explain by the additional glucose moiety present in rebaudioside D and M

TABLE A.1 (Continued)

Reference	Test system	Substance/purity	Test conditions	Sample analysed	Analysis	Results	Conclusions by the study authors
Purkayastha et al. (2015)	Human faecal homogenates from six Caucasian and six Asian donors (male and female subjects)	Rebaudioside E and Rebaudioside A purity not specified; at 0.2 and 2 mg/mL	Rebaudioside E and Rebaudioside A: as described in Purkayastha et al. (2014)	Human faecal homogenate	LC/MS/MS	Rebaudioside E at 0.2 mg/mL was metabolised to steviolbioside and steviol within 24 h incubation period. The intermediate metabolites steviolbioside was found at 4 and 8 h samples Incubation of rebaudioside E at 2 mg/mL showed slower metabolism compared to 0.2 mg/mL	No significant differences were identified in the rate or extent of metabolism between rebaudioside A and E, which are both degraded to steviol within 24 h. A metabolic intermediate of rebaudioside E, steviolbioside was identified. Steviolbioside is then hydrolysed into steviol. No apparent gender or ethnicity differences were found in the rate of metabolism of any of the rebaudiosides
Purkayastha et al. (2016)	Human faecal isolate from 12 volunteers (6/sex)	Rebaudioside A, B, C, D, E, F, M, steviolbioside and dulcoside A at 0.2 and 2 mg/mL	Anaerobic conditions up to 48 h	Human faecal homogenate	LC/MS	At 2 mg/mL, rebaudioside A, B and D were metabolised up to 17% at 8 h and at up to 102% of hydrolysis at 24 h. The rate of metabolism at 0.2 mg/mL was quicker At 2 mg/mL, Rebaudioside C has slower rate of hydrolysis compared to Rebaudioside A At 2 mg/mL, Rebaudioside M achieved complete hydrolysis at 16 h At 2 mg/mL, Rebaudioside E has similar rate of hydrolysis than rebaudioside A Rebaudioside F showed solubility issue at 2 mg/mL. At 0.2 and 2 mg/mL rate of hydrolysis was slower than rebaudioside A	At 2 mg/mL rebaudioside A, B, C and D were completely hydrolysed within 48 h, whether at lower concentration of 0.2 mg/mL the hydrolysis rate was more rapid For rebaudioside A, B and E at 24 h ~ 50%–80% of hydrolysis takes place, whether for rebaudioside C and D the level of hydrolisis ranges from 20% to 46% and 60%–100% respectively at 24 h Rebaudioside F has the lowest rate of hydrolysis with a range of 2.9%–6.6% at 24 h

(Continues)

TABLE A.1 (Continued)

Reference	Test system	Substance/purity	Test conditions	Sample analysed	Analysis	Results	Conclusions by the study authors
Purkayastha and Kwok (2020)	Human faecal homogenates from males, females and paediatric donors	Steviol glycoside materials derived from extraction, bioconversion and glycosylation (Total Steviol Glycosides (TSG) > 95%): 0.2 mg/mL and 0.4 mg/mL	Incubation up to 72 h	Human faecal homogenate samples	LC/MS	At 12–24 h of incubation: • Almost complete (> 95%) deglycosylation of Reb M and Reb D • Almost complete formation of steviol Reb M showed slower deglycosylation compared to Reb D at 4 h of incubation At 12h of incubation: • The minor steviol glycosides (tested at 0.2 and 0.4 mg/mL) were rapidly deglycosylated, going in parallel with the rapid formation of the steviol metabolite All glycosylated steviol glycosides, produced by enzymatic glucosylation of Reb A, exhibited rapid and near complete deglycosylation, not associated with a formation of steviol Rapid disappearance of Reb A and the parallel formation of the steviol metabolite was observed Each incubation of minor steviol glycosides with brain-heart infusion (BHI) broth samples was assayed and the steviol metabolite was not detected at any of the incubation time points over 48 h	All steviol glycosides produced by different technology display the same metabolic fate in the presence of colonic microbiota collected from adults and children The more rapid disappearance of Reb D was consistently observed, indicating that the conversion of steviol glycosides involves a stepwise deglycosylation of glucose units from the glycoside Paediatric group showed slower conversion of Reb M and formation of steviol in the early stage of incubation, mainly due to the complex constitution and relative abundance of microbes in the faecal homogenates These in vitro data showed a slower rate of deglycosylation at a higher concentration of steviol glycosides used for tests. Also, the quality and composition of faecal homogenates may have contributed to the variation of deglycosylation time The evaluation of the stability of steviol glycosides in BHI with no faecal homogenate was intended to demonstrate that the formation of the steviol metabolite in pooled human faecal homogenates was mediated by a metabolism-based deglycosylation process

TABLE A.2 In vivo animal studies.

Reference	Species	Substance/purity	Dosing	Samples analysed	Analysis	Results	Conclusions by the study authors
Wingard Jr et al. (1980) (see Table A.1 for the in vitro study)	Sprague Dawley Rats	Steviol-17-[^{14}C]	Oral in suspension 1 mL (1.7 uCi, 0.7 mg) of Steviol-17-[^{14}C]	Urine and faeces	Radioactive level	Steviol-17-[^{14}C] was completely absorbed from the bowel after oral administration. The radioactivity was largely excreted in the urine	After oral administration Steviol-17-[^{14}C] is readily absorbed by mammalian lower bowel
Nakayama et al., 1986	Wistar Rats. males	[^{3}H] Stevioside Purity not specified	Stomach tube in suspension [^{3}H] Stevioside 125 mL/5mL/kg (10.4–120 uCi/kg)	Blood (0.5,1, 2 and every 2 h up to 120 h) urine and faeces (at 24 h interval for 120 h) Tissues distribution	Liquid scintillation counter Thin-layer radio chromatogram of metabolites	In blood: [^{3}H] Stevioside reached C_{max} at 8 h and then decreased slowly with an elimination half-life of 24 h In urine and faeces: Faecal excretion was 68% and urinary excretion was 2.3% at 120 h respectively. Tissues distribution: in the stomach and small intestine the highest levels were reached at 1 h and in the cecum the highest concentration was reached at 4 h Less than 1% of [^{3}H] Stevioside was found in other tissues after 1 h Radio chromatogram of metabolites showed that in the stomach and faeces steviol was the main metabolites. Other metabolites were detected but not identified	[^{3}H] Stevioside concentration reached higher concentration at 1 and 4 h after the administration in the GI tract and in the blood respectively. Most of the compound stays in the GI tract with low distribution in other organs. Stevioside is hydrolysed into steviol in the intestine and mainly excreted via faeces,
Koyama, Sakai, et al. (2003) (see Table A.1 for the in vitro study)	Sprague Dawley Rats: males	Stevia mixture (main components: rebaudioside A 28.8%, stevioside 17.0%, rebaudioside C 25.2%, dulcoside A 10.2%); Steviol (purity not specified)	Stevia mixture 125 mg/kg single dose Steviol 45 mg/kg single dose	Plasma samples	LC/MS/MS LC-ESI/MS	A steviol peak concentration of 18 µg/mL in plasma was observed 15 min after oral administration of steviol. After oral administration of the stevia mixture, the steviol concentration in plasma increased steadily over 8 h	Rapid absorption of steviol by the rat gastrointestinal tract was demonstrated. Data also suggested that the Stevia mixture components are first metabolised and then absorbed as steviol in the rat intestine.

(Continues)

TABLE A.2 (Continued)

Reference	Species	Substance/purity	Dosing	Samples analysed	Analysis	Results	Conclusions by the study authors
Geuns, Malheiros, et al., 2003 (see Table A.1 for the in vitro study)	Pigs (female)	Stevioside (≥ 96% purity) for 14 days	Stevioside 0.07 g/kg bw per day (6 treated animals and 6 controls)	Faeces Blood samples	HPLC TLC	No stevioside could be detected in the faeces samples. Analysis of steviol in the faeces demonstrated huge amounts of steviol, in concentrations around 853 ± 48 μg/g dry weight. No stevioside or steviol could be detected in the blood of the animals	Results suggested that stevioside was completely converted into steviol by the bacteria of the colon
Geuns, Augustijns, et al. (2003)	Broiler chickens (Cobb); laying hens (Hisex brown)	Stevioside (≥ 96% purity)	Single dose: Stevioside 714 mg/kg bw or 714 mg/kg bw (gavage) Repeated dose: Stevioside 667 mg/kg diet for 2 weeks	Excreta (2 h interval from 0 until 48h) Blood samples (at 2, 4, 6,6,24 and 48h)	HPLC TLC	Single dose Considering the degree of recovery for low concentrations of steviol, the percentage of stevioside to steviol conversion would amount to about 2%. Neither stevioside nor steviol could be found in the blood. Repeated dose: Only about 21.5 and 7.3% of the administered stevioside was found as steviol in the excreta of chickens and laying hens, respectively.	Stevioside was recovered largely unchanged within the excreta. Only about 2%, 21.5% and 7.3% was converted into steviol by chickens after intubation or chickens and laying hens via feed (repeated doses), respectively. Neither stevioside nor steviol could be found in the blood Results also suggest that steviol was not taken up easily through the epithelia of the cecum or the colon since steviol formed in the excreta was not found in blood

TABLE A.2 (Continued)

Reference	Species	Substance/purity	Dosing	Samples analysed	Analysis	Results	Conclusions by the study authors
Roberts and Renwick (2008)	Sprague Dawley Rats (male and female)	^{14}C-labelled Rebaudioside A, ^{14}C- stevioside and ^{14}C-steviol (> 97% radiochem. purity) and unlabelled compounds	Rebaudioside A 5 mg/kg bw Stevioside 4.2 mg/kg bw Steviol 1.6 mg/kg bw Single dose by gavage	Plasma, urine, bile and faeces	LSC HPLC TLC	Plasma concentration- time profiles for Reb A and stevioside showed similar patterns for total radioactivity; C_{max} and AUC (0–72h) were higher in female than in male rats The main metabolite in plasma was steviol and in lower amount steviol-glucuronide Steviol was the main component in faeces	Rebaudioside A and stevioside are metabolised to steviol by gut microbioma and excreted in the faeces. Steviol-glucuronide was present in the urine
Nikiforov et al. (2013) (see Table A.1 for the in vitro study)	Sprague Dawley Rats 2 groups (3 animals/sex per group)	Rebaudioside D (93.5% purity with the remaining 6.5% comprising predominantly steviol glycosides) and Rebaudioside A (98.9% purity)	2000 mg/kg bw per day Rebaudioside D and Rebaudioside A	Plasma and urine Plasma samples collected at day 1 and at day 21/22, analysed for concentrations of Reb D, Reb A and/or their major hydrolysis and conjugation products	HPLC-MS/MS (LLOQ 10 ng/mL for all analytes)	At all sampling times, the dominating metabolites were steviol glucuronide and steviol aglycone, with only very small levels of parent compounds in rat plasma of the Reb D or Reb A animals. The results indicate common hydrolysis pathways of Reb D and Reb A, and also low systemic exposure to all analytes, particularly for both parent compounds (≤ 1.5 µg/mL), with the majority of the absorbed dose present as free (≤ 12 µg/mL) or conjugated (≤ 40 µg/mL) steviol	The data obtained illustrate the common hydrolysis pathways of Reb D and Reb A, with hydrolysis occurring slowly so that almost all parent material passes through the gut unchanged, and the small amount of steviol that is formed as the ultimate hydrolysis product is rapidly and efficiently conjugated to form steviol glucuronide, which is excreted in the urine

(Continues)

TABLE A.2 (Continued)

Reference	Species	Substance/purity	Dosing	Samples analysed	Analysis	Results	Conclusions by the study authors
Roberts et al. (2016) (see Table A.3 for the in vivo human study)	Sprague Dawley Rats (6 males and 6 females/time point/group)	Stevioside (> 95% purity)	Stevioside at 40 and 1000 mg/kg bw single oral gavage	Blood samples (plasma) before and 0.5, 1, 2, 4, 6, 8, 12, 24 up to 72 h post dosing	LC/MS/MS	After stevioside administration at 40 mg/kg bw, free steviol was detectable from 2 h post-dose and reached C_{max} between 4 and 6 h post-dose. Steviol was no longer detectable at 36 h. At 1000 mg/kg bw steviol was detected from 1 h post-dose reaching the C_{max} between 6 and 12 h post-dose followed by a strong decrease until 72 h post-dose. Steviol glucuronide was found at higher levels in plasma than steviol throughout	In the 40 mg/kg bw dose group, the kinetic parameters for steviol and for steviol glucuronide were similar for male and female rats, yet with slightly higher C_{max} and AUC_{last} values for the latter. In the 1000 mg/kg bw stevioside dose group, this difference was more pronounced, with C_{max} and AUC_{last} values in females being three to five-fold, and nearly three-fold higher than in male rats

TABLE A.3 In vivo human studies.

Reference	Substance/purity	Dosing	Samples analysed	Analysis	Result	Conclusions by the study authors
Kraemer and Maurer (1994) (Abstract)	Stevioside (purity not specified)	Not reported	Urine and faeces	HPLC GC–MS	Only small amounts of unchanged stevioside were excreted in faeces. Stevioside was readily metabolised to its aglycone steviol by human intestinal flora. The absorbed steviol was conjugated in the liver to an acyl-glucuronide which was excreted via bile and urine. 60% of the applied amount of stevioside was recovered from urine as steviol glucuronide over a period of about 100 h. This metabolite was also detected in the faeces during this period. Part of the glucuronide was metabolised by the intestinal flora to steviol, which can be reabsorbed and undergo an entero-hepatic circulation. Further phase I or phase II metabolites were not found in urine or faeces	Data demonstrated that the main metabolite of stevioside in plasma is steviol glucuronide. Steviol glucuronide is then excreted primarily via the urine. In addition, the presence of multiple peaks in time of plasma concentrations of steviol glucuronide indicates enterohepatic circulation
Simonetti et al. (2004) (9 male subjects)	Stevia extract (Stevioside 85% w/w)	Stevioside 375 mg (5.16 mg/kg per bw) single dose	Plasma samples, urine and faeces	LC/MS	Stevioside was detected in seven out nine subjects at 1 h after dosing	Stevioside is absorbed after oral administration and steviol-glucuronide was the only metabolite found in plasma and in urine. Steviol was present only in the faeces suggesting its formation after intestinal microflora metabolism
Geuns et al. (2006) (10 volunteers: 5 females and 5 males)	Stevioside (> 97% purity)	Stevioside 250 mg capsules × 3 time a day for 3 days	Urine	IR MS NMR UV	Steviol-glucuronide was the only metabolite detected in the urine. No free steviol was found	It is suggested that stevioside is degraded into steviol by colon microbiota and then transported to liver where steviol-glucuronide is formed

(Continues)

TABLE A.3 (Continued)

Reference	Substance/purity	Dosing	Samples analysed	Analysis	Result	Conclusions by the study authors
Geuns et al. (2007)	Stevioside (97% purity)	Stevioside 250 mg capsules × 3 time a day (8 h intervals) for 3 days. (~ 10–15 mg/kg per day) Volunteers: 5 males and 5 females	Blood, urine and faeces after 3 days of administration	HPLC	Blood: No stevioside or free steviol detected. Steviol glucoronide (SV glu) was found up to 33 µg/mL. After enzymatic hydrolysis steviol's concentrations ranged from 0.7 to 21.3 µg/mL plasma Urine: No stevioside or free steviol detected. SV glu was 318 mg/24 h. After enzymatic hydrolysis steviol's concentrations ranged from 28 to 205 mg per 24 h urine Faeces: No stevioside or steviol conjugates detected. The amounts of free steviol ranged from 13 to 40 mg per 24 h faeces	No uptake was found of stevioside by the GI tract, or the amounts taken up were very low and below the detection limit of the UV detector. Stomach juice did not degrade stevioside. All the stevioside reaching the colon was degraded by micro-organisms into steviol (free steviol), the only metabolite found in faeces In faeces, besides free steviol, no other steviol metabolites or conjugates were detected. Steviol was excreted as SV glu in urine
Wheeler et al. (2008)	Rebaudioside A (98.7% purity) and Stevioside (96.6% purity)	Rebaudioside A, 5 mg/kg and stevioside, 4.2 mg/kg; Single doses; Randomised, double-blind, cross-over study	Plasma, urine and faeces (males only)	LC/MS/MS	Steviol was detected only in one out of eight subjects following administration of both substances whilst steviol-glucuronide was found in plasma of all treated subjects. Steviol-glucuronide derived from rebaudioside A showed a peak at 12 h, steviol-glucuronide derived from stevioside showed a peak at 8 h Steviol-glucuronide was excreted in the urine and was not found in the faeces	Rebaudioside A and stevioside are hydrolysed to steviol in the lower GI tract. The main circulating metabolite is Steviol- glucuronide which is then excreted in the urine Rebaudioside A has one additional glucose moiety that must be removed by hydrolysis in the lower gut and this can explain the lower C_{max} and longer T_{max} than stevioside
Roberts et al. (2016) (See Table A.2 for in vivo animal study)	Stevioside (> 95% purity)	Stevioside at 40 mg/kg bw	Blood (10 male subjects)	LC/MS/MS	C_{max} of steviol was reached between 19- and 20-h post-dose after administration of stevioside. Steviol was below limit of quantification (20 ng/mL) at 48 h post-dose. Steviol glucuronide was present at far higher levels throughout the observation period	In humans, C_{max} for steviol occurred later than in rats. AUC values for steviol in humans were ~ 2.8 fold higher than in rats dosed with 40 mg/kg bw while the C_{max} values were comparable

ANNEX A

Exposure data and estimates

Table A.1 – Concentration levels of steviol glycosides (E 960a–d) used in the exposure assessment scenarios (mg/kg or mg/L).
Table A.2 – Summary of total estimated exposure to steviol glycosides (E 960a–d) from use as a food additive for the current regulatory maximum permitted level (MPL) exposure assessment scenario and the regulatory MPL exposure assessment scenarios considering the proposed extension of use and increase in the MPLs per population group (consumers only) and survey: mean and 95th percentile (mg steviol equivalents/kg bw per day).
Table A.3 – Summary statistics of total estimated exposure to steviol glycosides (E 960a–d) from use as a food additive for the current regulatory maximum permitted level (MPL) exposure assessment scenario and the regulatory MPL exposure assessment scenarios considering the proposed extension of use and increase in the MPLs per population group (consumers only) and survey: mean and 95th percentile (mg steviol equivalents/kg bw per day).

Table A.4 – Summary of total estimated exposure to steviol glycosides (E 960a–d) from use as a food additive for the current regulatory maximum permitted level (MPL) exposure assessment scenario and the regulatory MPL exposure assessment scenarios considering the proposed extension of use and increase in the MPLs per population group (general population) and survey: mean and 95th percentile (mg steviol equivalents/kg bw per day).

Table A.5 – Main food categories contributing to exposure to steviol glycosides (E960a–d) from use as a food additive for the current regulatory maximum permitted level (MPL) exposure assessment scenario and the regulatory MPL exposure assessment scenarios considering the proposed extension of use and increase in the MPLs per population group (consumers only) and survey (> 5% to the total mean exposure).

Annex A can be found in the online version of this output (in the 'Supporting information' section).