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SCIENTIFIC OPINION

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Safety of the proposed amendment of the specifications of the food additive E960c(i) or E960c(ii)

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

The EFSA Panel on Food Additives and Flavourings (FAF Panel) provides a scientific opinion on the safety of the proposed amendment of the EU specifications of Rebaudioside M produced via enzyme-catalysed bioconversion (E960c(i) or E 960c(ii)), to include a different microorganism strain in the definition. Rebaudioside M is produced via enzymatic bioconversion from Stevia leaf extract, using the genetically modified yeast strain *K. phaffii* CGMCC 7539. The final product is composed mostly of rebaudioside M (> 97%) and a mixture of rebaudiosides A, B and D at various concentrations. The Panel considered that the proposed amendment of the specifications is justified with respect to the inclusion of a new microorganism strain, taking into account that the manufacturing process and the submitted analytical data are already covered by the parameters listed in the existing EU specifications for E 960c(i) and E 960c(ii). The Panel considered that it is in the remit of the risk managers to decide whether the proposed changes in the specifications should result in an amendment of the already existing EU specifications of E960c(i) or E960c(ii). Viable cells and DNA from the production strain are not present in the final product; hence, the manufacturing process does not raise a safety concern. The Panel considered that the proposed food additive has the same physicochemical characteristics of E 960c(i) or E 960c(ii); therefore, the biological and toxicological data considered in previous evaluations will also apply to the safety assessment of Rebaudioside M produced from *K. phaffii* CGMCC 7539. The Panel concluded that there is no safety concern with respect to the proposed amendment to the EU specifications of E 960c(i) or E 960c(ii) related to the use of the new genetically modified strain *K. phaffii* CGMCC 7539 in the manufacturing process of the food additive Rebaudioside M produced via enzyme-catalysed bioconversion.

KEYWORDS

enzyme-catalysed bioconversion, *Komagaetella phaffi*, Rebaudioside M, stevia, Steviol glycosides

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SUMMARY

The European Commission requested the European Food Safety Authority (EFSA) to perform a safety assessment to provide a scientific opinion on the safety of the proposed modifications of the food additive Rebaudioside M produced via enzyme modification of steviol glycosides from Stevia (E 960c(i)) and the assessment of possible confidentiality requests in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.[1]

However, in the course of the risk assessment, the applicant revised its original proposal and requested a proposed amendment of the EU specifications of E 960c(ii). The Panel therefore also considered this last proposal in its assessment.

The proposed food additive is produced by enzymatic bioconversion of purified steviol glycoside of the *Stevia rebaudiana* Bertoni plant using the genetically modified strain *Komagatella phaffii* CGMCC 7539, hereinafter referred to also as Rebaudioside M (Reb M) produced from *K. phaffii* CGMCC 7539. The manufacturing process foresees the enzymatic bioconversion of purified *Stevia rebaudiana* Bertoni leaf extract (> 95% of Rebaudioside A (Reb A)) using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified yeast *K. phaffii* CGMCC 7539 that facilitates the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds. The final product is composed mostly of Reb M (> 97%) and contains a mixture of the following glycosides at various concentrations: rebaudiosides A, B and D.

Reb M produced from *K. phaffii* CGMCC 7539 meets the ≥ 95% purity assay for Rebaudioside M produced via enzymatic modification as established in the EU Specifications for both E 960c(i) and E 960c(ii). The Panel considered that the proposed amendment of the specifications is justified with respect to the inclusion of a new microorganism strain, taking into account that the manufacturing process and analytical data in support of this application are already covered by the parameters listed in the existing EU specifications for E 960c(i) and E 960c(ii).

The Panel considered that it is in the remit of the risk managers to decide whether the proposed changes in the specifications should result in an amendment of the already existing EU specifications of E960c(i) or E960c(ii). The Panel recommended specifying in the proposed definition the production strain *K. phaffii* CGMCC 7539.

Based on the data provided on the water solubility, the Panel concluded there is no concern with regard to the potential presence of small particles, including nanoparticles, in the proposed food additive and considered that the risk assessment can be performed following the EFSA Guidance for submission for food additive evaluations (EFSA ANS Panel, 2012) without nanospecific considerations.

Concerning the purity parameters, the applicant provided information on the levels of residual solvents (i.e. ethanol and methanol) and toxic elements ((i.e. lead (Pb)), cadmium (Cd), mercury (Hg) and arsenic (As)) in five batches of the food additive, which were in line with the proposed specifications. The Panel noted that, based on the analytical data, the proposed maximum limits for lead, mercury, cadmium and arsenic are adequate. The Panel performed a risk assessment on the presence of these toxic elements in the food additive at the specification limits and concluded that their presence does not give rise to safety concerns.

The absence of kaurenoic acid was demonstrated in five batches of the proposed food additive, using a method of analysis with a limit of detection (LOD) of 0.007 mg/kg. Based on the available data and the calculated potential exposure to kaurenoic acid applying a Threshold of Toxicological Concern (TTC) TTC approach for this contaminant (TTC value for a potential DNA-reactive mutagen and/or carcinogen, (EFSA Scientific Committee, 2019)), a genotoxic concern derived from kaurenoic acid in the final product could be ruled out. However, the Panel recommends introducing a specific entry for kaurenoic acid in the final food additive specifications.

Based on the data provided by the applicant, the Panel noted that the demonstrated absence of viable cells/residual DNA of the production microorganism in the final product is captured in the proposed definition. Since no viable cells nor DNA of the production microorganism remained in the final product, the manufacturing process does not raise a safety concern. Moreover, the Panel noted that adequate analytical data supporting compliance with the provision for residual protein specifications were submitted by the applicant.

Because the proposed uses and use levels of Reb M produced from *K. phaffii* CGMCC 7539 are the same as the already authorised food additive steviol glycosides (E 960a-d), the applicant did not provide a dietary exposure assessment. The Panel considers that if steviol glycosides would be replaced by the proposed food additive, the exposure to Reb M (expressed as steviol equivalents) will not be higher than the last EFSA estimate of exposure to steviol glycosides (E 960a-d) (EFSA FAF Panel, 2024).

Based on the data provided, the Panel considered that Rebaudioside M produced by enzyme-catalysed bioconversion from Stevia leaf extract using the genetically modified yeast *K. phaffii* CGMCC 7539 has the same physicochemical characteristics of E 960c(i) and E 960c(ii); therefore, the available biological and toxicological data considered in the previous evaluations by the ANS and FAF Panel (EFSA ANS Panel, 2010, 2015, 2018; EFSA FAF Panel, 2019, 2020, 2021, 2022a, 2022b, 2023, 2024) will also apply to the safety assessment of Rebaudioside M produced from *K. phaffii* CGMCC 7539.

The Panel concluded that there is no safety concern with respect to the proposed amendment to the EU specifications of E 960c(i) or E 960c(ii) related to the use of the new genetically modified strain *K. phaffii* CGMCC 7539 in the manufacturing process of the food additive Rebaudioside M produced via enzyme-catalysed bioconversion.

[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.

1 | INTRODUCTION

The present opinion deals with the safety assessment of the food additive Rebaudioside M (Reb M) produced by enzyme-catalysed bioconversion from Stevia leaf extract using a genetically modified strain of the yeast *Komagatella phaffii* (named *K. phaffi* CGMCC 7539), hereinafter referred to also as 'Reb M produced from *K. phaffii* CGMCC 7539'.

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 on food additives.[1] 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 modification of the specification of the food additive Rebaudioside M obtained from stevia leaves.

The proposed specifications are identical to the specification of the food additive Rebaudioside M produced via enzyme modification of steviol glycosides from Stevia (E960c(i)), apart from the use of a different microorganism strain to produce UDP-glucosyltransferase and sucrose synthase enzymes.

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to perform a safety assessment to provide a scientific opinion on the safety of the proposed modifications of the food additive Rebaudioside M produced via enzyme modification of steviol glycosides from Stevia, and the assessment of possible confidentiality requests in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes, and food flavourings.[3]

1.1.3 | Interpretation of Terms of Reference

Despite the background to this mandate from the European Commission indicating a proposed amendment of the specifications for the food additive E 960c(i), in the course of the risk assessment the applicant revised its original proposal and requested a proposed amendment of the specifications of E 960c(ii) (Documentation provided to EFSA No. 4). The Panel therefore considered also this last proposal in its assessment.

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 food, 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 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 the application of a 100-fold uncertainty factor to the no observed adverse effect level (NOAEL) from a 2-year carcinogenicity study in the rat (EFSA ANS Panel, 2010). Following the EFSA assessment in 2015 (EFSA ANS Panel, 2015), rebaudioside D and M were included in the specifications for steviol glycosides (E 960).

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

[2]Commission 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.

[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.

overall metabolic fate of these steviol glycosides is the same, and therefore, it would be acceptable to use a read-across 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 is based on the conclusions from EFSA 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 *K. phaffii* UGT-A and 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, 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 that 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 regarded rebaudioside D produced by enzymatic bioconversion of purified *Stevia 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).

In September 2024, the FAF Panel completed the safety evaluation 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 ADI. In the context of that opinion, the Panel updated the dietary exposure estimate to steviol, applying a methodology that 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 exposure to steviol calculated for the currently permitted uses at the current maximum permitted use levels represents the latest available dietary exposure estimates under the regulatory maximum level exposure assessment scenario for the food additive (E 960a-d).

The Panel concluded that there was insufficient justification to increase the current ADI for steviol glycosides (E960a–d) of 4 mg/kg bw per day (expressed as steviol equivalents) (EFSA FAF Panel, 2024).

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 *Stevia 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 its 87th 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 (FAO and WHO, 2020). The framework adopted in 2019 was subsequently revised by JECFA at its 91st meeting in February 2021, and

[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 listedin 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.

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 'Enzyme modified Steviol Glycosides' (FAO and WHO, 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), and on additional information, following requests by EFSA, submitted by the applicant in August 2024 (Documentation provided to EFSA No. 2) in February 2025 (Documentation provided to EFSA No. 3) and in March 2025 (Documentation provided to EFSA No. 4).

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 03 March to 24 March 2025.[11] No comments were received.[12]

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), 'Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011) and the 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel, 2021) have been followed by the FAF Panel for evaluating the proposed change in manufacturing process and changes in the specifications. The EFSA Scientific Committee 'Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles' (EFSA Scientific Committee, 2021) has been followed by the FAF Panel.

Provided that Rebaudioside M produced by enzyme-catalysed bioconversion from Stevia leaf extract using the genetically modified yeast *K. phaffii* CGMCC 7539 has the same physicochemical characteristics of E960c(i) or E 960c(ii), the biological and toxicological data for E 960a-d are considered by the Panel to support the safety of the food additives produced with the enzymatic bioconversion step subject of the present application. Therefore, no additional biological and toxicological data are required. The previous evaluations by the ANS and FAF Panel (EFSA ANS Panel, 2010, 2015, 2018; EFSA FAF Panel, 2019, 2020, 2021, 2022a, 2022b, 2023, 2024) will also apply to the safety assessment of Rebaudioside M produced by enzyme-catalysed bioconversion from Stevia leaf extract using the genetically modified yeast *K. phaffii* CGMCC 7539.

3 | ASSESSMENT

3.1 | Technical data

3.1.1 | Identity of the proposed food additive

The current EU specifications for the entry E 960c(i) correspond to the food additive Rebaudioside M produced via enzymatic bioconversion of purified steviol glycoside of the *Stevia rebaudiana* Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified yeasts *K. phaffi* (formerly known as *Pichia pastoris*)

[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-2023-17532.

[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/a0lTk000003rj6v/pc1344.

[12]https://open.efsa.europa.eu/consultations/a0cTk00000CexStIAJ?search=EFSA-Q-2023-00546.

UGT-a and *K. phaffi* UGT-b, which facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds.

The food additive E 960c(ii) is produced via enzymatic conversion, starting instead from highly purified steviol glycoside rebaudioside A extracts (95% steviol glycosides) and using the same enzymes produced by the genetically modified strains of *Escherichia coli* (pPM294, pFAF170 and pSK401).

The subject of the present application covers a proposed change in the enzymatic bioconversion step applied to the manufacturing, involving the use of the enzymes UDP-glucosyltransferase [redacted], UDP-glucosyltransferase [redacted] and sucrose synthase [redacted] from the genetically modified strain *K. phaffii* CGMCC 7539.

Reb M produced from *K. phaffii* CGMCC 7539 meets the ≥ 95% purity assay for Rebaudioside M produced via enzymatic modification of steviol glycosides from stevia as established in the EU Specifications for both E 960c(i) and E 960c(ii).[13] The steviol glycosides mixture was characterised using the high-performance liquid chromatography-ultraviolet detection (HPLC-UV) method proposed by JECFA for measuring steviol glycosides (FAO and WHO, 2021). The identity of the rebaudiosides was assigned according to the retention times of the pure standards (Documentation provided to EFSA No. 2). The corresponding chromatograms for five batches of the proposed food additive were provided along with the Certificates of Analysis (CoAs) (Documentation provided to EFSA No. 1–2). The food additive obtained via the proposed change in the manufacturing process contains ≥ 95% of Reb M, i.e. 97.2 ± 0.9% ($n = 5$) and other steviol glycosides: Rebaudioside A (Reb A) (0.45 ± 0.13%), Rebaudioside B (Reb B) (1.34 ± 0.24%) and Rebaudioside D (Reb D) (0.42 ± 0.28%).

According to the applicant, Reb M produced from *K. phaffii* CGMCC 7539 is a white powder with a characteristic sweet taste and odour (Documentation provided to EFSA No. 1).

3.1.2 | Proposed amendment to the EU specifications

The applicant provided proposals for amending the existing specifications for the already authorised food additive E 960c(i) or E 960c(ii) and data to demonstrate that the food additive resulting from the proposed changes to the manufacturing process is compliant with the proposed specifications (Documentation provided to EFSA No. 1–3).

A comparison between the existing EU specifications for E 960c(i) 'Rebaudioside M produced via enzyme modification of steviol glycosides from Stevia', E 960c(ii) 'Rebaudioside M produced via enzyme modification of highly purified rebaudioside A stevia leaf extracts' and the changes proposed by the applicant is presented in Table 1.

[13]Commission 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.

TABLE 1 Current EU specifications as set in Regulation (EU) No 231/2012 for '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' and as proposed by the applicant (Documentation provided to EFSA No. 1–4).

	EU specifications for E 960c(i) Rebaudioside M produced via enzyme modification of steviol glycosides from stevia (Regulation (EU) No 231/2012)			**EU specifications for E 960c(ii) Rebaudioside M produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts (Regulation (EU) No 231/2012)**			**Proposed specifications by the applicant for Rebaudioside M produced by enzyme-catalysed bioconversion from Stevia leaf extract using the genetically modified strain *K. phaffii* CGMCC 7539**
Definition	Rebaudioside M is a steviol glycoside composed predominantly of rebaudioside M with minor amounts of other steviol glycosides such as rebaudioside A, rebaudioside B, rebaudioside D, rebaudioside I, and stevioside. Rebaudioside M is obtained via enzymatic bioconversion of purified steviol glycoside leaf extracts (95% steviol glycosides) of the *Stevia rebaudiana* Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified yeasts *K. phaffi* (formerly known as *Pichia pastoris*) UGT-a and *K. phaffii* UGT-b that facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds. After removal of the enzymes by solid–liquid separation and heat treatment, the purification involves concentration of the rebaudioside M by resin adsorption, followed by recrystallisation of rebaudioside M resulting in a final product containing not less than 95% of rebaudioside M. Viable cells of the yeasts *K. phaffii* UGT-a and *K. phaffii* UGT-b and their DNA shall not be detected in the food additive.			Rebaudioside M produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts is a steviol glycoside composed predominantly of rebaudioside M with minor amounts of other steviol glycosides such as rebaudioside A and rebaudioside D. Rebaudioside M is produced via enzymatic conversion of highly purified steviol glycoside rebaudioside A extracts (95% steviol glycosides) obtained from Stevia rebaudiana Bertoni plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified strains of E. coli (pPM294, pFAF170 and pSK401) that facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds. After removal of the enzymes by solid–liquid separation and heat treatment, the purification involves concentration of the rebaudioside M by resin adsorption, followed by recrystallisation of the steviol glycosides resulting in a final product containing not less than 95% of rebaudioside M. Viable cells of *E. coli* (pPM294, pFAF170 and pSK401) and their DNA shall not be detected in the food additive.			Rebaudioside M produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts is a steviol glycoside composed predominantly of rebaudioside M with minor amounts of other steviol glycosides, such as rebaudioside A, rebaudioside B and rebaudioside D. Rebaudioside M is produced via enzymatic conversion of highly purified steviol glycoside rebaudioside A extracts (95% steviol glycosides) obtained from *Stevia rebaudiana Bertoni* plant using UDP-glucosyltransferase and sucrose synthase enzymes produced by the genetically modified strains of E. coli (pPM294, pFAF170 and pSK401) or of *K. phaffii* (CGMCC 7539) that facilitate the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds. After removal of the enzymes by solid–liquid separation and heat treatment, the purification involves concentration of the rebaudioside M by resin adsorption, followed by recrystallisation of rebaudioside M resulting in a final product containing not less than 95% of rebaudioside M. Viable cells of *E. coli* (pPM294, pFAF170 and pSK401) or *K. phaffii* (CGMCC 7539) and their DNA shall not be detected in the food additive.
Chemical names	Rebaudioside **M**: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D- glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester			Rebaudioside **M**: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D- glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester			No proposed changes
Molecular formula	**Trivial name**	**Formula**	**Conversion factor**	**Trivial name**	**Formula**	**Conversion factor**	
	Rebaudioside M	$C_{56}H_{90}O_{33}$	0.25	Rebaudioside M	$C_{56}H_{90}O_{33}$	0.25	No proposed changes

TABLE 1 (Continued)

	EU specifications for E 960c(i) Rebaudioside M produced via enzyme modification of steviol glycosides from stevia (Regulation (EU) No 231/2012)			EU specifications for E 960c(ii) Rebaudioside M produced via enzymatic conversion of highly purified rebaudioside A stevia leaf extracts (Regulation (EU) No 231/2012)			Proposed specifications by the applicant for Rebaudioside M produced by enzyme-catalysed bioconversion from Stevia leaf extract using the genetically modified strain *K. phaffii* CGMCC 7539
Molecular weight and CAS No	**Trivial name**	**CAS Number**	**Molecular weight (g/mol)**	**Trivial name**	**CAS Number**	**Molecular weight (g/mol)**	
	Rebaudioside M	1220616-44-3	129,129	Rebaudioside M	1220616-44-3	129,129	No proposed changes
Assay	Not less than 95% rebaudioside M on the dried basis.			Not less than 95% rebaudioside M on the dried basis.			No proposed changes
Description	White to light yellow powder, approximately between 200 and 350 times sweeter than sucrose (at 5% sucrose equivalency).			White to light yellow powder, approximately between 150 and 350 times sweeter than sucrose (at 5% sucrose equivalency).			No proposed changes compared to E 960c(i)
Identification							
Solubility	Freely soluble to slightly soluble in water			Freely soluble to slightly soluble in water			No proposed changes
pH	Between 4.5 and 7.0 (1 in 100 solution)			Between 4.5 and 7.0 (1 in 100 solution)			No proposed changes
Purity							
Total ash	Not more than 1%			Not more than 1%			No proposed changes
Loss on drying	Not more than 6% (105°, 2 h)			Not more than 6% (105°, 2 h)			No proposed changes
Residual solvent	Not more than 5000 mg/kg ethanol			Not more than 5000 mg/kg ethanol			No proposed changes
Arsenic	Not more than 0.015 mg/kg			Not more than 0.015 mg/kg			No proposed changes
Lead	Not more than 0.2 mg/kg			Not more than 0.2 mg/kg			No proposed changes
Cadmium	Not more than 0.015 mg/kg			Not more than 0.015 mg/kg			No proposed changes
Mercury	Not more than 0.07 mg/kg			Not more than 0.07 mg/kg			No proposed changes
Residual protein	Not more than 5 mg/kg			Not more than 5 mg/kg			No proposed changes
Particle size	Not less than 74 μm [using a mesh #200 sieve with a particle size limit of 74 μm]			Not less than 74 μm [using a mesh #200 sieve with a particle size limit of 74 μm]			No proposed changes

The applicant submitted analytical data from the analyses of five batches of Reb M produced from *K. phaffii* CGMCC 7539 (Documentation provided to EFSA No. 1–3). Based on these data, the Panel considered that the proposed food additive is consistently produced and compliant with the proposed specifications, as outlined in Table 1.

The proposed food additive contains not less than 95% of Reb M. The Panel noted that the data submitted from the analysis of steviol glycosides in five batches of the proposed food additive (see Section 3.1.1) fulfil such declared purity (Documentation provided to EFSA No. 1). The Panel considered adequate the proposed purity assay of Reb M to account for not less than 95% of the final steviol glycoside product from *K. phaffi* CGMCC 7539 (dry basis).

The current EU specs for E 960c(i) and E960c(ii) describe the food additive as 'freely soluble to slightly soluble' and the applicant did not propose any changes with respect to this parameter. Upon EFSA's request, the applicant provided information on the water solubility for five batches of the proposed food additive, determined by applying the OECD Test guideline (TG) 105 (OECD, 1995) (shake flask method) (Documentation provided to EFSA No. 3). The solubility of the tested steviol glycosides at 20°C, at pH circa 4.6 ranged from 1.79 to 1.81 g/L across the five samples. The Panel considered that the water solubility tests submitted by the applicant have not been performed in full accordance with the requirements of the EFSA Guidance on Particle-TR (EFSA Scientific Committee, 2021) as the filter used in the test was 0.1 μm instead of 3–10 kDa. Based on the data provided, the Panel noted that the water solubility in the proposed specifications would be better described as 'slightly soluble'.

Concerning the purity parameters, the applicant provided information on the levels of residual solvents (i.e. ethanol and methanol) and toxic elements (i.e. lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As)) in five batches of the proposed food additive, which were in line with the proposed specifications. The analyses were performed by headspace gas chromatography with flame ionisation detection (HS-GC-FID) and inductively coupled plasma mass spectrometry (ICP-MS) for residual solvents and toxic elements, respectively. CoAs were submitted (Documentation provided to EFSA No. 1). Concerning the potential presence of residual solvents, ethanol was present at levels of 578 ± 241 mg/kg ($n = 4$; limit of quantification (LOQ): 200 mg/kg), whereas methanol was reported '*not detected*' in five batches LOQ 10 mg/kg; LOD not reported) (Documentation provided to EFSA No. 2). Concerning the toxic elements, all five batches had Pb, Cd and Hg below the LOQ which were 0.05, 0.005 and 0.003 mg/kg, respectively. Regarding As, it was quantified only in one batch at 0.008 mg/kg, whereas in the other four batches, it resulted below the LOQ (i.e. 0.005 mg/kg) (Documentation provided to EFSA No. 1).

Following an additional data request from EFSA concerning the potential presence of kaurenoic acid, the applicant submitted a certificate of analysis reporting that kaurenoic acid was not detected in five batches of the proposed food additive using a method of analysis with a limit of detection (LOD) of 0.007 mg/kg. The analysis was through liquid chromatography with tandem mass spectrometry (LC–MS/MS) operated in negative electrospray ionisation (ESI) mode with selective ion monitoring (SIM) of the deprotonated molecular ion for kaurenoic acid at *m/z* 301.27 (Documentation provided to EFSA No. 2). The LOD and LOQ were established using authentic standards of kaurenoic acid. Upon sample dissolution, the LOD and LOQ were equivalent to 0.007 mg/kg and 0.024 mg/kg in the test samples, with S/N ratios of 6:1 and 19:1, respectively. When test samples were spiked with kaurenoic acid at 0.024 mg/kg (corresponding to the LOQ), the analytical recovery was 80–92%. The Panel considered this recovery range as acceptable.

Five batches of the proposed food additive were analysed for the presence of microbiological contaminants (Documentation provided to EFSA No. 1). The total (aerobic) plate count was < 10 CFU/g ($n = 4$), yeasts and moulds < 10 CFU/g ($n = 5$), *E. coli* absent in 1 g ($n = 4$), Salmonella spp. absent in 25 g ($n = 4$) and *Staphylococcus aureus* absent in 1 g ($n = 4$).

Data on the absence of viable cells and recombinant DNA from the production strain in the final product were provided by the applicant (Documentation provided to EFSA No. 1) and summarised in Section 3.1.3. The Panel noted that the absence of viable cells/residual DNA of the production microorganism in the final product is captured in the proposed definition, where it is stated 'Viable cells of *K. phaffii* CGMCC 7539 and their DNA shall not be detected in the food additive'.

In the proposed specifications, the applicant reported that 'not more than 5 mg/kg' of residual protein are expected to be found in the proposed food additive. The Panel noted that the analytical data provided by the applicant comply with the proposed specification limit for residual protein (i.e. < 5 mg/kg) (Documentation provided to EFSA No. 1).

3.1.3 | Manufacturing process

Following a request from EFSA, the applicant provided the information regarding the raw materials and processing aids used to manufacture the proposed food additive (Documentation provided to EFSA No. 2).

3.1.3.1 | *Description of manufacturing process*

A schematic overview of the manufacturing process was provided by the applicant (Documentation provided to EFSA No. 1–2).

Dried and crushed leaves of the plant *S. rebaudiana* Bertoni are extracted with hot water [redacted] to obtain a crude extract containing [redacted] of Reb A. This crude extract is subjected to several steps of purification (i.e. crystallisation, filtration, centrifugation, rinsing and sifting) in order to obtain crystals with ≥ 95% of Reb A.

When submitting the revised proposed amendment of the specifications, the applicant highlighted that the starting extract used in the manufacturing process is aligned with the current description applicable to E 960c(ii), i.e. highly purified steviol glycoside rebaudioside A extracts (95% steviol glycosides).

In subsequent steps, Reb A is enzymatically converted to Reb M. The enzymes required for the bioconversion process, UDP-glucosyltransferase [redacted] and UDP-glucosyltransferase [redacted] and sucrose synthase [redacted], are produced by the genetically modified *K. phaffii* strain CGMCC7539; its characterisation data are reported in Section 3.1.3.2.

UDP-glucosyltransferase facilitates the transfer of glucose from an activated donor molecule (e.g. UDP-glucose) to the acceptor molecule steviol transfer glucose (Richman et al., 2005). Sucrose synthase ensures the availability of UDP-glucose by catalysing the conversion of UDP and sucrose to fructose and UDP-glucose (Wang et al., 2016).

Upon heat treatment, the crude Reb M solution is subjected to a series of purification and concentration steps (i.e. washing with ethanol, filtration, crystallisation, centrifugation, sieving, etc.), which lead to the final Reb M product (≥ 95% of Reb M).

3.1.3.2 | *Characterisation of the production organism*

Characteristic of the GMM production strain

The production strain of the three enzymes necessary for the manufacturing of the proposed food additive is the genetically modified yeast *Komagataella phaffii* CGMCC 7539, which was deposited in the China General Microbiological Culture Collection Center (CGMCC, China) with deposition number CGMCC 7539 (Documentation provided to EFSA No. 1). The production strain was taxonomically identified as *K. phaffii* by phylogenetic analysis based on the [redacted] (Documentation provided to EFSA No. 1).

Characteristics of the parental/recipient strain

The parental strain, which is directly used as recipient, is [redacted]. *K. phaffii* is a species included in the Qualified presumption of safety (QPS) list with the qualification 'for production purposes'. However, the production strain contains a bleomycin resistance gene, which is considered to be a hazard.

Characteristics of the inserted sequences

Four different expression cassettes were introduced in the vector [redacted]. The [redacted]) and [redacted]) gene sequences, each coding for a glycosyltransferase and the [redacted] sequence coding for a sucrose synthase are expressed using the promoter and terminator sequences of the [redacted] gene. Between the 5' of the coding sequence and the [redacted] tags are introduced for possible enzyme-linked immunoassay and for possible affinity purification, respectively. The fourth expression cassette contains the bleomycin resistance gene expressed from [redacted].

Description of the genetic modification

The plasmid [redacted] was introduced into the recipient strain [redacted] and integrated into the genome of the strain [redacted].

Safety aspects of the genetic modification

The presence in the production strain of multiple copies of a gene conferring resistance to bleomycin is considered a hazard. However, since the absence of viable cells and DNA of the production strain are demonstrated in the final product (see Sections 3.1.3.3 and 3.1.3.4), this is not considered to be a risk.

3.1.3.3 | *Absence of viable cells of the production strain in the final product*

A total of five batches of steviol glycosides were tested for the presence of viable cells of *K. phaffii* CGMCC 7539, each tested in triplicate. A total of 10 g per sample was diluted in 90 mL of saline water, and 10 mL of this dilution, corresponding to 1 g of the sample, was poured in 10 non-selective agar plates (1 mL per plate) which were incubated for 4 days at 28°C. A positive control was included. No viable cells were detected.

3.1.3.4 | *Absence of DNA of the production strain in the final product*

A total of five batches of steviol glycosides were tested for the presence of DNA of *K. phaffii* CGMCC 7539, each tested in triplicate. The DNA was extracted starting from 1 g of the samples, and a lysis method was used. No residual DNA was detected by qPCR, amplifying a target sequence of *K. phaffii* genomic DNA, with an LOD of at least 0.1 ng/g of sample. The Panel noted that the analysis provided is not in line with the method requested by the EFSA CEP Panel (2021), i.e. PCR; however, it was considered acceptable by the Panel.

3.1.4 | Method(s) of analysis in food

No information on a method of analysis for this proposed additive in food was provided by the applicant. However, the Panel assumes that the methods of analysis in food available for the other steviol glycosides preparations (e.g. Park et al., 2021) will be applicable.

3.1.5 | Stability, reaction and fate in food of the proposed food additive

The applicant submitted two studies with the proposed food additive to evaluate its stability under (i) accelerated (for 6 months) and (ii) long-term (up to 24 months) storage conditions. The studies were performed with five non-consecutive batches of the proposed food additive, stored in commercial packaging of low-density polyethylene bags (Documentation provided to EFSA No. 1).

The first study consisted of an accelerated stability study carried out at 40 ± 2°C and at 75 ± 5% of relative humidity. Samples were stored for 6 months. The parameters of moisture and Reb M (wt%) content were analysed at 0, 1, 2, 3 and 6 months. The results of the analyses demonstrated that the proposed food additive remained stable for up to 6 months under accelerated storage conditions (overall ≥ 95% Reb M, with approximately 1 wt% loss).

The second study consisted of a long-term storage stability study carried out at 25 ± 2°C and 60 ± 10% relative humidity. Samples were stored for up to 24 months. The parameters of moisture and Reb M (wt%) content were tested at 0, 3, 6, 12, 18 and 24 months. The results of the analyses demonstrated that the proposed food additive remained stable for up to 24 months under long-term storage conditions (overall ≥ 95% Reb M, with approximately 1 wt% loss).

3.2 | Proposed uses and use levels

Maximum levels of steviol glycosides (E 960a-d) expressed as steviol equivalents are defined in Annex II to Regulation (EC) No 1333/2008.[14]

Reb M produced from *K. phaffii* CGMCC 7539 is proposed for use in food and beverages under the same conditions as those already approved for steviol glycosides (E 960a-d) in the EU (Documentation provided to EFSA No. 1).

3.3 | Exposure data

Because the proposed uses and use levels of Reb M produced from *K. phaffii* CGMCC 7539 are the same as the already authorised food additive steviol glycosides (E 960a-d), the applicant did not provide a dietary exposure assessment.

The Panel considers that if steviol glycosides would be replaced by Rebaudioside M produced from *K. phaffii* CGMCC 7539, the exposure to Rebaudioside M (expressed as steviol equivalents) will not be higher than the last EFSA estimate of exposure to steviol glycosides (E 960a-d) (EFSA FAF Panel, 2024). At that time, based on the MPLs, the FAF Panel concluded that the conservative estimates of the exposure (mean, 95th percentile) to steviol glycosides (E 960) were below the ADI of 4 mg/kg bw per day in all population groups, except for infants and toddlers at the upper range of the exposure estimates in one country (4.1 and 4.8 mg steviol equivalents/kg bw per day, respectively).

3.3.1 | Anticipated exposure to impurities

As indicated in Section 3.1.2 (Table 1), Reb M produced from *K. phaffii* CGMCC 7539 may contain the toxic elements As, Cd, Pb, Hg and kaurenoic acid as impurities. The potential exposure to these impurities from the use of the proposed food additive can be calculated by assuming that they are present up to a certain limit value, and then by calculating pro-rata to the estimates of exposure to the food additive itself.

For the current assessment, previous exposure estimates performed by the FAF Panel (EFSA FAF Panel, 2024) were considered. The highest exposure levels to steviol glycosides for the mean and 95th percentile among the different population groups were considered, i.e. 1.3 and 4.8 mg/kg bw per day, respectively, for toddlers, expressed as steviol equivalents.

The current application concerns Reb M produced from *K. phaffii* CGMCC 7539 that contains predominantly Reb M with minor amounts of Reb A, Reb B and Reb D. The proposed specifications include that there should be not less than 95% rebaudioside M on a dried basis. Taking a conservative approach, Reb M has the lowest steviol equivalency conversion factor at 0.25 (JECFA, 2021) and also assuming the lowest assay at 95%, then exposure expressed as steviol equivalents corresponds to a 4.2-fold higher exposure when expressed as the proposed food additive itself. Therefore, an exposure of 1.3 and 4.8 mg/kg bw per day expressed as steviol equivalents equates to 5.5 and 20.2 mg/kg bw per day for the highest mean and highest 95th percentile of the proposed food additive produced using the proposed production process.

[14]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.

The level of impurities in the food additive combined with these estimated exposure levels to Reb M produced from *K. phaffii* CGMCC 7539 could result in an exposure which can be compared with the reference points (RP) or health-based guidance values (HBGV) in Table 2. It is assumed that any arsenic in the food additive corresponds to the element in the inorganic form rather than the organic form. Consequently, the RP for inorganic arsenic was used for the comparison.

TABLE 2 Reference points/health-based guidance value for impurities present in Reb M produced from *K. phaffii* CGMCC 7539.

Impurity/constituent/HBGV/ RP (μg/kg bw)	Basis/reference
Lead (Pb)/0.5 ($BMDL_{01}$)	The reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. The EFSA CONTAM Panel mentioned that a 1-point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a 1-point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the $BMDL_{01}$ (MOE lower than 1). EFSA CONTAM Panel (2010)
Mercury (Hg)/4 (TWI)	The HBGV was set using kidney weight changes in male rats as the pivotal effect. Based on the $BMDL_{10}$ of 0.06 mg/kg bw per day, expressed as mercury, and an uncertainty factor of 100 to account for inter- and intraspecies differences, with conversion to a weekly basis and rounding to one significant figure, a TWI for inorganic mercury of 4 μg/kg bw per week, expressed as mercury was established. EFSA CONTAM Panel (2012)
Cadmium (Cd)/2.5 (TWI)	The derivation of the reference point is based on a meta-analysis to evaluate the dose–response relationship between selected urinary cadmium and urinary beta-2-microglobulin as the biomarker of tubular damage recognised as the most useful biomarker in relation to tubular effects. A group-based $BMDL_5$ of 4 μg Cd/g creatinine for humans was derived. A chemical-specific adjustment factor of 3.9 was applied to account for human variability in urinary cadmium within each dose-subgroup in the analysis resulting in a reference point of 1.0 μg Cd per g creatinine. In order to remain below 1 μg Cd/g creatinine in urine in 95% of the population by age 50, the average daily dietary cadmium intake should not exceed 0.36 μg Cd/kg bw, corresponding to a weekly dietary intake of 2.5 μg Cd/kg bw. EFSA CONTAM Panel (2009)
Inorganic arsenic (iAs)/0.06 mg/kg bw per day ($BMDL_{05}$)	The reference point is based on a benchmark dose lower confidence limit ($BMDL_{05}$) of 0.06 μg/kg bw per day identified for skin cancer. The reference point is considered to cover lung cancer, bladder cancer, skin lesions, ischaemic heart disease, chronic kidney disease, respiratory disease, spontaneous abortion, stillbirth, infant mortality and neurodevelopmental effects. An MOE of 1 would correspond to the exposure level that is associated with a 5% increase relative to the background incidence for skin cancer, based on the available data". An MOE of 1 raises a health concern. Because there are no precedents in EFSA for identification of an MOE of low concern, when using a BMDL derived from human cancer data the CONTAM Panel decided not to determine a value for an MOE of low concern. EFSA CONTAM Panel (2024)

Abbreviations: BMDL01, benchmark dose (lower confidence limit); bw, body weight; HBGV, health-based guidance value; MOE, margin of exposure; RP, reference point; TDI, Tolerable Daily Intake; TWI, Tolerable Weekly Intake.

The risk assessment of the impurities helps to determine whether there could be a possible health concern if these impurities would be present at a certain level in the proposed food additive. The assessment is then performed by calculating the MOE (margin of exposure) by dividing the RP (i.e. BMDL, Table 2) by the exposure estimate (Section 3.3), or by estimating the contribution of the use of the food additive to the HBGV (expressed as percentage of the HBGV).

3.3.1.1 | *Toxic elements*

The Panel noted that the occurrence data on toxic elements submitted by the applicant are lower than the limits in the proposed specifications (Table 1; Documentation provided to EFSA No. 1).

The results of the analyses for lead, mercury, cadmium and arsenic in five samples of the food additive were reported (Section 3.1.2). Information on the LOQs and the analytical method used to quantify the toxic elements was provided.

The Panel performed the risk assessment that would result if these toxic elements were present in the food additive at the maximum limits as proposed in the specifications by the applicant.

The outcome of the risk assessment of the Panel is illustrated in Table 3.

TABLE 3 Risk assessment for toxic elements.

Exposure to Reb M produced from *K. phaffii* CGMCC 7539 (mg/kg bw per day)	Considering the presence of toxic elements at the proposed specifications limits in Reb M produced from *K. phaffii* CGMCC 7539			
	MOE for Pb at 0.2 mg/kg	% of the TWI for Hg at 0.07 mg/kg	% of the TWI for Cd at 0.015 mg/kg	MOE for iAs at 0.015 mg/kg
Mean: 5.5[a]	455	0.07	0.02	727
95th percentile: 20.2[a]	124	0.20	0.08	198

[a]Estimated exposure converted from steviol equivalents (EFSA FAF Panel, 2024) taking into account Reb M at 95% and a conversion factor of 0.25.

When considering the limits proposed for the specifications, the Panel concluded that the presence of the toxic elements in the proposed food additive would not give rise to a concern.

The Panel considered that the choice of maximum limits for toxic elements in the specifications is in the remit of risk manager(s). The numbers used here were merely taken to support the risk assessment of these toxic elements as presented above.

3.3.1.2 | *Kaurenoic acid*

Several publications assessing the in vitro and in vivo genotoxicity of kaurenoic acid were retrieved from the literature. In bacterial reverse mutation assays, kaurenoic acid showed negative results (Damasceno et al., 2019; Pezzuto et al., 1985; Pezzuto et al., 1986). In other in vitro studies (micronucleus and comet assay), positive results occurred at high concentrations; however, the level of cytotoxicity was not appropriately estimated (Cardoso et al., 2017; Cavalcanti et al., 2006; Cavalcanti et al., 2010; Rocha et al., 2019). When non-cytotoxic concentrations of kaurenoic acid were tested, negative results were observed (Cano et al., 2017; Dalenogare et al., 2019; Damasceno et al., 2019; Pezzuto et al., 1985, 1986). Only the in vitro micronucleus assay by Cano et al. (2017) was performed according to a modified OECD TG 487 (OECD, 2014), while the other studies were not performed according to OECD test guidelines. In the absence of a complete and reliable battery of in vitro assays, the Panel could not conclude on the in vitro genotoxic potential of kaurenoic acid.

In the in vivo study by Dalenogare et al. (2019), kaurenoic acid was administered at 1 mg/kg bw by gavage for 7 days to male and female Swiss mice. The study assessed comet assay parameters in liver and blood and the presence of micronuclei in bone marrow. No evidence of bone marrow exposure was provided. No genotoxicity was observed at the dose used in the study.

Cavalcanti et al. (2010) reported positive results in vivo in a micronucleus test and in a comet assay in Swiss male mice. The Panel noted the shortcomings of the study, which included that kaurenoic acid was administered by intraperitoneal injection, a route not recommended by OECD TG; that the high doses tested in the micronucleus assay (25, 50 and 100 mg/kg bw) caused high bone marrow toxicity; and that in the comet assay, no information on local toxicity (histopathological analysis) was reported. Notwithstanding these shortcomings, the Panel could not dismiss the positive findings in the in vivo micronucleus assay.

In the light of the uncertainties in the genotoxicity data, the Panel considered the threshold of toxicological concern (TTC) approach to conduct a risk assessment for kaurenoic acid, as an impurity, based on the absence of compound-specific toxicity data and given the indications of a possible genotoxic potential reported in the Cavalcanti et al. (2010) publication.

The Panel considered kaurenoic acid as a potential DNA-reactive mutagen and/or carcinogen, for which a TTC of 0.15 μg/person per day or 0.0025 μg/kg bw per day is applicable (EFSA Scientific Committee, 2019).

The Panel performed the calculation of the potential exposure to kaurenoic acid in the food additive at the LOD (0.0071 mg/kg) of the analytical method (Documentation provided to EFSA No. 1). The outcome of this calculation is given in Table 4.

TABLE 4 Potential exposure to kaurenoic acid from the use of the food additive Reb M produced from *K. phaffii* CGMCC 7539 (Documentation provided to EFSA No. 1).

Exposure to Reb M produced from *K. phaffii* CGMCC 7539 (mg/kg bw per day)	Exposure to kaurenoic acid if present at the LOD of 0.0071 mg/kg in the proposed food additive (μg/kg bw per day)
Mean: 5.5[a]	0.00004
95th percentile: 20.2[a]	0.00014

[a]Estimated exposure converted from steviol equivalents (EFSA FAF Panel, 2024) taking into account Reb M at 95% and a conversion factor of 0.25.

The calculated mean potential exposure to kaurenoic acid is 0.00004 μg/kg bw per day and at the 95th percentile is 0.00014 μg/kg bw per day. The Panel noted that these calculations indicate a potential for exposure at levels much lower than the TTC value of 0.0025 μg/kg bw per day.

The Panel considered that the absence of kaurenoic acid in the proposed food additive was demonstrated, using a method with an LOD of 0.0071 mg/kg; therefore, it dispels the uncertainties and concerns for potential genotoxicity.

The Panel recommends introducing a specific entry for kaurenoic acid in the proposed specifications.

4 | DISCUSSION

The present opinion deals with the proposal for amending the existing EU specifications of the food additive E 960c(i) to include a different microorganism strain in the definition of the current EU specifications. In the course of the risk assessment, the applicant revised its original proposal and requested a proposed amendment of the EU specifications of E 960c(ii).

The new proposed food additive is produced by enzyme-catalysed bioconversion from a Stevia leaf extract using the genetically modified strain *K. phaffii* CGMCC 7539, hereinafter referred to also as Reb M produced from *K. phaffii* CGMCC 7539. The manufacturing process foresees the enzymatic bioconversion of purified *Stevia rebaudiana* Bertoni leaf extract (≥ 95% of Reb A) using UDP-glucosyltransferase and sucrose synthase enzymes produced by *K. phaffi* CGMCC 7539, that

facilitates the transfer of glucose from sucrose and UDP-glucose to steviol glycosides via glycosidic bonds. The final product is composed mostly of Reb M (> 97%) and contains a mixture of the following glycosides at various concentrations: Reb A, B and D.

The Panel considered that the proposed amendment of the specifications is justified with respect to the inclusion of a new microorganism strain, taking into account that the manufacturing process and analytical data in support of this application are already covered by the parameters listed in the existing EU specifications for E 960c(i) and E 960c(ii).

The Panel considered that it is in the remit of the risk managers to decide whether the proposed changes in the specifications should result in an amendment of the already existing EU specifications of E960c(i) or E960c(ii).

However, the Panel recommended specifying in the proposed definition the production strain *K. phaffii* CGMCC 7539, which was deposited in the China General Microbiological Culture Collection Center (CGMCC) with deposition number 7.539, i.e. CGMCC 7539.

The applicant provided data on the water solubility of the proposed food additive, measured at approximately 1.8 g/L. In line with previous assessment (EFSA FAF Panel, 2023), taking into account the MPLs, the reported solubility and the volume of gastric secretion, the Panel considered that full dissolution of the food additive is to be expected in foods and/or in the gastrointestinal (GI) tract and that ingested particles (if any) would not persist. Therefore, the Panel concluded there is no concern with regard to the potential presence of small particles, including nanoparticles, in the proposed food additive and considered that the risk assessment can be performed following the EFSA Guidance for submission for food additive evaluations (EFSA ANS Panel, 2012), without nanospecific considerations.

The Panel noted that adequate analytical data supporting the compliance with the provision for residual protein specifications were submitted by the applicant. Since no viable cells nor DNA of the production strain remained in the final product, the manufacturing process does not raise a safety concern.

Analytical data on levels of toxic elements (As, Pb, Cd, Hg) in five samples of the food additive were provided by the applicant. The Panel noted that the data on toxic elements submitted by the applicant are lower than the limits in the proposed specifications. The Panel noted that, based on the analytical data, the proposed maximum limits for Pb, Hg, Cd, As are adequate. The potential exposure to these impurities was compared against the available HBGVs and RPs (Section 3.3.1, Tables 2 and 3). The Panel performed a risk assessment on the presence of these toxic elements in the proposed food additive at the specification limits and concluded that the presence of the toxic elements does not give rise to safety concerns.

The absence of kaurenoic acid was demonstrated in five batches of the proposed food additive, in which kaurenoic acid, analysed by LC–MS/MS, was not detected in the tested samples (LOD of 0.007 mg/kg). Based on the available data and the calculated potential exposure to kaurenoic acid applying a TTC approach for this contaminant (TTC value for a potential DNA-reactive mutagen and/or carcinogen), a genotoxic concern derived from kaurenoic acid in the final product could be ruled out. However, the Panel recommends introducing a specific entry for kaurenoic acid in the final food additive specifications.

Based on the data provided, the Panel considered that Rebaudioside M produced by enzyme-catalysed bioconversion from Stevia leaf extract using the genetically modified yeast *K. phaffii* CGMCC 7539 has the same physicochemical characteristics of E 960c(i) and E960c(ii); therefore; the available biological and toxicological data considered in the previous evaluations by the ANS and FAF Panel (EFSA ANS Panel, 2010, 2015, 2018; EFSA FAF Panel, 2019, 2020, 2021, 2022a, 2022b, 2023, 2024) will also apply to the safety assessment of Reb M produced from *K. phaffii* CGMCC 7539.

5 | CONCLUSIONS

The Panel concluded that there is no safety concern with respect to the proposed amendment to the EU specifications of E 960c(i) or E 960c(ii) related to the use of the new genetically modified strain *K. phaffii* CGMCC 7539 in the manufacturing process of the food additive Rebaudioside M produced via enzyme-catalysed bioconversion.

6 | DOCUMENTATION AS PROVIDED TO EFSA

1. Application to modify the specifications of the already authorised food additive Rebaudioside M obtained from stevia leaf extract. Technical dossier. January 2024. Submitted by Ingia Bio.[9]
2. Additional information submitted by Ingia Bio following a request from EFSA. August 2024.
3. Additional information submitted by Ingia Bio following a request from EFSA. February 2025.
4. Additional information submitted by Ingia Bio following a request from EFSA. March 2025.

ABBREVIATIONS

ADI	acceptable daily intake
ANS Panel	Panel on Food Additives and Nutrient Sources added to Food
As	arsenic
ATCC	American Type Culture Collection
BMDL	benchmark dose (lower confidence limit)

bw	body weight
CAS	Chemical Abstract Service
Cd	cadmium
CEP Panel	Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
CGMCC	China General Microbiological Culture Collection Center
CoA	colony forming units
CONTAM Panel	Panel on Contaminants in the Food Chain
DNA	Deoxyribonucleic acid
ESI	Electrospray ionisation
FAF Panel	Panel on Food Additives and Flavourings
FAO/WHO	Food and Agriculture Organisation/World Health Organisation
GI	Gastrointestinal
GMO	Genetically Modified Organisms
GRAS	Generally Recognized As Safe
HBGV	health-based guidance value
Hg	mercury
HPLC/MS	High-performance liquid chromatography/mass spectrometry
HPLC-UV	High-performance liquid chromatography – ultraviolet
HS-GC-FID	headspace gas chromatography
ICP-MS	Inductively coupled plasma – mass spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC/MS	Liquid chromatography-mass spectrometry
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MOE	margin of exposure
MPLs	maximum permitted levels
NOAEL	no observed adverse effect level
OECD	Organization for Economic Co-operation and Development
PCR	Polymerase Chain Reaction
Pb	lead
pH	Potential of Hydrogen
QPS	Qualified presumption of safety
Reb A	Rebaudioside
Reb B	rebaudioside B
Reb D	rebaudioside D
Reb M	Rebaudioside M
RP	reference point
SC	Scientific Commission
TDI	Tolerable Daily Intake
TG	test guideline
TTC	threshold of toxiciological concern
TWI	Tolerable weekly intake
UDP	Uridine diphosphate
UGT	UDP glucosyltransferase
wt	weight

REQUESTOR

European Commission

QUESTION NUMBER

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REFERENCES

Cano, B. L., Moreira, M. R., Goulart, M. O., Dos Santos Gonçalves, N., Veneziani, R. C., Bastos, J. K., Ambrósio, S. R., & Dos Santos, R. A. (2017). Comparative study of the cytotoxicity and genotoxicity of kaurenoic acid and its semi-synthetic derivatives methoxy kaurenoic acid and kaurenol in CHO-K1 cells. *Food and Chemical Toxicology*, *102*, 102–108. https://doi.org/10.1016/j.fct.2017.02.003

Cardoso, P. C. D. S., Rocha, C. A. M. D., Leal, M. F., Bahia, M. O., Alcântara, D. D. F. Á., Santos, R. A. D., Gonçalves, N. D. S., Ambrósio, S. R., Cavalcanti, B. C., Moreira-Nunes, C. A., Pessoa, C. D. Ó., & Burbano, R. M. R. (2017). Effect of diterpenoid kaurenoic acid on genotoxicity and cell cycle progression in gastric cancer cell lines. *Biomedicine & Pharmacotherapy*, *89*, 772–780. https://doi.org/10.1016/j.biopha.2017.02.085

Cavalcanti, B. C., Costa-Lotufo, L. V., Moraes, M. O., Burbano, R. R., Silveira, E. R., Cunha, K. M., Rao, V. S., Moura, D. J., Rosa, R. M., Henriques, J. A., & Pessoa, C. (2006). Genotoxicity evaluation of kaurenoic acid, a bioactive diterpenoid present in copaiba oil. *Food and Chemical Toxicology*, *44*(3), 388–392. https://doi.org/10.1016/j.fct.2005.08.011

Cavalcanti, B. C., Ferreira, J. R., Moura, D. J., Rosa, R. M., Furtado, G. V., Burbano, R. R., Silveira, E. R., Lima, M. A., Camara, C. A., Saffi, J., Henriques, J. A., Rao, V. S., Costa-Lotufo, L. V., Moraes, M. O., & Pessoa, C. (2010). Structure-mutagenicity relationship of kaurenoic acid from Xylopia sericeae (Annonaceae). *Mutation Research*, *701*(2), 153–163. https://doi.org/10.1016/j.mrgentox.2010.06.010

Dalenogare, D. P., Ferro, P. R., De Prá, S. D. T., Rigo, F. K., de David Antoniazzi, C. T., de Almeida, A. S., Damiani, A. P., Strapazzon, G., de Oliveira Sardinha, T. T., Galvani, N. C., Boligon, A. A., de Andra, V. M., da Silva Brum, E., Oliveira, S. M., & Trevisan, G. (2019). Antinociceptive activity of Copaifera officinalis Jacq. L oil and kaurenoic acid in mice. *Inflammopharmacology*, *27*(4), 829–844. https://doi.org/10.1007/s10787-019-00588-3

Damasceno, J. L., Arnet, Y. F., Fortunato, G. C., Girotto, L., Marena, G. D., Rocha, B. P., Resende, F. A., Ambrosio, S. R., Veneziani, R. C. S., Bastos, J. K., & Martins, C. H. G. (2019). Investigation of safety profile of four *Copaifera* species and of Kaurenoic acid by *salmonella*/microsome test. *Evidence-Bbased Complementary and Alternative Medicine*, *2019*, 7631531. https://doi.org/10.1155/2019/7631531

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources). (2010). Scientific opinion on safety of steviol glycosides for the proposed uses asa food additive. *EFSA Journal*, *8*(4), 1537. https://doi.org/10.2903/j.efsa.2010.1537

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). (2012). Guidance for submission for food additive evaluations. *EFSA Journal*, *10*(7), 2760. https://doi.org/10.2903/j.efsa.2012.2760

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food). (2015). Scientific opinion on the safety of the proposed amendment of the specifications for steviol glycosides (E 960) as a food additive. *EFSA Journal*, *13*(12), 4316. https://doi.org/10.2903/j.efsa.2015.4316

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Dusemund, B., Filipič, M., Frutos, M. J., Galtier, P., Gundert-Remy, U., Kuhnle, G. G., Lambré, C., Leblanc, J.-C., Lillegaard, I. T., Moldeus, P., Mortensen, A., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., … Gott, D. (2018). Scientific Opinion on the safety of the proposed amendment of the specifications of the food additive steviol glycosides (E 960). *EFSA Journal*, *16*(3), 5236. https://doi.org/10.2903/j.efsa.2018.5236

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Herman, L., … Chesson, A. (2021). Scientific guidance for the submission of dossiers on food enzymes. *EFSA Journal*, *19*(10), 6851. https://doi.org/10.2903/j.efsa.2021.6851

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). (2009). Scientific opinion on cadmium in food. *EFSA Journal*, *7*(10), 980. https://doi.org/10.2903/j.efsa.2009.980

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). (2010). Scientific opinion on lead in food. *EFSA Journal*, *8*(4), 1570. https://doi.org/10.2903/j.efsa.2010.1570

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). (2012). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA Journal*, *10*(12), 2985. https://doi.org/10.2903/j.efsa.2012.2985

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. R., Leblanc, J.-C., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Vleminckx, C., Wallace, H., Barregård, L., Benford, D., … Schwerdtle, T. (2024). Update of the risk assessment of inorganic arsenic in food. *EFSA Journal*, *22*(1), 8488. https://doi.org/10.2903/j.efsa.2024.8488

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes, M., Aquilina, G., Engel, K.-H., Fowler, P., Frutos Fernandez, M. J., Fürst, P., Gürtler, R., Gundert-Remy, U., Husøy, T., Mennes, W., Moldeus, P., Oskarsson, A., Shah, R., Waalkens-Berendsen, I., Wölfle, D., Degen, G., Gott, D., Leblanc, J.-C., … Castle, L. (2019). Scientific Opinion on the safety of the proposed amendment of the specifications for steviol glycosides (E 960) as a food additive: Rebaudioside M produced via enzyme-catalysed bioconversion of purified stevia leaf extract. *EFSA Journal*, *17*(10), 5867. https://doi.org/10.2903/j.efsa.2019.5867

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes, M., Aquilina, G., Engel, K.-H., Fowler, P., Frutos Fernandez, M. J., Fürst, P., Gürtler, R., Gundert-Remy, U., Husøy, T., Manco, M., Mennes, W., Moldeus, P., Passamonti, S., Shah, R., Waalkens-Berendsen, I., Wölfle, D., Wright, M., Degen, G., … Castle, L. (2020). Scientific Opinion on the safety of a proposed amendment of the specifications for steviol glycosides (E 960) as a food additive: To expand the list of steviol glycosides to all those identified in the leaves of stevia Rebaudiana Bertoni. *EFSA Journal*, *18*(4), 6106. https://doi.org/10.2903/j.efsa.2020.6106

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes, M., Aquilina, G., Castle, L., Engel, K.-H., Fowler, P., Frutos Fernandez, M. J., Fürst, P., Gürtler, R., Gundert-Remy, U., Husøy, T., Manco, M., Mennes, W., Passamonti, S., Moldeus, P., Shah, R., Waalkens-Berendsen, I., Wölfle, D., Wright, M., … Castle, L. (2021). Scientific opinion on the safety evaluation of steviol glycoside preparations, including rebaudioside AM, obtained by enzymatic bioconversion of highly purified stevioside and/or rebaudioside A stevia leaf extracts. *EFSA Journal*, *19*(8), 6691. https://doi.org/10.2903/j.efsa.2021.6691

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes, M., Aquilina, G., Engel, K.-H., Fowler, P. J., Frutos Fernandez, M. J., Fürst, P., Gürtler, R., Gundert-Remy, U., Husøy, T., Manco, M., Mennes, W., Moldeus, P., Passamonti, S., Shah, R., Waalkens-Berendsen, I., Wölfle, D., Wright, M., Barat, J. M., … Castle, L. (2022a). Scientific Opinion on the safety evaluation of glucosylated steviol glycosides as a food additive in different food categories. *EFSA Journal*, *20*(2), 7066. https://doi.org/10.2903/j.efsa.2022.7066

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes, M., Aquilina, G., Engel, K.-H., Fowler, P. J., Frutos Fernandez, M. J., Fürst, P., Gürtler, R., Gundert-Remy, U., Husøy, T., Manco, M., Mennes, W., Moldeus, P., Passamonti, S., Shah, R., Waalkens-Berendsen, I., Wright, M., Barat Baviera, J. M., Degen, G., … Castle, L. (2022b). Scientific Opinion on the safety of the proposed amendment of the specifications for enzymatically produced steviol glycosides (E 960c): Rebaudioside D produced via enzymatic bioconversion of purified stevia leaf extract. *EFSA Journal*, *20*(5), 7291. https://doi.org/10.2903/j.efsa.2022.7291

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes, M., Aquilina, G., Degen, G., Engel, K.-H., Fowler, P., Frutos Fernandez, M. J., Fürst, P., Gundert-Remy, U., Gürtler, R., Husøy, T., Manco, M., Mennes, W., Passamonti, S., Moldeus, P., Shah, R., Waalkens-Berendsen, I., Wright, M., Barat Baviera, J. M., … Castle, L. (2023). Safety evaluation of the food additive steviol glycosides, predominantly Rebaudioside M, produced by fermentation using Yarrowia lipolytica VRM. *EFSA Journal*, *21*(12), 8387. https://doi.org/10.2903/j.efsa.2023.8387

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Castle, L., Andreassen, M., Aquilina, G., Bastos, M. L., Boon, P., Fallico, B., FitzGerald, R., Frutos Fernandez, M. J., Grasl-Kraupp, B., Gundert-Remy, U., Gürtler, R., Houdeau, E., Kurek, M., Louro, H., Morales, P., Passamonti, S., Barat Baviera, J. M., Degen, G., … Vermeiren, S. (2024). 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. *EFSA Journal*, *22*(11), 9045. https://doi.org/10.2903/j.efsa.2024.9045

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2011). Scientific opinion on guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. *EFSA Journal*, *9*(6), 2193. https://doi.org/10.2903/j.efsa.2011.2193

EFSA Scientific Committee. (2009). Guidance of the scientific committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General principles. *EFSA Journal*, *7*(5), 1051. https://doi.org/10.2903/j.efsa.2009.1051

EFSA Scientific Committee. (2019). Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. *EFSA Journal*, *17*(6), 5708.

EFSA Scientific Committee, More, S., Bampidis, V., Benford, D., Bragard, C., Halldorsson, T., Hernández-Jerez, A., Bennekou, S. H., Koutsoumanis, K., Lambré, C., Machera, K., Naegeli, H., Nielsen, S., Schlatter, J., Schrenk, D., Silano, V., Turck, D., Younes, M., Castenmiller, J., … Schoonjans, R. (2021). Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles. *EFSA Journal*, *19*(8), 6769. https://doi.org/10.2903/j.efsa.2021.6769

FAO and WHO (Food and Agriculture Organization of the United Nations and World Health Organization). (2020). Compendium of Food Additive Specifications. Joint FAO, WHO Expert Committee on Food Additives (JECFA), 87th Meeting, June 2019. FAO JECFA Monographs 23. Rome.

JECFA (Joint FAO, WHO Expert Committee on Food Additives). (2009). Steviol glycosides (addendum). In: Toxicological evaluation of certain food additives. Sixty-ninth report of JECFA. June 17-26-29, 2008, Rome. WHO Food Additives series, No. 60, 183–219.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2008). Steviol glycosides. In: Compendium of food additive specifications. 69th meeting of JECFA, June 17–26, 2008, Rome. FAO/JECFA Monographs no. 5, 75–78.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2017). Monograph 20. Combined compendium of food additive specifications. Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 84th meeting 2017. Steviol Glycosides from Stevia rebaudiana Bertoni, pp. 50–69. https://openknowledge.fao.org/server/api/core/bitstreams/b0904756-1a75-444f-83fd-88f47673501d/content

JECFA (Joint FAO and WHO Expert Committee on Food Additives). (2021). Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives, 91st Meeting – Virtual meeting, 1–12 February 2021. FAO JECFA Monographs No. 26. Rome.

OECD (Organization for Economic Co-operation and Development). (1995). Test No. 105: Water Solubility, OECD Guidelines for the Testing of Chemicals, Section 1. https://doi.org/10.1787/9789264069589-en

OECD (Organization for Economic Co-operation and Development). (2014). Test No. 487: In Vitro Mammalian Cell Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4. https://ntp.niehs.nih.gov/sites/default/files/iccvam/suppdocs/feddocs/oecd/oecd-tg487-2014-508.pdf?sequence=1&isAllowed=y

Park, J.-M., Lee, J.-H., Koh, J.-H., & Kim, J.-M. (2021). Pretreatment methods for analyzing steviol glycosides in diverse food samples. *Journal of Food Science*, *86*(7), 3075–3081.

Pezzuto, J. M., Compadre, C. M., Swanson, S. M., Nanayakkara, D., & Kinghorn, A. D. (1985). Metabolically activated steviol, the aglycone of stevioside, is mutagenic. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(8), 2478–2482.

Pezzuto, J. M., Nanayakkara, N. P., Compadre, C. M., Swanson, S. M., Kinghorn, A. D., Guenthner, T. M., Sparnins, V. L., & Lam, L. K. (1986). Characterization of bacterial mutagenicity mediated by 13-hydroxy-ent-kaurenoic acid (steviol) and several structurally-related derivatives and evaluation of potential to induce glutathione S-transferase in mice. *Mutation Research*, *169*(3), 93–103. https://doi.org/10.1016/0165-1218(86)90088-1

Richman, A., Swanson, A., Humphrey, T., Chapman, R., McGarvey, B., Pocs, R., & Brandle, J. (2005). Functional genomics uncovers three glucosyltransferases involved in the synthesis of the major sweet glucosides of Stevia rebaudiana. *Plant Journal*, *41*(1), 56–67.

Rocha, S. M. M. D., Cardoso, P. C. D. S., Bahia, M. O., Pessoa, C. D. Ó., Soares, P. C., Rocha, S. M. D., Burbano, R. M. R., & Rocha, C. A. M. D. (2019). Effect of the kaurenoic acid on genotoxicity and cell cycle progression in cervical cancer cells lines. *Toxicology In Vitro*, *57*, 126–131. https://doi.org/10.1016/j.tiv.2019.02.022

Wang, Y., Chen, L., Li, Y., Li, Y., Yan, M., Chen, K., Hao, N., & Xu, L. (2016). Efficient enzymatic production of rebaudioside A from stevioside. *Bioscience, Biotechnology, and Biochemistry*, *80*, 67–73.

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