Approved: 1 July 2026

DOI: 10.2903/j.efsa.2026.10259

efsa JOURNAL

SCIENTIFIC OPINION

Re-evaluation of salt of aspartame-acesulfame (E 962) as food additive

EFSA Panel on Food Additives and Flavourings (FAF) | Laurence Castle | Monica Andreassen | Gabriele Aquilina | Maria Lourdes Bastos | Polly Boon | Biagio Fallico | Rex FitzGerald | Maria Jose Frutos Fernandez | Bettina Grasl-Kraupp | Ursula Gundert-Remy | Rainer Gürtler | Eric Houdeau | Marcin Kurek | Henriqueta Louro | Patricia Morales | Sabina Passamonti | Ellen Bruzell | Cristina Fortes | Kevin James Chipman | Riccardo Crebelli | Peter Fürst | Jean-Charles Leblanc | Evangelia Ntzani | Andrea Terron | Matthew Wright | Stefania Barmaz | Costanza Bonnici | Consuelo Civitella | Elena Mazzoli | Josef Daniel Rasinger | Ana Rincon | Camilla Smeraldi | Alexandra Tard | Federica Lodi

Correspondence: Ask a Question

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

Abstract

The present opinion deals with the re-evaluation of salt of aspartame-acesulfame (E 962) as a food additive. The Panel considered that upon ingestion, E 962 dissociates into aspartame and acesulfame ions which correspond to those of the authorised sweeteners aspartame (E 951) and acesulfame K (E 950). The Panel therefore assessed the safety of E 962 by a separate assessment of E 950 and E 951. The Panel considered that the 2025 EFSA re-evaluation of E 950 in which an acceptable daily intake (ADI) of 15 mg/kg bw per day was established, was sufficiently recent for the assessment of E 962. Exposure to E950 resulting from the use of both E 950 and E 962 was generally below the ADI in all population groups. For E 951, an update of the exposure and toxicological assessments of the 2013 EFSA re-evaluation of aspartame was performed. For E 951 or its main degradation product 5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP), no concerns for genotoxicity were identified. Based on the synthesis of systematically appraised evidence of human and animal studies, for E 951 the Panel concluded that there are no new studies suitable for identification of a reference point (RP). Consequently, the Panel confirmed the ADI of 40 mg/kg bw per day previously established by the EFSA ANS Panel. Dietary exposure to E 951 from the use of both E 951 and E 962 did not exceed the ADI. Therefore, the Panel concluded that there is no safety concern at the reported uses and use levels for E 950, E 951 and E 962. The Panel recommended updating the EU specifications for E 962 to ensure that it is produced from E 950 and E 951 that comply with their respective specifications and reviewing the limits for arsenic and lead in the specifications for E 951.

KEYWORDS

E 962, food additive, salt of aspartame-acesulfame, sweetener

CONTENTS

1 | INTRODUCTION

The present opinion deals with the re-evaluation of the salt of aspartame-acesulfame (E 962) when used as a food additive. The generic term 'salt of aspartame-acesulfame' will be used in the body of this opinion unless more specific information is reported (i.e. E number) in biological and toxicological studies.

The Scientific Committee on Food (SCF, 2000a) considered that the salt of aspartame-acesulfame (E 962) dissociates into aspartame and acesulfame upon ingestion; ions released after dissociation of the aspartame-acesulfame salt correspond to those of the authorised sweeteners aspartame (E 951) and acesulfame K (E 950) (see Section 1.1.3). For this reason, in the present opinion, the FAF Panel assessed the safety of the salt (E 962) by a separate assessment of aspartame and acesulfame (see Section 2.2).

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background

Regulation (EC) No 1333/2008[1] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union (EU). In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010.[2] This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU of 2001. The report "Food additives in Europe 2000" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority. In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

1.1.2 | Terms of Reference

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No. 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.1.3 | Information on existing authorisations and evaluations

1.1.3.1 | *Salt of aspartame-acesulfame (E 962)*

The salt of aspartame-acesulfame (E 962) is authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and specifications for the substance are defined in the Commission Regulation (EU) No 231/2012.[3] The EU Scientific Committee on Food (SCF) (SCF, 2000a) considered that the salt of aspartame-acesulfame is a true salt that dissociates into aspartame and acesulfame ions upon ingestion. The Committee noted the ions released after dissociation of the salt of aspartame-acesulfame (E 962) correspond to those of the authorised sweeteners aspartame (E 951) and acesulfame K (E 950), and judged the use of the salt to pose no additional safety concerns beyond those associated with its two components. The SCF highlighted that intakes of the salt of

[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]Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19.

[3]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 Text with EEA relevance. OJ L 83, 22.3.2012, p. 1–295.

aspartame-acesulfame (E 962) should be accounted for when estimating exposure to aspartame and acesulfame K relative to their respective acceptable daily intakes (ADI). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered that the ADIs established for aspartame and acesulfame K also cover the respective aspartame and acesulfame moieties released upon dissociation of the aspartame-acesulfame salt (JECFA, 2000).

1.1.3.2 | *Aspartame (E 951)*

Aspartame (E 951) was previously evaluated by SCF (1985a, 1985b, 1989, 1997, 2002), EFSA (2006, 2009a, 2009b, 2011), EFSA ANS Panel (2011, 2013) and JECFA (1975, 1980, 1981, 2023). In 2013, the EFSA ANS Panel conducted a comprehensive re-evaluation of aspartame (E 951), identifying a no observed adverse effect level (NOAEL) of 4000 mg/kg bw per day from chronic toxicity studies in rats and confirming the ADI of 40 mg/kg bw per day previously set by SCF and JECFA. The EFSA ANS Panel also concluded that aspartame is not of safety concern at current exposure estimates and that the ADI does not apply to individuals with phenylketonuria (PKU) (EFSA ANS Panel, 2013). More recently, JECFA confirmed in its latest evaluation an ADI of 0–40 mg/kg bw per day, based on a NOAEL of 4000 mg/kg bw per day (JECFA, 2023).

In 2023 aspartame was also evaluated by the International Agency for Research on Cancer (IARC) which classified the substance as 'possibly carcinogenic to humans' (Group 2B), based on limited evidence in humans and limited evidence in experimental animals (IARC, 2023, 2024).

1.1.3.3 | *Acesulfame k (E 950)*

Acesulfame K (E 950) was previously evaluated by SCF (SCF, 1985a, 1985b, 2000b) that established an ADI of 0–9 mg/kg bw per day, based on the NOAEL in a 2-year study in dog. Acesulfame K (E 950) was also evaluated by JECFA (1983, 1991) and in 1990 an ADI of 15 mg/kg bw per day was established, based on the NOAEL of 1500 mg/kg bw per day in a 2-year study in rat (JECFA, 1991). More recently, EFSA re-evaluated the safety of acesulfame K (E 950), confirming an ADI of 15 mg/kg bw per day, based on a NOAEL of 1500 mg/kg bw per day, the highest dose tested in chronic dietary studies in rats (EFSA FAF Panel, 2025).

2 | DATA AND METHODOLOGIES

The current risk assessment was carried out by the EFSA Panel on Food Additives and Flavourings (FAF Panel) in the context of Regulation (EC) No 257/2010. Structured protocols on hazard identification and characterisation (EFSA, 2020a; EFSA FAF Panel, 2023[4]) and on exposure assessment (EFSA, 2020b; EFSA FAF Panel, 2024a[5]) were developed in line with the principles of the EFSA PROMETHEUS project (PROmoting METHods for Evidence Use in Scientific assessments) (EFSA, 2015a). The protocols define the strategy to be applied for collecting and selecting data, appraising the relevant evidence, and analysing and integrating the evidence in order to draw conclusions that will form the basis for the scientific opinions.

The draft protocol for the hazard identification and characterisation of sweeteners was published on EFSA's website for comments, and the online public consultation was made available until 19 September 2019. A technical report on the outcome of this public consultation with the overview of the comments received and the general responses from EFSA was published (EFSA, 2020a). During the implementation phase, some amendments and further elaborations to the original protocol were introduced. The changes introduced are documented in the revised version published in April 2023[4] (EFSA FAF Panel, 2023) and were followed for the preparation of the present opinion.

The draft protocol for assessing dietary exposure to sweeteners was published on EFSA's website for comments, and the online public consultation was made available until 22 November 2019. A technical report on the outcome of this public consultation with the overview of the comments received and the general responses from EFSA was published (EFSA, 2020b). The protocol was revised, and the changes introduced are documented in the revised version published in December 2024 (EFSA FAF Panel, 2024a).

2.1 | Data

The FAF Panel was not provided with a newly submitted dossier for the re-evaluation of the salt of aspartame-acesulfame (E 962). In accordance with Regulation (EU) No 257/2010, EFSA launched public calls for data[6,7,8,9,10,11] and contacted interested parties that had replied to the call for data to collect additional clarification or supplemental information.

[4]Available online: https://doi.org/10.5281/zenodo.7788969.
[5]Available online: https://zenodo.org/records/13912094.
[6]Available online: https://www.efsa.europa.eu/en/data/call/170621.
[7]Available online: https://www.efsa.europa.eu/en/consultations/call/call-technical-data-sweeteners-authorised-food-additives-eu.
[8]Available online: https://www.efsa.europa.eu/en/call/call-data-genotoxicity-data-sweeteners.
[9]Call for food additives usage level and/or concentration data in food and beverages intended for human consumption (Batch 7).
[10]Available online: https://www.efsa.europa.eu/en/consultations/call/call-aspartame-e-951-use-level-andor-analytical-data-food-and.
[11]Available online: https://www.efsa.europa.eu/en/call/open-call-food-additive-occurrence-data-food-and-beverages-intended-human-consumption-2.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature obtained through extensive literature searches including any relevant literature published since the previous evaluation by EFSA or SCF, allowing 1 year of overlap, and up to 4 and 6 June 2025 (Appendix A). In line with the established methodology, literature published after the cut-off date was not considered in the assessment. Clarifications on the data to be submitted by the IBOs were also provided in a teleconference held on 10 August 2021. The steps followed for the acquisition of data and their selection are documented in Appendix A.

Food consumption data used to estimate the dietary exposure to aspartame and acesulfame, from both aspartame (E 951) and acesulfame K (E 950) and salt of aspartame-acesulfame (E 962), were derived from the EFSA Comprehensive European Food Consumption Database[12] (Comprehensive Database). The Mintel's Global New Products Database (GNPD) was checked to identify the uses of aspartame (E 951) and acesulfame K (E 950) and salt of aspartame-acesulfame (E 962) in food and beverage products and food supplements. The Mintel's GNPD is an online database that contains the compulsory ingredient information present on the label of numerous products. Mintel's GNPD is not a database managed by EFSA; data retrieved are presented in their original form and are not further scrutinised by the Panel.

2.2 | Methodologies

This opinion was formulated following the principles described in the EFSA Guidance 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. In line with these principles, this risk assessment was carried out based on structured protocols on hazard identification and characterisation of sweeteners (EFSA, 2020a; EFSA FAF Panel, 2023) and on exposure assessment (EFSA, 2020b; EFSA FAF Panel, 2024a).

The FAF Panel assessed the safety of the salt of aspartame-acesulfame (E 962) as a food additive in line with the principles laid down in Regulation (EU) No. 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the SCF (SCF, 2001) and the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

The SCF considered that the salt of aspartame-acesulfame (E 962) dissociates into aspartame and acesulfame when added to foods (aqueous food and also in the mouth); ions released after dissociation of the aspartame-acesulfame salt correspond to those of the authorised sweeteners aspartame (E 951) and acesulfame K (E 950) (see Section 1.1.3). For this reason, in the present opinion, the FAF Panel assessed the safety of the salt of aspartame-acesulfame (E 962) by a separate assessment of the two components. The Panel considered that for acesulfame K (E 950), the EFSA opinion of 2025 (EFSA FAF Panel, 2025) is sufficiently recent to be used in the re-evaluation of the salt of aspartame-acesulfame (E 962). For aspartame (E951), an update of the exposure and toxicological assessment of the EFSA opinion of 2013 (EFSA ANS Panel, 2013) was performed in the current opinion (Appendix A). Additionally, data specifically referring to the salt of aspartame-acesulfame (E 962) were also considered as part of the assessment, when available.

In animal studies, when the test substance is administered in the feed or in the drinking water, but doses are not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake is calculated by the Panel using the relevant default values. In case of rodents, the values indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) are applied. The Panel considered it appropriate to use the generic default values from the EFSA Scientific Committee Guidance document (2012) for studies in which both males and females were treated, while for studies in which only males or females were treated the respective sex specific default values were used. As regard to the duration of the studies, the Panel considered appropriate to use the default values for sub-acute studies for all studies of duration lower than 8 weeks, the default values for sub-chronic studies for studies of duration equal or longer than 8 weeks up to 1 year and the default value for chronic studies for studies of duration equal or longer than 1 year, as reported in the revised protocol on hazard identification and characterisation of sweeteners (EFSA FAF Panel, 2023). In the case of other animal species, the default values used by JECFA (2000) are used. In these cases, the dose is expressed as 'equivalent to mg/kg bw per day'. If a concentration in feed or drinking water was reported and the dose in mg/kg bw per day was calculated (by the authors of the study report or by the Panel) based on these reported concentrations and on reported consumption data for feed or drinking water, the dose is expressed as 'equal to mg/kg bw per day'. When in adult human studies (aged above 18 years) the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day is calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012).

In the case of genotoxicity, studies were evaluated according to the approach outlined in the revised protocol (EFSA FAF Panel, 2023). For the other toxicological endpoints, the methods for hazard identification, including the assessment of internal validity for individual studies (Risk of Bias, RoB) and the assessment of the body of evidence across all health outcomes, are described in the revised protocol and also detailed in Appendix A. In brief, following data retrieval and screening for relevance, RoB was performed and studies were classified into tiers from 1 (low risk of bias) to 3 (high risk of bias). In the current opinion, relevant studies retrieved from the literature with low to moderate RoB (tiers 1 and 2) were considered and included in the weight of evidence (WoE) evaluation. In accordance with the revised protocol, studies

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

previously evaluated by the SCF or EFSA and considered for setting an ADI were also subjected to a RoB evaluation and eventually in the WoE.

During the WoE evaluation, ratings of initial confidence (expressed as 'high', 'moderate', 'low' or 'very low') were assigned to all studies based on study design for each relevant, reported outcome. For each outcome across studies, the initial confidence rating could be downgraded based on either a concern for bias across studies, unexplained inconsistency, relevance of studies and/or imprecision; similarly, it could be upgraded based on the magnitude of effect, dose–response, consideration of residual confounding (human studies only) and consistency across study designs and experimental model systems (NTP-OHAT, 2019). The confidence ratings for an outcome or group of outcomes were translated into levels of evidence for adverse effects or no adverse effects on health. The following descriptors were used to rate the level of evidence for the presence of adverse effects on health: 'high', 'moderate', 'low' or 'inadequate when the confidence in the body of evidence is 'high', 'moderate', 'low' or 'very low', respectively. When no adverse effects on health are identified, the following descriptors for level of evidence were used: 'high', 'moderate' and 'inadequate' corresponding to confidence in the body of evidence ratings of 'high', 'moderate' and 'low/very low', respectively. More details on the WoE procedure are outlined in step 1.14 of the revised protocol on hazard identification and characterisation, and in the US National Toxicology Program (NTP) Handbook for conducting a literature-based health assessment (NTP-OHAT, 2019) with some modifications. The integration of animal and human data was based on the highest level of evidence rating for an adverse or no adverse effect on health. If relevant, evidence from the earlier assessment (EFSA ANS Panel, 2013) was considered in the final integration. Hazard identification conclusions i.e. expressions of likelihood of an association between intake of aspartame (E 951) and adverse effect on health, were reached on groups of toxicological outcomes following guidance developed by the FAF Panel (EFSA FAF Panel, 2023).

Dietary exposure to the salt of aspartame-acesulfame (E 962), aspartame (E 951) and acesulfame K (E 950) from their use as food additives was estimated by combining food consumption data available within the EFSA Comprehensive Database with the maximum levels according to Annex II to Regulation (EC) No 1333/2008 and with reported use levels and analytical data submitted to EFSA following public calls for data. The exposure was calculated according to several different scenarios (see Section 2).

Finally, uncertainties in the hazard identification, characterisation and exposure assessment were identified and discussed (see Section 4).

3 | ASSESSMENT

3.1 | Technical data

3.1.1 | Identity and specifications and identity of E 962

Salt of aspartame-acesulfame (E 962) is the salt prepared by heating an approximately 2:1 ratio (w/w) of aspartame and acesulfame K. The chemical structure of the salt of aspartame-acesulfame (E 962) is given in Figure 1.

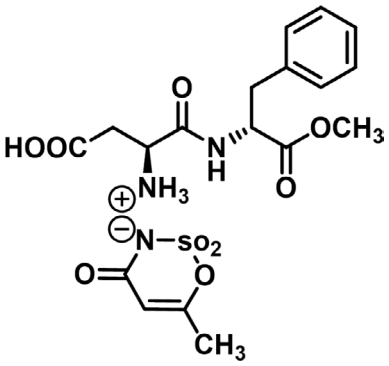


FIGURE 1 Chemical structure for the salt of aspartame-acesulfame (E 962).

Specifications for the salt of aspartame-acesulfame (E 962) as defined in Commission Regulation (EU) No 231/2012 and the specifications as defined by JECFA (2005) are listed in Table 1.

TABLE 1 Specifications for the salt of aspartame-acesulfame (E 962) according to Commission Regulation (EU) No 231/2012 and the JECFA (2005).

	Commission regulation (EU) No 231/2012	JECFA (2005)
Synonyms	Aspartame-acesulfame; Aspartame-acesulfame salt	Aspartame-acesulfame, INS No. 962
Definition	The salt is prepared by heating an approximately 2:1 ratio (w/w) of aspartame and acesulfame K in solution at acidic pH and allowing crystallisation to occur. The potassium and moisture are eliminated. The product is more stable than aspartame alone.	The salt is prepared by heating an approximately 2:1 ratio (w/w) of aspartame and acesulfame K in solution at acidic pH and allowing crystallisation to occur. The potassium and moisture are eliminated. The product is more stable than aspartame alone.
CAS No.		106372-55-8
Chemical names	6-Methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide salt of L-phenylalanyl-2-methyl-L-α-aspartic acid	6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide salt of L-phenylalanyl-2-methyl-L-α-aspartic acid. [2-carboxy-β-(N-(b-methoxycarbonyl-2- phenyl) ethylcarbamoyl)]ethanaminium-6-methyl-4-oxo-1,2,3-oxathiazin3-ide-2,2-dioxide
Chemical formula	$C_{18}H_{23}O_9N_3S$	$C_{18}H_{23}O_9N_3S$
Molecular weight	457.46	457.46
Description	A white, odourless, crystalline powder	A white, odourless, crystalline powder
Assay	63.0%–66.0% aspartame (dry basis) and 34.0%–37.0% acesulfame (acid form on a dry basis)	63.0%–66.0% aspartame (dried basis) and 34.0%–37.0% acesulfame (acid form on a dried basis)
Identification		
Solubility	Sparingly soluble in? water; slightly soluble in ethanol	Sparingly soluble in water and slightly soluble in ethanol.
Transmittance	The transmittance of a 1% solution in water determined in a 1 cm cell at 430 nm with a suitable spectrophotometer using water as a reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022.	The transmittance of a 1% solution in water determined in a 1 cm cell at 430 nm with a suitable spectrophotometer using water as a reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022.
Specific rotation	$[\alpha]_D^{20}$ + 14.5° to +16.5° Determine at a concentration of 6.2 g in 100 mL formic acid (15N) within 30 min of preparation of the solution. Divide the calculated specific rotation by 0.646 to correct for the aspartame content of the salt of aspartame-acesulfame	$[\alpha]_D^{20}$ + 14.5 to +16.5. After preparing a solution of 6.2 g of sample in 100 mL formic acid (15N), make the measurement within 30 min of preparation of the solution. Divide the calculated specific rotation by 0.646 to correct for the aspartame content of the aspartame-acesulfame salt.
Purity		
Loss on drying	Not more than 0.5% (105°C, 4 h)	No more than 0.5% (105°, 4 h)
5-Benzyl-3,6-dioxo-2-piperazineacetic acid	Not more than 0.5%	Not more than 0.5%
Lead	Not more than 1 mg/kg	Not more than 1 mg/kg

No information on the salt of aspartame-acesulfame (E 962) has been submitted to EFSA by any interested business operator (IBO) or other interested party following two calls for data.[13,14]

The Panel noted that the current EU specifications for the salt of aspartame-acesulfame (E 962) indicate the use of aspartame and acesulfame K as source materials. However, it is not specified whether the aspartame and acesulfame K used in the production of the salt of aspartame-acesulfame (E 962) have to comply with the specifications for aspartame (E 951) and acesulfame K (E 950), respectively.

As no technical information or details on the manufacturing process of the salt of aspartame-acesulfame (E 962) were submitted to EFSA, the Panel was unable to evaluate any analytical data related to this food additive. Nonetheless, it was observed that the existing purity criteria for the salt of aspartame-acesulfame (E 962) do not align with the listed impurities and limits defined in the specifications for aspartame (E 951), nor with the recommendation for the amendment of the specifications for acesulfame K (E 950) as proposed by the Panel in its 2025 re-evaluation (EFSA FAF Panel, 2025).

In the absence of analytical data on the salt of aspartame-acesulfame (E 962) and on its production, the Panel considers that, to ensure its safety by conducting a dedicated risk assessment, the definition of the salt of aspartame-acesulfame (E 962) in the EU specifications should be modified. It should be required that the food additive E 962 is prepared by reacting an approximately 2:1 ratio (w/w) of aspartame and acesulfame K, which should meet the specifications laid down for aspartame (E 951) in Commission Regulation (EU) No. 231/2012, and for acesulfame K (E 950) according to the proposed revision for the specifications as recommended in the Panel's re-evaluation (EFSA FAF Panel, 2025), until such revisions are formally incorporated into Commission Regulation (EU) No 231/2012.

[13]Available online: https://www.efsa.europa.eu/en/data/call/170621.

[14]Available online: https://www.efsa.europa.eu/en/consultations/call/call-technical-data-sweeteners-authorised-food-additives-eu.

3.1.2 | Manufacturing process

No information on the salt of aspartame-acesulfame (E 962) has been submitted to EFSA by any IBO following calls for data.

According to the current EU specifications, the salt of aspartame-acesulfame (E 962) is prepared by heating an approximately 2:1 ratio (w/w) of aspartame and acesulfame K in aqueous solution at acidic pH and allowing crystallisation to occur.

General information on a manufacturing process of the salt of aspartame-acesulfame (E 962) is described by Fry et al. (2012) where it is indicated that the salt is formed in aqueous acid solution and it is subsequently crystallised, separated, washed and dried. It is also mentioned that aspartame and acesulfame K used in the production of the salt of aspartame-acesulfame are 'food grade'. No organic solvents are used in the manufacturing process.

At the time of the re-evaluation (EFSA ANS Panel, 2013), IBOs indicated that aspartame, when used as a food additive, was manufactured by chemical synthesis. The Panel is aware that synthesis can be achieved through enzymatic peptide bond formation. However, the use of any enzyme in the manufacture of aspartame (E 951) has not been evaluated.

3.1.3 | Methods of analysis in food

No information on analysis of the salt of aspartame-acesulfame (E 962) in food has been submitted to EFSA by any IBO following calls for data. To analyse the food additive E 962 in food, it should be extracted from the food matrix, during which dissociation of the salt is expected to occur. Therefore, the analytical methods developed for acesulfame (EFSA FAF Panel, 2025) and aspartame (EFSA ANS Panel, 2013) are considered suitable for determining the individual components of the salt of aspartame-acesulfame (E 962). However, these methods do not allow differentiation between acesulfame and aspartame originating from the direct use of acesulfame K (E 950) and aspartame (E 951), respectively, and those derived from the use of the salt of aspartame-acesulfame (E 962).

3.1.4 | Stability of the substance, and reaction and fate in food

No information on salt of aspartame-acesulfame (E 962) has been submitted to EFSA by any IBO following calls for data. Wherever the salt of aspartame-acesulfame (E 962) is dissolved in food, it is expected to be dissociated into its individual components, aspartame and acesulfame. Information on the stability of acesulfame K is available in the EFSA Opinion on the re-evaluation of E 950 (EFSA FAF Panel, 2025) and on aspartame in the EFSA Opinion on the re-evaluation of E 951 (EFSA ANS Panel, 2013).

According to Fry et al. (2012), the salt of aspartame-acesulfame is stable in its dry solid form and does not degrade into its components, aspartame or acesulfame, even after prolonged storage at elevated temperatures, e.g. up to a year at 60°C. It has been suggested that the salt may be more stable than aspartame alone. This increased stability is considered to result from the blocking of the amino group of aspartame by acesulfame. This prevents the amino group from participating in self-cyclisation to form 5-benzyl-3,6-dioxo-2-piperazineacetic acid (DKP), and the subsequent breakdown of DKP into aspartylphenylalanine (AP). Additionally, the formation of Maillard-type reactions involving aspartame is inhibited in the salt.

Screening of publications on the stability of aspartame, identified in the literature search conducted for this assessment, corroborated what was already concluded at the time of the re-evaluation of aspartame (E 951) (EFSA FAF Panel, 2013). The only new evidence obtained was a study by Trawiński and Skibiński (2023), which investigated the phototransformation of aspartame in soft drinks and, for the first time, reported the formation of 'phenolic derivatives of aspartame' as novel degradation products. Under simulated sunlight, aspartame degradation was markedly accelerated in commercial beverages compared with buffered solutions, likely due to interactions with co-occurring ingredients acting as photosensitisers. The phototransformation experiments simulated the full solar UV–Vis spectrum for direct sunlight exposure in summer months (290–800 nm), at 750 W/m^2 for up to 240 h, corresponding to 1 month of a day/night cycle. Six transformation products were detected: the three known degradation products (DKP, a-aspartylphenylalanine and phenylalanine methyl ester) along with much lower concentrations of three new 'phenolic derivatives of aspartame' (ortho-, meta- and para-hydroxyaspartame), formed via aromatic hydroxylation. These novel photodegradation products were observed only in certain beverages (three of the eight commercial beverages studied in the phototransformation experiments), suggesting a dependence on specific formulation factors. However, the study has several important limitations. Structural elucidation of the phenolic compounds was not fully confirmed and relied primarily on mass spectrometric data (UHPLC-ESI-QTOF analysis), and the experimental set-up may not accurately reflect typical storage and exposure conditions. An in silico QSAR analyses of the proposed structures of the 'phenolic derivatives of aspartame' (ortho-, meta- and para-hydroxyaspartame), performed by the Panel (Appendix B), did not indicate any alerts for genotoxicity.

3.2 | Authorised uses and use levels

The salt of aspartame-acesulfame (E 962) dissociates in food into its individual components, aspartame and acesulfame (see Section 3.1.4 technical part). Maximum levels of the salt of aspartame-acesulfame (E 962) have been defined in Annex II, Part E, to Regulation (EC) No 1333/2008 on food additives, as amended. In this document, these levels are called

maximum permitted levels (MPLs). These MPLs in food are expressed as 'acesulfame K equivalent' or 'aspartame equivalent' (Regulation (EC) No 1333/2008). Currently, the salt of aspartame-acesulfame (E 962) is an authorised food additive in the EU in 34 food categories (FCs) (corresponding to 44 authorised uses). It is authorised at MPLs ranging from 25 to 2000 mg/kg in 31 foods categories and at *quantum satis* (QS) in 3 food categories of table-top sweeteners (FCs 11.4.1, 11.4.2 and 11.4.3). Currently, aspartame (E 951) is authorised in the same 34 food categories as the salt of aspartame-acesulfame (E 962). In this case, however, there are 46 authorised uses, with MPLs ranging from 25 to 6000 mg/kg, and use at QS in the same three FCs (FC 11.4 – Table-Top Sweeteners in liquid, powder and tablet form) as E 962. Acesulfame K (E 950) is likewise authorised in the same 34 food categories as the salt of aspartame-acesulfame (E 962), and detailed information on its authorised uses is provided in the re-evaluation of acesulfame K (E 950) (EFSA FAF Panel, 2025). Table 2 lists the food categories, including any restrictions or exceptions, in which aspartame (E 951) and the salt of aspartame-acesulfame (E 962) are permitted, together with their corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

TABLE 2 Food categories that are permitted to contain aspartame (E 951) and the salt of aspartame -acesulfame (E 962) according to Annex II to Regulation (EC) No 1333/2008 and the MPLs.

Food category number	Food category name	E number	Restrictions/exception	MPL of E 951/ E 962 (mg/L or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	E 951/E 962	Only energy-reduced products or with no added sugar	1000/350[a,c,d]
03	Edible ices	E 951/E 962	Only energy-reduced or with no added sugar	800/800[b,c,d]
04.2.2	Fruit and vegetables in vinegar, oil or brine	E 951/E 962	Only sweet–sour preserves of fruit and vegetables	300/200[a,c,d]
04.2.3	Canned or bottled fruit and vegetables	E 951/E 962	Only fruit energy-reduced or with no added sugar	1000/350[a,c,d]
04.2.4.1	Fruit and vegetable preparations excluding compote	E 951/E 962	Only energy-reduced	1000/350[a,c,d]
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EC	E 951/E 962	Only energy-reduced jams, jellies and marmalades	1000/1000[b,c,d]
04.2.5.2	Jam jellies and marmalades and similar products	E 951/E 962	Only energy-reduced jams, jellies and marmalades	1000/1000[b,c,d]
04.2.5.3	Other similar fruit or vegetable spreads	E 951/E 962	Only energy-reduced fruit or vegetable spreads and dried fruit based sandwich spreads, energy-reduced or with no added sugar	1000/1000[b,c,d]
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	E 951/E 962	Only energy-reduced or with no added sugar	2000/500[a,c,d]
05.2	Other confectionery including breath refreshening microsweets	E 951/E 962	Only cocoa, milk, dried fruit or fat based sandwich spreads, energy-reduced or with no added sugar	1000/1000[b,c,d]
05.2	Other confectionery including breath refreshening microsweets	E 951/E 962	Only starch-based confectionery energy-reduced or with no added sugar	2000/1000[a,c,d]
05.2	Other confectionery including breath refreshening microsweets	E 951/E 962	Only confectionery with no added sugar	1000/500[a,c,d]
05.2	Other confectionery including breath refreshening microsweets	E 951/E 962	Only cocoa or dried fruit based, energy-reduced or with no added sugar	2000/500[a]
05.2	Other confectionery including breath refreshening microsweets	E 951/E 962	Only breath-freshening microsweets, with no added sugar	6000/2500[a,c,d]
05.2	Other confectionery including breath refreshening microsweets	E 951	only strongly flavoured freshening throat pastilles with no added sugar	2000
05.3	Chewing gum	E 951/E 962	Only with no added sugar	5500/2000[a,c,d]
05.3	Chewing gum	E 951	Only with added sugar or polyols, as flavour enhancer	2500[e]
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	E 951/E 962	Only sauces	350/350[b,c,d]
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	E 951/E 962	Only starch-based confectionery energy-reduced or with no added sugar	2000/1000[a,c,d]
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	E 951/E 962	Only cocoa or dried fruit based, energy-reduced or with no added sugar	2000/500[a,c,d]

TABLE 2 (Continued)

Food category number	Food category name	E number	Restrictions/exception	MPL of E 951/E 962 (mg/L or mg/kg as appropriate)
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	E 951/E 962	Only confectionery with no added sugar	1000/500[a,c,d]
06.3	Breakfast cereals	E 951/E 962	Only breakfast cereals with a fibre content of more than 15%, and containing at least 20% bran, energy-reduced or with no added sugar	1000/1000[b,c,d]
07.2	Fine bakery wares	E 951/E 962	Only essoblaten – wafer paper	1000/1000[b,c,d]
09.2	Processed fish and fishery products including molluscs and crustaceans	E 951/E 962	Only sweet–sour preserves and semi-preserves of fish and marinades of fish, crustaceans and molluscs	300/200[a]
11.4.1	Table Top Sweeteners in liquid form	E 951/E 962		*quantum satis*
11.4.2	Table Top Sweeteners in powder form	E 951/E 962		*quantum satis*
11.4.3	Table Top Sweeteners in tablets	E 951/E 962		*quantum satis*
12.4	Mustard	E 951/E 962		350/350[b,c,d]
12.5	Soups and broths	E 951/E 962	Only energy-reduced soups	110/110[b,c,d]
12.6	Sauces	E 951/E 962		350/350[b,c,d]
12.7	Salads and savoury based sandwich spreads	E 951/E 962	Only *Feinkostsalat*	350/350[b,c,d]
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	E 951/E 962		1000/450[a,c,d]
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)	E 951/E 962		800/450[a,c,d]
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	E 951/E 962	Only energy-reduced or with no added sugar	600/350[a,c,d]
14.1.4	Flavoured drinks	E 951/E 962	Only energy-reduced or with no added sugar	600/350[a,c,d]
14.2.1	Beer and malt beverages	E 951/E 962	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 'Obergäriges Einfachbier'; Beers with a minimum acidity of 30 milli-equivalents expressed as NaOH; Brown beers of the 'oud bruin' type	600/350[a,c,d]
14.2.1	Beer and malt beverages	E 951/E 962	Only energy-reduced beer	25/25[b,c,d]
14.2.3	Cider and perry	E 951/E 962	Excluding cydr jakościowy, perry jakościowe, cydr lodowy, perry lodowe	600/350[a,c,d]
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	E 951/E 962		600/350[a,c,d]
15.1	Potato-, cereal-, flour- or starch-based snacks	E 951/E 962		500/500[b,c,d]
15.2	Processed nuts	E 951/E 962		500/500[b,c,d]
16	Desserts excluding products covered in category 1, 3 and 4	E 951/E 962	Only energy-reduced or with no added sugar	1000/350[a,c,d]
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children	E 951/E 962		2000/500[a,c,d]
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children	E 951/E 962	Only food supplements in chewable form	5500/2000[a,c,d]

(Continues)

TABLE 2 (Continued)

Food category number	Food category name	E number	Restrictions/exception	MPL of E 951/ E 962 (mg/L or mg/kg as appropriate)
17.2	Food supplements sup Synthesis of systematically appraised evidence plied in a liquid form, excluding food supplements for infants and young children	E 951/E 962		600/350[a,c,d]
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children	E 951/E 962	Only food supplements in syrup form	5500/2000[a,c,d]

Abbreviation: MPL, maximum permitted level.

[a]Limits are expressed as acesulfame K equivalent.

[b]Limits are expressed as aspartame equivalent.

[c]The maximum usable levels are derived from the maximum usable levels for its constituent parts, aspartame (E 951) and acesulfame-K (E 950).

[d]The levels for both E 951 (aspartame) and E 950 (acesulfame K) are not to be exceeded by use of the salt of aspartame-acesulfame (E 962), either alone or in combination with E 950 or E 951.

[e]If E 950, E 951, E 955, E 957, E 959 and E 961 are used in combination in chewing gum, the maximum level for each is reduced proportionally.

The Panel noted that for FCs 05.2, only cocoa or dried fruit based, energy-reduced or with no added sugar, and 09.2, the MPL for the salt of aspartame-acesulfame (E 962) does not have the footnotes (c) and (d). As it seems likely that this may be an omission, it was assumed that footnotes (c) and (d) are also applicable to this food category.

The salt of aspartame-acesulfame (E 962) and aspartame (E 951) are not authorised to be used according to Annex III to Regulation (EC) No. 1333/2008 i.e. these additives cannot be added to other food additives, food enzymes, food flavourings or nutrients.

3.3 | Exposure data

The salt of aspartame-acesulfame (E 962) dissociates in food into aspartame and acesulfame; thus, contributing to the total exposure to its two constituents. The overall exposure to acesulfame from the salt of aspartame-acesulfame (E 962) and acesulfame K (E 950) was already evaluated in the recently published acesulfame K (E 950) opinion (EFSA FAF Panel, 2025). Therefore, the present opinion considers only the exposure to aspartame, updating its previous exposure assessment performed in 2013 (EFSA ANS Panel, 2013), resulting from the use of the salt of aspartame-acesulfame (E 962) and aspartame (E 951).

3.3.1 | Concentration data

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, actual concentration data are required to perform a more realistic exposure assessment as well as to obtain data for those food categories authorised with an MPL at QS.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[15] for concentration data (use level and/or analytical data) on some sweeteners, including salt of aspartame-acesulfame (E 962). A specific call for concentration data on aspartame (E 951) was launched in 2020.[16]

In addition, analytical data on the salt of aspartame-acesulfame (E 962) and aspartame (E 951) have been submitted yearly to EFSA through the open calls for occurrence data on food additives in food and beverages intended for human consumption.[17]In response to these calls, use levels on E 962 and E 951 in food and beverages were made available by industry and analytical data were made available by Member States. Use levels and analytical data as available in EFSA's Data Warehouse on 5 February 2025 and 3 June 2025 respectively, were considered for the present assessment.

Reported use levels in foods of salt of aspartame-acesulfame (E 962) and aspartame (E 951)

Industry provided EFSA with six use levels of the salt of aspartame-acesulfame (E 962) in foods and beverages for 3 out of the 47 authorised uses, corresponding to three out of the 34 authorised food categories according to Annex II to Regulation

[15]15 January 2018: Call for food additives usage level and/or concentration data in food and beverages intended for human consumption (Batch 7): 180122.pdf.

[16]Call for Aspartame (E 951) use level and/or analytical data in food and beverages intended for human consumption, 2020. https://www.efsa.europa.eu/sites/default/files/consultation/callsfordata/Call_for_data_on_food_additives_2020.pdf.

[17]5 March 2024: Open call for food additive occurrence data in food and beverages intended for human consumption: https://www.efsa.europa.eu/en/call/open-call-food-additive-occurrence-data-food-and-beverages-intended-human-consumption-2.

(EC) No. 1333/2008 (Table 2). These use levels were provided by International Chewing Gum Association (ICGA Europe), Food Drink Europe (FDE) and European Fruit Juice Association (AIJN) (Documentation provided to EFSA No 1–3).

Additionally, industry provided 312 use levels of aspartame (E 951) in foods and beverages for 13 out of 46 authorised uses, corresponding to 13 out of 34 authorised food categories[18] according to Annex II to Regulation (EC) No 1333/2008 (Table 2).

The use levels of aspartame (E 951) were provided by Specialised Nutrition Europe (SNE), Food Drink Europe (FDE), Chocolat Frey, Food Supplement Europe (FSE), European Dairy Association (EDA), International Chewing Gum Association (ICGA Europe), International Sweetener Association (ISA), Italian Association of Confectionary and Pasta Industries, Active Nutrition International and MOWI (Documentation provided to EFSA n. 4–13).

The Panel noted that industry indicated that the use level provided for FC 4.2.2 'Fruit and vegetables in vinegar, oil or brine' and for five use levels for FC 14.1.4 'Flavoured drinks' referred to niche products. Since analytical data were also available for these food categories, the Panel did not use these use levels in the exposure assessment (EFSA FAF Panel, 2024a). Moreover, three use levels from FC 01.4 'Flavoured fermented milk products including heat-treated products' were excluded due to insufficient information, which prevented accurate categorisation. One use level from FC 14.1.4 'Flavoured drinks' was excluded after the data provider indicated that aspartame is no longer used in the product. Ten other use levels from FC 14.1.4 'Flavoured drinks' were excluded due to a reporting error. Finally, the sample submitted under FC 17.2 'Food supplements supplied in a liquid form' was re-categorised under FC 17.1 'Food supplements supplied in a solid form' after receiving supplementary information on the product from the data provider. Overall, the dataset available for the exposure assessment comprised 298 use levels for 12 authorised food categories.

Annex A, Table A2 summarises the reported use levels of the salt of aspartame-acesulfame (E 962) and aspartame (E 951) in foods and beverages.

Analytical results of salt of aspartame-acesulfame (E 962) and aspartame (E 951) provided by Member States

Four analytical results of the salt of aspartame-acesulfame (E 962) were reported to EFSA between 2013 and 2024. These results were all left-censored (< LOD). According to the sweeteners protocol of exposure, left-censored data are not included in the exposure assessment (EFSA FAF Panel, 2024a).

Regarding aspartame (E 951), a total of 48,629 analytical results were reported to EFSA by 17 EU Member States between 2014 and 2023. The Panel considered data for foods and beverages sampled since the last 2013 EFSA opinion on aspartame (EFSA ANS Panel, 2013).

The reporting countries were Germany ($n = 35{,}020$), Hungary ($n = 3586$), Slovakia (2897), Austria ($n = 2278$), Italy ($n = 1351$), Ireland ($n = 935$), Belgium ($n = 609$), Lithuania ($n = 456$), Cyprus ($n = 405$), Luxembourg ($n = 267$), Croatia ($n = 257$), Spain ($n = 213$), Portugal ($n = 196$), Czechia ($n = 65$), Denmark (n = 60), Greece ($n = 29$) and France ($n = 5$).

In total, 88.5% of the analytical results of E 951 were left-censored (either < LOD or < LOQ) and were excluded from the exposure assessment (EFSA FAF Panel, 2024a). In addition, three results were excluded as they were 'above the working limit' of the analytical method or a qualitative value was provided. This resulted in 5613 analytical results for E 951 to be considered.

With the removal of 22 analytical levels from a non-accredited laboratory, an additional 708 analytical levels reported at the level of LOQ and 72 duplicates samples, 4811 analytical results were left.

The data were further cleaned, and some clarifications were requested from the data providers.

The Panel noted that 42 quantified analytical results were reported food categories in which E 951 is not authorised for direct addition according to Annex II of Regulation (EC) No. 1333/2008, including FC 07.2 'Fine bakery wares' other than essoblaten – wafer paper (authorised for use up to 2019), FC 04.2.3 'Canned or bottled fruits and vegetables' other than fruits, FC 14.2.2 'Wine and other products defined in Part II of Annex VII to Regulation (EU) No 1308/2013', and FC 14.2.4 'Fruit wine and made wine'. In addition, four analytical results of food additives preparations were reported. These results were not considered in the exposure assessment. A further 10 results were discarded, because no clarifications were provided in response to a request for more information.

In addition, 59 analytical results for authorised food categories were above the MPL and were not considered. These results were for FC 14.1.4 Flavoured drinks ($n = 20$), FC 17.1 and 17.2 Food supplements supplied in solid and liquid form (respectively 26 and 2 samples), FC 12.7 Salads and savoury based sandwich spreads ($n = 3$), FC 05.2 Confectionary ($n = 3$), FC 04.2.2 Fruit and vegetables in vinegar, oil or brine ($n = 2$), FC 12.6 Sauces ($n = 1$) and FC 14.2.1 Beer and malt beverages ($n = 2$).

Overall, 4700 analytical results for aspartame (E 951) in foods and beverages were considered for the exposure assessment corresponding to 30 out of 34 food categories in which E 951 is authorised as a food additive (Annex II to Regulation (EU) No 1333/2008).

The majority of these 4700 analytical values were for FC 14.1.4 'Flavoured drinks' ($n = 2903$), followed by FC 05.3 'Chewing gum' ($n = 324$), FC 04.2.2 Fruit and vegetables in vinegar, oil or brine ($n = 249$), FC 01.4 Flavoured fermented milk products including heat-treated products ($n = 237$), FC 05.2 'Other confectionery including breath freshening micro-sweets' ($n = 218$), FC 14.2.8 Other alcoholic beverages ($n = 116$) and FC 17.1 Food supplements supplied in a solid form, excluding

[18]Term 'authorised uses' refers to each single authorised use of the food additive considering each restriction/exception. Term 'authorised food categories' refers to the food categories in which the use of the food additive is authorised regardless of the restriction/exception.

food supplements for infants and young children ($n = 110$). For the other food categories, the number of analytical values varied from 1 to 90.

Details on the analytical results available for the exposure assessment are provided in Annex A, Table A3.

3.3.2 | Summarised data extracted from Mintel's Global New Products Database

Mintel's GNPD contains one food product (chewing gum) labelled to contain the salt of aspartame-acesulfame (E 962) in 2025. The Panel noted that six use levels of this additive were received from IBO on four food categories of which one was chewing gum.

According to Mintel's GNPD, between January 2020 and October 2025, aspartame (E 951) was labelled on 2853 products. These products belong mainly to the Mintel subcategories 'Carbonated Soft Drinks' ($n = 945$), 'Gum' ($n = 449$), 'Vitamins & Dietary Supplements' ($n = 220$), 'Prepared Meals' ($n = 157$) and 'Spoonable Yogurt' ($n = 119$).

Annex A, Table A4 lists the percentages of the food products labelled to contain aspartame (E 951) out of the total number of food products per food subcategory according to Mintel's GNPD food classification. The percentages ranged from less than 0.1% in many food subcategories to 52% in Mintel's GNPD food subcategory 'Gum', followed by 'Liquid Dairy Other' with 30%. The overall percentage of foods labelled to contain E 951 was 1.2%. However, these percentages do not consider the market share of the products listed per food subcategory.

Annex A, Table A4 also contains the list of corresponding food categories according to Annex II to Regulation (EC) No. 1333/2008. The information from Mintel's GNPD indicated the use of aspartame (E 951) in two authorised food categories (FC 15.2 'Processed nuts' and FC 05.4 'Decorations and coating', only starch-based/only confectionary with no added sugar/only cocoa and dried fruit based), for which no use levels or analytical data were reported. The Panel considered that the impact of not including these two food categories into the exposure assessment is low. Foods belonging to FC 05.4 may be widely consumed but only in small quantities and only three food products belonging to this food category contain E 951 according to Mintel's GNPD. For FC 15.2 'Processed nuts', only five food products were indicated to contain the sweetener, but foods belonging to this food category are not widely consumed.

The Panel noted that for eight subcategories in which the use of aspartame (E 951) is not authorised, foods were found labelled to contain it (for instance, FC 14.1.2 'Juice'). As a one-to-one linkage between Mintel's GNPD food subcategories and the food categories according to Annex II to Regulation No. 1333/2008 was not possible, these results should be considered indicative. Furthermore, the Panel noted that the percentage of products containing E 951 from non-authorised food categories out of the total number of products labelled as containing E 951 on the market was 0.5%.

3.3.3 | Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

Food consumption data of different population groups, infants, toddlers, children, adolescents, adults and the elderly, in the Comprehensive Database were used for this exposure assessment. Food consumption data were available from 46 different dietary surveys carried out in 23 European countries.[19] The details of the population groups considered and the countries with food consumption surveys available are presented in Annex A, Table A1.

Specific considerations on table-top sweeteners

An examination of the consumption records of the table-top sweeteners in tablet form in the Comprehensive Database showed peaks in the consumption distributions at integer numbers of 1 and above in certain dietary surveys. These amounts very likely reflect the number of tablets consumed instead of the consumption in the requested unit of grams. EFSA has asked the data providers of the 17 relevant surveys if they could confirm this and, to date, has received an affirmative response from the data providers of seven dietary surveys. No response has yet been received for the other nine surveys.

For the current assessment, the consumed amounts of table-top sweetener in tablet form in integer numbers of 1 and above in all dietary surveys were converted to grams by multiplying the reported consumption with the highest unit weight of tablets reported to contain aspartame (E 951) in Mintel's GNPD, i.e. 0.085 g per tablet. The same correction was applied to the consumption records of unspecified table-top sweeteners (i.e. those for which the form, tablet or otherwise, was not reported) in the same 16 dietary surveys, as the same consumption pattern was found for these table-top sweeteners.

For this opinion, this ad-hoc correction was applied to the dataset extracted from the Comprehensive database in October 2025.

[19]Not all Member States provided consumption information for all population groups, and in some cases the same country provided food consumption data from more than one consumption survey. In most cases, when, for one country and age class, different dietary surveys were available, only the most recent 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.

Food categories considered for the exposure assessment of aspartame from E 962 and E 951

Food categories for which concentration data for the salt of aspartame-acesulfame (E 962) and aspartame (E 951) were provided, were selected from the nomenclature of the Comprehensive Database (FoodEx2 classification system), at the most detailed level possible (up to FoodEx2 Level 7) (EFSA, 2015b).

Facets were used to identify eating events referring to foods reported to contain sweeteners (i.e. energy-reduced or with no added sugar) and to foods related to the specific restrictions/exceptions as defined in the legislation for the use of the salt of aspartame-acesulfame (E 962) and aspartame (E 951) (see details in Annex A, Table A5). Facets were not used to identify relevant eating events for FCs 11.4 'Table-top sweeteners' and 05.3 'Chewing gum', for gum drops in FC 05.2 'Other confectionery including breath refreshening microsweets', for energy drinks in FC 14.1.4 'Flavoured drinks' and for vitamin and mineral supplements in FC 17 'Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children' (EFSA FAF Panel, 2024a).

As FC 17 Food supplements does not consider food supplements for infants and toddlers as defined in the legislation, the exposure estimates of aspartame (E 951) for these two population groups do not include the exposure via food supplements.

Eating occasions belonging to FCs 13.2 Dietary foods for special medical purposes and 13.3 Dietary foods for weight control diets intended to replace total daily food intake or an individual meal were reclassified under food categories in accordance with their main component (e.g. gluten-free pasta reclassified as pasta). The concentration data from these two food categories were not considered in the exposure assessment; instead, the concentration data for the food categories into which they were reclassified were used.

Some restrictions/exceptions of certain food categories are not referenced in the Comprehensive Database and therefore the whole food category was considered in the exposure assessment (Annex A, Table A5). This was the case for FC 05.2 'Other confectionery including breath refreshening microsweets' for the restrictions 'only strongly flavoured throat pastilles with no added sugar' and 'only energy-reduced tablet form confectionary' and for FC 06.3 'Breakfast cereals' for the restriction 'only breakfast cereals with a fibre content of more than 15%, and containing at least 20% bran, energy-reduced or with no added sugar'. Restriction for FC 14.2.3 'Cider and Perry' was also not considered and all foods belonging to this food category were included in the exposure assessment.

In addition, the consumption of FC 04.2.5.1 'Extra jam and extra jelly as defined by Directive 2001/113/EC' cannot be distinguished from that of FC 04.2.5.2 'Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC' in the Comprehensive Database and therefore, the consumption of FC 04.2.5.1 was considered in the exposure assessment via the consumption of jam (FC 04.2.5.2).

Food supplements in solid and liquid form have a different MPL which is applicable to food supplements in a chewable or syrup form, respectively (see Table 2). According to Mintel GNPD, out of 220 food supplements labelled with E 951, only three are in a chewable form and three in a syrup form. Therefore, these restrictions were not considered and the MPLs for FCs 17.1 and 17.2 used in the exposure assessment were respectively 2000 and 600 mg/kg.

The Panel noted that for some food categories, the number of concentration data for a specific food category was limited (< 5), but these data were considered anyway in the exposure assessment.

Overall, out of the 34 food categories in which the salt of aspartame-acesulfame (E 962) and aspartame (E 951) are authorised, 32 food categories were included in the *regulatory maximum level exposure scenario* (29 food categories with an MPL and the highest reliable percentile of reported analytical level for the three FCs 11.4.1, 11.4.2 and 11.4.3 ('Table-top sweeteners')). For the refined scenarios (i.e. *refined regulatory maximum level exposure assessment scenario* and *refined brand-loyal exposure assessment scenario*), 29 food categories were included. Compared to the *regulatory maximum level exposure scenario*, FCs 04.2.4.1 'Fruit and vegetable preparations excluding compote', 05.4 'Decorations coatings and fillings' (for three restrictions: 'only cocoa or dried fruit based, energy-reduced or with no added sugar', 'only confectionery with no added sugar' and 'only starch-based confectionery energy-reduced or with no added sugar') and 15.2 'Processed nuts' was not included in the refined exposure scenarios due to lack of concentration data.

The assigned concentrations to each food category in each scenario are detailed in Annex A, Table A5.

3.4 | Dietary exposure estimates

The Panel considered appropriate, in the remit of the re-evaluation of sweeteners, to estimate the chronic exposure to aspartame (E 951) according to the sweetener exposure protocol (EFSA FAF Panel, 2024a). As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011), dietary surveys with only 1 day per subject were not considered as they are not adequate to assess repeated exposure. Similarly, subjects who participated only 1 day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment.

Exposure assessments of sweeteners under the re-evaluation programme are carried out by the Panel based on two different sets of concentration data: (a) MPLs set down in the EU legislation (in the *regulatory* and *refined regulatory maximum level exposure assessment scenario*) and (b) use levels and/or analytical data provided through the calls for data (in the *refined brand-loyal exposure assessment scenario*).

To calculate the chronic dietary exposure to aspartame, food consumption and body weight data at the individual level were extracted from the Comprehensive Database and linked to the concentration data as described in section 5.2.1 of the

sweetener exposure protocol (EFSA FAF Panel, 2024a). Three different scenarios were performed according to the sweetener exposure protocol (EFSA FAF Panel, 2024a):

- Regulatory maximum level exposure assessment scenario: exposure calculations based on the MPLs according to Annex II to Regulation (EC) No 1333/2008.
- Refined brand-loyal exposure assessment scenario: exposure calculations based on the maximum (highest reliable percentile) or mean/median levels.
- Refined regulatory maximum level exposure assessment scenario: exposure calculations based on the MPL according to Annex II to Regulation (EC) No 1333/2008 for the same FCs as in the refined brand-loyal exposure assessment scenario.

These scenarios are performed for only consumers of at least one food category that could contain E 951. Exposure estimates for these population groups are assumed to be the best approximation reflecting the exposure levels in diabetics, i.e. the population with the highest expected exposure to sweeteners (EFSA FAF Panel, 2024a). Depending on the food categories considered in the exposure assessment, the exposure was estimated based on different numbers of consumers. Exposure estimates based on fewer food categories could be higher than those based on a larger number of food categories due to the higher number of non-consumers within certain food categories.

In order to evaluate if consumers only of a single food category could have a higher exposure than consumers only of at least one food category, the exposure to aspartame for consumers only of each single food category (but still considering their whole diet) was also calculated for the *refined brand-loyal exposure assessment scenario*. These exposure estimates are discussed if they are higher than the exposure estimates for consumers only of at least one food category for this refined scenario.

Annex A, Table A5 summarises the concentration levels of aspartame used in the different exposure assessment scenario.

3.4.1 | Dietary exposure to aspartame from the use of E 962 and E 951

Table 3 summarises the estimated dietary exposure to aspartame from the use of aspartame-acesulfame (E 962) and aspartame (E 951) as food additives in six population groups according to three exposure scenarios among consumers only of at least one food category containing E 962 and/or E 951.

TABLE 3 Summary of chronic dietary exposure to aspartame from the use of E 962 and E 951 as food additives in the *regulatory maximum level exposure assessment scenario*, *refined regulatory maximum level exposure assessment scenario* and *refined brand-loyal exposure scenario*, in six population groups among consumers only of at least one food category containing aspartame (minimum–maximum across the dietary surveys in mg/kg bw per day and, in brackets, number of surveys).

	Infants (4–12 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[a]						
• Mean[b]	0.1–3.4 (12)	0.4–8.8 (17)	0.5–5.6 (21)	0.3–2.9 (23)	0.3–2.0 (23)	0.1–1.4 (25)
• 95th percentile[c]	0.5–7.3 (4)	1.3–30.5 (16)	1.5–19.7 (21)	1.1–10.2 (22)	0.8–7.1 (23)	0.5–5.4 (24)
Refined regulatory maximum level exposure assessment scenario						
• Mean[b]	0.3–8.5 (8)	0.8–12.1 (15)	0.3–6.2 (20)	0.1–3.3 (22)	0.3–2.5 (23)	0.2–2.5 (23)
• 95th percentile[c]	1.6 (1)	2.2–33.2 (7)	1.3–20.2 (18)	1.5–10.5 (17)	1.3–7.4 (20)	0.8–13.1 (16)
Refined brand-loyal exposure assessment scenario						
• Mean[b]	0.1–7.7 (8)	0.5–7.6 (15)	0.2–4.8 (20)	0.1–2.7 (22)	0.3–2.3 (23)	0.2–2.3 (23)
• 95th percentile[c]	0.9 (1)	1.5–26.7 (7)	1.0–15.1 (18)	1.2–9.3 (17)	1.2–6.7 (20)	0.7–13.1 (16)

[a]Results of the regulatory maximum level exposure assessment scenario and the two refined exposure assessment scenarios are not comparable as the underlying populations of consumers are different. This is due to a difference in the number of food categories considered ($n = 31$ and 29, respectively) and because facets are only considered in the regulatory maximum level exposure assessment scenario when the restriction 'only energy-reduced products or with no added sugar' applies (EFSA FAF Panel, 2024).

[b]Mean estimates based on dietary surveys/population groups up to and including 5 consumers may not represent the population group and are thus not included in this table.

[c]95th percentile estimates based on dietary surveys/population groups up to and including 59 consumers may not be statistically robust (EFSA, 2011) and are thus not included in this table.

Detailed results per population group and survey for all three exposure scenarios are presented in Tables A6 and A7 of Annex A.

Main food categories contributing to the exposure to aspartame (from E 962 and E 951)

In the *regulatory maximum level exposure assessment scenario*, the main contributing food category to the total mean exposure estimates was FC 14.1.4 Flavoured drinks for all population groups except for infants, where FC 12.6 Sauces contributed

most. In addition, FC 15.2 Processed nuts contributed also considerately for infants, FC 12.6 Sauces for toddlers, FC 11.4.3 Table-top sweeteners in tablet form for adults and FC 04.2.2 Fruit and vegetables in vinegar, oil or brine for the elderly.

In the *refined brand-loyal exposure assessment scenario*, the main contributor was FC 14.1.4 'Flavoured drinks' in all population groups. Then, the food categories contributing most for infants were FC 05.2 'Other confectionary' and FC 16 'Desserts including breath freshening microsweets'; while for toddlers, these were FC 01.4 'Flavoured fermented milk products' and FC 05.2 'Other confectionary including breath freshening microsweets. For children, FC 05.2 'Other confectionary including breath freshening microsweets' was the 2nd most contributing food category. For adolescents, the two FCs 17 and 17.1 'Food supplements' and 'Food supplements in solid form' were the next most contributing food categories and finally, for adults and the elderly, FCs 11.4 and 11.4.2 'Tabletop sweeteners in tablets' and 'Tabletop sweeteners' without form were main contributors to the total dietary exposure.

A similar pattern to the *refined brand-loyal scenario* was observed in the *refined regulatory maximum level exposure assessment scenario*.

For details on the contribution of each food category to the exposure to aspartame in the three scenarios, see Tables A8, A9 and A10 in Annex A.

Dietary exposure for consumers of a single food category containing aspartame (from E 962 and E 951)

Exposure to aspartame was also calculated for consumers only of each food category separately, while still considering their whole diet, for the *refined brand-loyal exposure assessment scenario*. Annex A, Table A11 lists the maximum mean and P95 exposure estimates that exceeded the highest corresponding exposure estimates of consumers only of at least one food category in the *refined brand-loyal exposure assessment scenario*.

For most of the exposure estimates, mean and P95 exposure for consumers only of one food category were lower than those for consumers only of at least one food category. The mean exposure estimates for consumers of six food categories exceeded the highest mean exposure estimates of consumers of at least one food category (Table 3): FCs 05.2 'Other confectionery including breath freshening microsweets' in toddlers (12.6 mg/kg bw per day), 11.4 'Table top sweeteners' in adults (12.2 mg/kg bw per day), 11.4.2 'Tabletop sweeteners in powder form' in children and adolescents (10.5 and 9.8 mg/kg bw per day, respectively), 11.4.3 'Tabletop sweeteners in tablets' in adults (9.2 mg/kg bw per day), 14.1.4. 'Flavoured drinks' in infants and toddlers (9.6 and 11.1 mg/kg bw per day) and 16 'Desserts' in children (7.7 mg/kg bw per day). For the 95th percentile of exposure, this was only true for FC 14.1.4 'Flavoured drinks' in toddlers (29.4 mg/kg bw per day).

3.4.2 | Uncertainty analysis related to the exposure assessment

Potential sources of uncertainty in the exposure assessment of aspartame have been presented above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 4.

TABLE 4 Qualitative evaluation of influence of uncertainties on the dietary exposure estimate.

Sources of uncertainties	Direction[a]
Consumption data	
Different methodologies/representativeness/underreporting/misreporting/no portion size standard/only a few days	+/–
Underreporting of food descriptors (facets) concerning the presence or potential presence of sweeteners	–[b]
Not considering some of the restrictions specified in the legislation (e.g. restrictions related to FC 06.3)	+
Tabletop sweeteners in tablets and unspecified form: food consumption data assumed to be reported in units were adjusted with the highest tablet size in Mintel's GNPD	+
Unspecified tabletop sweeteners in the consumption database matched with the occurrence data for tablet top sweeteners in tablets (highest available among the tabletop sweeteners categories)	+
Concentration data	
Correspondence of reported use levels and analytical data to the food items in the Comprehensive Database: uncertainties to which types of food the levels refer	+/–
Uncertainty in possible national differences in use levels of food categories	+/–
Regulatory maximum level exposure assessment: 32 food categories out of the 34 authorised to contain aspartame from the use of E 951 or E 962 were considered in the exposure assessment	+/–
Refined regulatory maximum level exposure assessment and brand-loyal scenario: 29 out of the 34 authorised food categories to contain aspartame were considered in the exposure assessment	–
Refined regulatory maximum level exposure assessment and *brand-loyal exposure assessment scenario*: 55 out of 77 food subcategories in Mintel, representing 91% of the products labelled with aspartame (E 951) were included in the current exposure assessment	–

(Continues)

TABLE 4 (Continued)

Sources of uncertainties	Direction[a]
Concentration levels/MPLs considered applicable to all foods for the majority of food categories, while the percentage of foods out of the total number of foods in a corresponding food subcategory were labelled to contain aspartame (E 951) in Mintel was maximally 52% (FC 05.2)	+
Methodology	
Regulatory and *refined regulatory maximum level exposure assessment scenario*:	+
– Exposure calculations based on the MPL according to Annex II to Regulation (EC) No 1333/2008	
Refined brand-loyal exposure assessment scenario:	+/–
– Exposure calculations based on the maximum (highest reliable percentile) or mean/median levels	
Use of data from food consumption survey covering only a few days to estimate high percentile (95th) of long-term (chronic) exposure	+

[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 dietary exposure to aspartame from the use of aspartame (E 951) or the salt of aspartame-acesulfame (E 962) estimated for the *refined regulatory maximum level exposure assessment* and *brand-loyal exposure assessment scenario* covers the majority of food categories in which the sweeteners are authorised (29 out of the 34 authorised food categories and 55 out of 77 food subcategories in recorded in Mintel).

The Panel acknowledged that the assumption that all foods meeting the facets' criteria (see Section 3.3.3) within a food category for which concentrations were available, contain aspartame has resulted in an overestimation of the exposure to this sweetener. This observation is supported by the fact that a maximum of 52% of foods out of the total number of foods in a corresponding food subcategory (0.9% on average) were labelled to contain aspartame (E 951) in Mintel.

Overall, the Panel considered the dietary exposure to aspartame from the use of E 962 and E 951 as food additives, to be overestimated by the *regulatory maximum* and the two *refined exposure assessment scenarios* (i.e. *maximum* and *brand-loyal*).

The Panel considered the *refined brand-loyal exposure assessment scenario* the most appropriate exposure scenario for the risk assessment of aspartame from the use of the salt of aspartame-acesulfame (E 962) and aspartame (E 951) as food additives.

3.4.3 | Concentrations of and dietary exposure to aspartame (E 951) in the literature

A literature search (Appendix A) was carried out to gather information on levels of the salt of aspartame-acesulfame (E 962) and aspartame (E 951) in food and beverages, and on dietary exposure estimates of aspartame in Europe published between 2013 and 2025.

Occurrence data

Several European studies have analysed aspartame in food and beverages. The results of these studies are discussed below. The mean concentrations reported are based on quantified values.

In Diogo et al. (2013), a total of 78 samples of drinks were purchased from supermarkets in Portugal and analysed including 59 traditional soft drinks and soft drinks based on mineral waters, 3 sport/energy drinks and 16 nectars. For 21 out of 78 samples (27%), aspartame was reported at concentrations ranging from 6.1 to 568 mg/L and a mean concentration of 155 mg/L. The maximum reported concentration referred to a sample of soft drinks based on mineral waters. In another Portuguese study, a total of 56 samples of drinks were analysed including 27 traditional soft drinks, 10 soft drinks based on tea extracts, 4 soft drinks based on mineral waters, 6 sport/energy drinks and 9 nectars (Basílio et al., 2020). In 21 of these samples (37.5%), aspartame was reported at concentrations ranging from 60 to 679.8 mg/L. The maximum reported concentration referred to a sample of energy drinks. In a third Portuguese study, Silva et al. (2021) analysed 68 samples of soft drinks that were targeted to contain the sweetener according to the label, and grouped them as colas ($n = 16$), juice drinks ($n = 28$), iced teas ($n = 13$) and lemon- flavoured drinks ($n = 11$). Aspartame was found in all samples, at concentrations ranging from 19 to 231 mg/L.

In a study from Romania, Oroian et al. (2013) analysed 30 samples of carbonated drinks, not targeted to contain aspartame. Aspartame was reported in all samples at concentrations ranging from 42 to 883 mg/L, with a mean concentration of 510 mg/L.

Concentrations of aspartame in food and food supplements ($n = 290$) on the Italian market were reported by Janvier et al. (2015). Foods included were flavoured drinks ($n = 57$), fruit nectars ($n = 18$), syrups ($n = 3$), jams ($n = 14$), ketchups ($n = 1$), confectionery ($n = 84$), yogurt ($n = 42$), ice creams ($n = 3$), table-top sweeteners ($n = 14$) and food supplements ($n = 54$), with no detection of aspartame in syrups, jams and ketchup. In 20 samples of flavoured drinks (35%), aspartame was reported at a mean concentration of 162 mg/L. In two of the 14 samples of table-top sweetener formulations (14%), aspartame was found at a mean concentration of 388 mg/kg. Aspartame was found in all 42 samples of chewing gum at a mean

concentration of 1922 mg/kg and in 11 of the 54 samples of solid food supplements (20%) at a mean concentration of 5797 mg/kg for which 8 (72%) exceeded the MPL of 2000 mg/kg set out by the EU regulation.

Buffini et al. (2018) analysed 377 samples of foods and beverages from the Irish market, which comprised 17 food categories. Aspartame was not reported in edible ices, ice-cream cornet and wafers, soups and broths, sauces and mixtures of alcoholic and non-alcoholic drinks. In the other food categories, aspartame was reported at a mean concentration of 226 mg/kg for dairy products, 415 mg/kg for jams, jellies and marmalade, 255 mg/kg for cocoa and chocolate products, 1295 mg/kg for non-chocolate confectionary, 306 g/kg for table-top sweeteners 179 mg/L for flavoured drinks, 277 mg/L for carbonated flavoured drinks, 126 mg/L for cider and perry, 45 mg/kg for potato, cereals or flour based snacks, 135 mg/kg for desserts, and 10 and 18.9 g/kg for solid food supplements and syrup-type or chewable foods supplements, respectively. It is noted that measured concentration data for all 21 solid food supplements and 3 syrup-type or chewable foods supplements exceeded the MPL set out by the EU regulation with 3 to 5 times higher levels than the MPL of 2000 mg/kg and 5500 mg/L (see Table 2), respectively.

Van Vliet et al. (2020) analysed 111 samples of soft drinks from 10 European countries (Belgium, Denmark, Finland, France, Germany, Spain, Sweden, the Netherlands, Turkey and the United Kingdom) that were divided into four subgroups; orange drinks, lemon drinks, cola drinks and other drinks. In 95 of these samples (86%), aspartame was reported at concentrations ranging from 30 to 527 mg/L.

Knezovic et al. (2025) analysed 121 samples of foods that are regularly consumed in Croatia. The samples were categorised into seven groups: carbonated and non-carbolated soft drinks ($n=47$), fruit juices/nectars ($n=26$), syrups for dilution ($n=3$), instant drinks ($n=6$), energy drinks ($n=7$), high-protein milk products ($n=20$) and chewing gum ($n=12$). Aspartame was not reported in energy drinks, instant drinks and high-protein milk samples. It was reported in 35 samples of carbonated and non-carbonated soft drinks (31.7%) at concentrations ranging from 1 to 494 mg/L with a mean concentration of 36 mg/L. Aspartame was also reported in 14 samples of juice/nectars (54%) in concentrations ranging from 1 to 10 mg/L with a mean concentration of 1.9 mg/L, in all samples of syrups in concentrations ranging from 1 to 37.1 mg/L with a mean concentration of 24.2 mg/L and in 8 samples of chewing gum (67%) at concentrations ranging from 1 to 1792 mg/kg with a mean concentration of 859 mg/kg.

In summary, the quantified levels of aspartame in food and beverages reported in the above-mentioned studies in Europe are comparable to the analytical data reported to EFSA. The Panel noted that for solid food supplements, there were analytical data above the MPL in the reported literature, this is also the case in the analytical data submitted to EFSA (see Section 3.3.1).

Dietary Exposure

Below, publications that have assessed the dietary exposure to aspartame considering the whole diet are reported. The Panel noted that the methodologies used in these studies to assess the exposure differ from the one described in the protocol on exposure assessment of sweeteners.

An European research study conducted in the frame of the FACET project, assessed the exposure to aspartame (E 951) for France, Italy, the UK and Ireland (Vin et al., 2013). The assessment was based on individual food consumption data with use levels of aspartame in foods and beverages provided by food industry. The mean exposure to aspartame, across countries, ranged from 0.62 to 3.1 mg/kg bw per day in children (1–17 years) and from 0.18 to 1.0 mg/kg bw per day in adults. The P97.5 estimates ranged from 2.6 to 8.7 and 1.2 to 3.7 mg/kg bw per day, respectively.

Aspartame exposure of Belgian children aged 4–6, 7–12 and 13–18 years with type 1 diabetes was assessed based on analytical data and a specific food survey (De Winter et al., 2015). The mean exposure to aspartame for the three age groups ranged from 0.68 to 1.07 mg/kg bw per day with children aged 7–12 years having the highest exposure levels. The P95 estimates for consumers only was between 2.72 to 3.89 mg/kg bw per day, with children aged 4–6 years having the highest exposure levels. An exposure assessment combining individual food consumption data of the Belgian adult population with MPLs resulted in mean aspartame exposure of 6.9 mg/kg bw per day and a P95 estimate of 14.7 mg/kg bw per day for the total adult population (Van Loco et al., 2015).

Aspartame (E 951) exposure of French children aged less than 3 years was assessed based on individual food consumption data and MPLs (Mancini et al., 2015). The mean exposure to aspartame within four age groups (1–4, 5–6, 7–12 and 13–36 months) ranged from 0.19 to 14.1 mg/kg bw per day with children aged 13–36 months having the highest exposure levels. The P90 estimates were between 0 and 23.9 mg/kg bw per day, also with children aged 13–36 months having the highest exposure levels. Chazelas et al. (2021) reported a mean exposure to aspartame (E 951) of 0.13 mg/kg bw per day and a P95 exposure of 0.72 mg/kg bw per day for French adults that participated in the NutriNet-Santé cohort. The concentration data used was a mix of analytical data, use levels and MPLs.

Estimates of aspartame (E 951) exposure in Irish children of 1–4 years were reported by Martyn et al. (2016) combining food survey data and analytical data in foods and beverages available on the Irish market. Mean aspartame exposure of consumers only was 0.76 mg/kg bw per day and 2.8 mg/kg bw per day at the P95.

Estimates of aspartame (E 951) exposure in a 3 to >65 years old Italian population ranged from 0.2 (mean of total population) to 0.6 mg/kg bw per day (P95 of total population) (Le Donne et al., 2017). The estimates were based on analytical data and a food label survey.

Based on the analytical data and food consumption data from the National Adults Nutrition Survey described by Buffini et al. (2018; see above), the mean exposure to aspartame (E 951) in consumers only of the adult Irish population was 0.80 mg/kg bw per day and 7.1 mg/kg bw per day at the P99.

Carvalho et al. (2022) estimated the exposure to aspartame (E 951) based on the Portuguese dietary survey IAN-AF 2015–2016. Using analytical data for food categories containing foods that were labelled to contain aspartame (E 951), exposure estimates varied within the age groups (> 3–84 years) between 0.08 and 0.30 mg/kg bw per day at the mean and from 0.37 to 1.31 mg/kg bw per day at the P95.

A dietary exposure of aspartame via foods and beverages in the Dutch population was performed in a refined assessment using branded data (product labels, use levels and consumption shares of brands) and scenarios for brand-loyalty (Sprong et al., 2025). The median exposure of consumers only to aspartame within age groups (toddlers, children, adolescents and adults) ranged from 0.16 mg/kg bw per day in adults to 0.28 mg/kg bw per day in adults. The P95 ranged from 1.3 mg/kg bw per day in toddlers to 4.1 mg/kg bw per day in adults.

In summary, the Panel noted that estimates from the current opinion tended to be higher than those reported in the literature. However, a direct comparison is not possible because (i) more food categories are considered in this opinion, (ii) the approach is different (consumers only approach vs. whole population), (iii) concentration data used in the opinion are from across all European countries vs. country specific data in the literature, (iv) and/or different population groups are considered.

3.5 | Biological and toxicological data

The FAF Panel assessed the safety of the salt of aspartame-acesulfame (E 962) by a separate assessment of aspartame and acesulfame; additionally, data specifically referring to the salt of aspartame-acesulfame (E 962) were also considered as part of the assessment, when available (see Section 2).

No data were received on the salt of aspartame-acesulfame (E 962) in response to the call for biological and toxicological data.[20] One study on a binary mixture of aspartame (E 951) and acesulfame k (E 950) was identified from the updated literature search.

For aspartame (E 951), all relevant studies published since the previous evaluation by EFSA (EFSA ANS Panel, 2013), allowing 1 year of overlap, and up to 4 and 6 June 2025 (Appendix A) were considered in this assessment. In addition, the Panel considered data on DKP, a major degradation product of aspartame (EFSA ANS Panel, 2013; JECFA, 2023). The biological and toxicological studies were assessed according to the inclusion criteria established in the revised protocol on hazard identification and characterisation of sweeteners (EFSA FAF Panel, 2023). An evaluation of the risk of bias (RoB) of studies considered relevant was performed (Annex D) and a weight of evidence (WoE) approach for the reliable studies (for which RoB was scored as tier 1 or tier 2) was applied for each health outcome for both human and animal studies (Annex E1, Annex E2). A narrative synthesis of the WoE analysis is reported in Section 3.5.4.

Studies on absorption, distribution, metabolism and excretion (ADME) were not subject to RoB assessment but were evaluated independently by two experts. Information from mechanistic studies, and studies not directly relevant for the identification of a reference point were considered as supporting information (Appendix C).

Genotoxicity studies were evaluated according to the approach outlined in the revised protocol (EFSA FAF Panel, 2023).

Regarding acesulfame, the Panel refers to the assessment outlined in the latest EFSA scientific opinion on acesulfame K (E 950) of 2025 (EFSA FAF Panel, 2025).

3.5.1 | Absorption, distribution, metabolism and excretion

The Panel considered that upon ingestion, the salt of aspartame-acesulfame (E 962) will be fully dissociated into the two components, aspartame and acesulfame, which would be expected to have the same fate whether they result from dissociation of the salt or from the ingestion of the food additives aspartame (E 951) and acesulfame K (E 950), respectively (see Section 3.1).

3.5.1.1 | *The salt of aspartame-acesulfame (E 962)*

No data on the ADME of the salt of aspartame-acesulfame (E 962) were received in response to the call for biological and toxicological data.[21] No new relevant data regarding ADME were identified from the updated literature search (Appendix A).

3.5.1.2 | *Aspartame*

The ADME of aspartame was previously evaluated by the ANS Panel in 2013 based on the results of the unpublished studies received following a public call for data and the published literature identified until 15 November 2013. The conclusion of the ANS Panel was as follows: '*the Panel noted that no unchanged aspartame was identified in body fluids or in tissues from*

[20]Call for technical and toxicological data on sweeteners authorised as food additives in the EU - Extended deadline for submitting data: 30/6/2018. https://www.efsa.europa.eu/en/data/call/170621.

[21]Call for technical and toxicological data on sweeteners authorised as food additives in the EU - Extended deadline for submitting data: 30/6/2018. https://www.efsa.europa.eu/en/data/call/170621.

experimental animals or humans in any of the studies. Therefore, the Panel concluded that after oral ingestion, aspartame was hydrolysed in the gastrointestinal tract to yield aspartic acid, phenylalanine and methanol. These metabolites are then absorbed and enter normal endogenous metabolic pathways'. Humans heterozygous for phenylalanine hydroxylase mutations, show a slightly reduced capacity to metabolise phenylalanine compared to normal individuals. Individuals homozygous for phenylalanine hydroxylase mutations (phenylketonuria (PKU) patients) have a markedly reduced capacity for phenylalanine metabolism. After birth, homozygous PKU babies show severe impairment in development and cognition if the phenylalanine intake via *the diet is not strictly controlled* (EFSA ANS Panel, 2013).

Since the adoption of the EFSA opinion on aspartame (E 951) (EFSA ANS Panel, 2013), two new relevant studies were retrieved through an extensive literature search.

In an in vitro study from Sjostedt et al. (2017), interactions between the major intestinal efflux transporters belonging to the ATP-binding cassette (ABC) family, namely breast cancer resistance protein (BCRP), multidrug resistance-associated protein 2 (MRP2), P-glycoprotein (P-gp) and several food additives, including aspartame, were investigated. The authors concluded that there was no biologically significant interaction of aspartame with human BCRP, MRP2 or P-gp. The Panel agreed with this conclusion.

In a human study by Sylvetsky et al. (2015), 20 lactating women each provided one sample of breast milk. Aspartame along with several other sweeteners was measured using liquid chromatography-mass spectrometry. Aspartame was reported as not detected.

The Panel considered that no aspartame would be expected in body fluids or in tissues from experimental animals or humans.

3.5.1.3 | *5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP)*

The ADME of the aspartame degradation product DKP was previously evaluated by the ANS Panel in 2013 based on the results of the unpublished studies received following a public call for data and the published literature identified until 15 November 2013. The conclusion of the ANS Panel was as follows: '*Studies in animals and man have shown that DKP was not well absorbed from the gut and was recovered in the faeces, primarily as unchanged DKP. Following oral administration of DKP metabolism to phenylacetic acid occurs in the gastrointestinal tract, phenylacetic acid can then be absorbed and is rapidly excreted in the urine as phenylacetic acid and following conjugation with glutamate to phenylacetylglutamine. The urinary excretion of phenylacetic acid and phenylacetylglutamine accounts for 20% (rat) to 50% (monkey and man) of the orally administered DKP. Phenylacetylglutamine is a naturally occurring compound that is also generated during the metabolism of phenylalanine*'.

Since the adoption of the EFSA opinion on aspartame (EFSA ANS Panel, 2013), no relevant new studies on the ADME of DKP were retrieved through an extensive literature search.

3.5.1.4 | *Acesulfame K*

In the recent re-evaluation of acesulfame K (E 950), the FAF Panel considered that '*acesulfame K is almost fully absorbed (at least at doses up to 430 mg/kg bw in humans; 2000 mg/kg bw in rats). The Panel noted that acesulfame K is not metabolised, has a half-life of 2 to 4 h and is primarily excreted in the urine. Newly available data from humans focused on foetal and breast-fed infant exposure. Acesulfame K can pass through the placenta, as indicated by its detection in amniotic fluid and cord blood samples and enter foetal circulation. Acesulfame K also transfers into breast milk, exposing the breast-fed infant to a low extent, accounting for 1.6% of the mother's dose*' (EFSA FAF Panel, 2025).

3.5.1.5 | *Conclusion on the ADME of the salt of aspartame-acesulfame (E 962)*

The Panel considered that no aspartame would be expected in body fluids or in tissues from experimental animals or humans. The Panel concluded that after oral ingestion, aspartame in the salt of aspartame-acesulfame (E 962) will be hydrolysed in the gastrointestinal tract to yield aspartic acid, phenylalanine and methanol. These metabolites would then be absorbed and enter normal endogenous metabolic pathways. In humans, PKU patients show a reduced capacity for phenylalanine metabolism, therefore in this population the phenylalanine intake via the diet should be strictly controlled (EFSA ANS Panel, 2013).

Any DKP present in the salt of aspartame-acesulfame (E 962) would not be expected to be well absorbed from the gut. A minor proportion of DKP would be expected to be metabolised to phenylacetic acid in the gastrointestinal tract, absorbed and excreted in the urine as either phenylacetic acid – or as the glutamate conjugate – phenylacetylglutamine. The urinary excretion of phenylacetic acid and phenylacetylglutamine accounts for 20% (rat) to 50% (monkey and man) of orally administered DKP. Phenylacetylglutamine is a naturally occurring compound that is also generated during the metabolism of phenylalanine (EFSA ANS Panel, 2013).

Acesulfame from the salt of aspartame-acesulfame (E 962) would be expected to be almost fully absorbed; not metabolised, primarily excreted in urine and to have a half-life of 2–4 h. Acesulfame present in the salt of aspartame-acesulfame (E 962) would be able to pass through the placenta and enter foetal circulation as well as transfer into breast milk to a low extent (EFSA FAF Panel, 2025).

Overall, as outlined above, taking into consideration the few and limited new data available, the Panel reiterates the conclusions on ADME for aspartame of the ANS Panel in the 2013 opinion (EFSA ANS Panel, 2013) on the re-evaluation of

aspartame (E 951), and for acesulfame of the FAF Panel in the 2025 opinion (EFSA FAF Panel, 2025) on the re-evaluation of acesulfame K (E 950).

3.5.2 | Animal toxicity

No data on acute toxicity of the salt of aspartame-acesulfame (E 962) were received in response to the call for biological and toxicological data. No new data on acute toxicity were identified in the literature for the salt of aspartame-acesulfame (E 962) and for aspartame. Repeated dose toxicity studies on aspartame were assessed systematically and are discussed in Section 3.5.4.1.

3.5.3 | Genotoxicity

3.5.3.1 | *The salt of aspartame-acesulfame (E 962)*

No data on genotoxicity of the salt of aspartame-acesulfame (E 962) were received in response to the calls for data[22,23] No new data regarding genotoxicity were identified from the updated literature search for the salt of aspartame-acesulfame (E 962) (Appendix A).

3.5.3.2 | *Aspartame*

The genotoxicity of aspartame (E 951) was previously evaluated by the EFSA ANS Panel in 2013 based on the results of the unpublished studies received following a public call for data and the published literature identified until 15 November 2013.

The ANS Panel noted that the majority of the available studies were performed before the implementation of GLP and relevant OECD test guidelines but considered that these limitations would not per se preclude their consideration for risk assessment, provided that the study design and reporting of the data were appropriate. To this aim the quality of all studies was evaluated by the ANS Panel. The FAF Panel considered that the criteria adopted by the ANS Panel in 2013 (Appendix I to EFSA ANS Panel, 2013) were generally consistent with the recent criteria for the assessment of the reliability and relevance of genotoxicity studies to be considered in the WoE approach (EFSA, 2023; EFSA FAF Panel, 2023). Therefore, no re-evaluation of the studies considered by the ANS Panel in 2013 was deemed necessary in the current assessment.

According to the ANS Panel, the studies for which methods implemented were sufficiently robust to support the results reported included: (i) two Ames tests performed with a limited battery of tested strains considered sufficiently robust (i.e. lacking the TA102/E.coli WP2 strain); (ii) an in vitro unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes (of limited relevance); (iii) an in vivo chromosomal aberration test in rat bone marrow with repeated (five daily) oral administration; (iv) an in vivo micronucleus test in rat bone marrow with repeated (three daily) oral administration; (v) micronucleus tests in peripheral blood cells of transgenic mice strains after 9 months dietary exposure; (vi) an in vivo comet assay in various organs (stomach, colon, liver, kidney, bladder, lung, brain, bone marrow) of mice treated intragastrically with a single dose; (vii) two dominant lethal assays in rats. All in vitro and in vivo studies provided negative results, apart from the micronucleus assay in p53 haploinsufficient mice where an equivocal result was obtained in female animals.

The conclusion of the ANS Panel was as follows: *'The Panel concluded that the* in vitro *genotoxicity data on bacterial reverse mutation exhibited some limitations (*e.g. *absence of TA102 and WP2 uvrA Escherichia coli). However, the Panel considered the weight of evidence was sufficient to conclude that aspartame was not mutagenic in bacterial systems. Concerning mammalian systems* in vitro, *the Panel concluded that, apart from the valid UDS study that was negative, no conclusion could be drawn at the gene and chromosomal level because no studies dealing with these endpoints were available*.

In vivo, *the majority of investigations on systemic genotoxicity reported negative findings. Equivocal positive findings were only described in a NTP study, positive in female but not in male p53 haploinsufficient mice; in two other transgenic mouse strains the results were negative. Concerning the possible site of first contact effects* in vivo, *limited data are available. However, the available* in vitro *data do not indicate a direct genotoxic activity of aspartame that might predispose to a site of first contact effect* in vivo.

Overall, the Panel concluded that the available data do not indicate a genotoxic concern for aspartame'.

Concerning the possible effects at the site of first contact in vivo, the Panel noted that negative results were reported in an in vivo comet assay in several organs, including the stomach, in mice treated intragastrically with a single dose with aspartame to the maximum recommended dose (Sasaki et al., 2002, cited in EFSA ANS Panel, 2013).

Studies on aspartame genotoxicity published after the adoption of the last EFSA opinion (EFSA ANS Panel, 2013) were retrieved through an extensive literature search (Appendix A). Thirteen papers, reporting the results of ten in vitro, and

[22]Call for technical and toxicological data on sweeteners authorised as food additives in the EU – Extended deadline for submitting data: 30/6/2018. https://www.efsa.europa.eu/en/data/call/170621.

[23]Call for data on genotoxicity data on sweeteners. Deadline for submitting data: 31 March 2022 https://www.efsa.europa.eu/en/call/call-data-genotoxicity-data-sweeteners.

three in vivo studies and two papers reporting both in vitro and in vivo studies were deemed suitable for data extraction. The studies, described in detail in Annex C, were evaluated according to the EFSA harmonised criteria for the evaluation of genotoxicity evidence (EFSA, 2023; EFSA FAF Panel, 2023). The results of studies evaluated as of low relevance were not further considered in the WoE. The results of the remaining two papers, evaluated as of sufficient (high or limited) relevance are summarised in Tables 5 and 6.

In vitro, aspartame was negative in an Ames test performed with the whole OECD recommended battery of tester strains (Table 5). Negative results were also reported in a study evaluating the phosphorylation of the histone H2AX, as a marker of DNA double strand breaks, in a set of human cell lines of colon, liver, kidney and neuronal origin (Table 5).

TABLE 5 Summary table of the in vitro genotoxicity studies on Aspartame. A comprehensive summary of the extracted data and detailed account of the evaluations performed is provided in Annex C.

Test system	Exposure conditions (concentration/ testing conditions)	Information on the characteristics of the test substance	Result	Reliability/ comments[24]	Relevance of the test system/ relevance of the result[25]	Reference
Bacterial reverse mutation in Salmonella Typhimurium TA98, TA100, TA1535 and TA1537, and *E. coli* WP2 *uvrA*	Concentrations: 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate. With and without metabolic activation (S9 mix from liver of rats)	Aspartame (99.5% purity), supplied by Ajinomoto Co., Inc.	Negative	Reliable with restrictions	High/Limited	Otabe et al. (2019)
γH2AX/pH3 assay in four cell lines: LS-174T, HepG2, ACHN and SH-SY5Y	0.1, 0.3, 1, 3, and 10 mg/mL	Aspartame from supplier in France (E 951; CAS RN® 22,839–47-0)	Negative	Reliable with restrictions	Limited/ Limited	Recoules et al. (2025)

In vivo, negative results were obtained in the micronucleus test in mouse bone marrow after intragastric administration of aspartame (single dose at three dose levels) to the maximum dose recommended by OECD. Despite the lack of evidence of bone marrow toxicity, the results of this study were considered of high relevance, as toxicokinetic data demonstrate the systemic exposure to aspartame metabolites (phenylalanine, aspartic acid and methanol) after oral administration of aspartame to mice (EFSA ANS Panel, 2013).

TABLE 6 Summary table of the in vivo genotoxicity studies on Aspartame. A comprehensive summary of the extracted data and detailed account of the evaluations performed is provided in Annex C.

Test system	Exposure conditions (dose/ testing conditions) (administration)	Information on the characteristics of the test substance	Result	Reliability/ Comments[19]	Relevance of the test system/ relevance of the result[20]	Reference
Micronucleus analysis in bone marrow Crlj:CD1(ICR) strain SPF male mice	Single dose of 0, 500, 1000 or 2000 mg/ kg bw by gavage Twelve mice/group Exposure: 24 and 48 h	Aspartame (99.5% purity), supplied by Ajinomoto Co., Inc.	Negative	Reliable without restrictions	High/High	Otabe et al. (2019)

Conclusion on aspartame genotoxicity

The newly available Ames test data (Otabe et al., 2019) confirm the previous conclusion on the lack of mutagenicity of aspartame in bacterial systems. There was no indication of potential clastogenic activity of aspartame in a recent study evaluating H2AX histone phosphorylation in human cell lines (Recoules et al., 2025).

In vivo, a highly relevant micronucleus test in mice (Otabe et al., 2019) supports the previous conclusions on the lack of systemic genotoxicity of orally administered aspartame.

Overall, the Panel concluded that the available data do not raise a concern for genotoxicity for aspartame.

[24]See Annex C for comments.

[25]According to EFSA supporting publication 2023:EN-8270. Harmonised approach for reporting reliability and relevance of genotoxicity studies.

3.5.3.3 | *5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP)*

The genotoxicity of the aspartame degradation product DKP was previously evaluated by the ANS Panel in 2013 based on the results of the unpublished studies received following a public call for data and the published literature identified until 15 November 2013. The conclusion of the ANS Panel was as follows: *'The genotoxicity data on bacterial reverse mutation exhibit some limitations (e.g. absence of TA102 and WP2 uvrA Escherichia coli) but were considered sufficient to conclude that DKP was not mutagenic in bacterial systems. The in vivo studies on genotoxicity of DKP comprise chromosome aberration in bone marrow erythrocytes and dominant lethal assays, all of which gave negative results. Taken together, the Panel considered that the data available in vitro and in vivo did not indicate a genotoxic potential of DKP'*.

No relevant data regarding genotoxicity of the main degradation product DKP were identified from the updated literature search (Appendix A). In the present assessment, a QSAR analysis was performed using the rule-based OECD QSAR Toolbox profilers to further evaluate the potential genotoxicity of DKP and in silico predicted metabolites. The potential genotoxicity of DKP was evaluated also using a suite of VEGA statistical models. No relevant indication of potential genotoxicity was provided by the in silico assessment of DKP or its metabolites (Appendix B).

The QSAR analysis supports the previous conclusion of the ANS Panel that the data available did not indicate a genotoxic potential of DKP.

3.5.3.4 | *Acesulfame K*

In the recent re-evaluation of acesulfame K (E 950), the FAF Panel concluded that acesulfame K does not raise a concern for genotoxicity (EFSA FAF Panel, 2025).

However, the FAF Panel identified 5-chloro-acesulfame as a possible impurity in acesulfame K (E 950) and a QSAR analysis indicated genotoxicity alerts for this chemical (EFSA FAF Panel, 2025). In the absence of experimental toxicity data to dismiss these alerts, a maximum limit of 0.1 mg/kg for 5-chloro-acesulfame was recommended to be included in the specifications for acesulfame K (E 950). Alternatively, appropriate genotoxicity data should be generated to clarify the safety concern with 5-chloro-acesulfame (EFSA FAF Panel, 2025).

3.5.3.5 | *Conclusion on the genotoxicity of the salt of aspartame-acesulfame (E 962)*

The available data did not raise a concern for genotoxicity of the salt of aspartame-acesulfame (E 962) based on the individual assessment of the two components (aspartame and acesulfame).

3.5.4 | Synthesis of systematically appraised evidence and weight of evidence assessment

3.5.4.1 | *Animal studies*

3.5.4.1.1 | *The salt of aspartame-acesulfame (E 962)*

No data on animal experimental studies of the salt of aspartame-acesulfame (E 962) were received.[26] No studies were identified from the updated literature search for the salt of aspartame-acesulfame (E 962) (Appendix A).

3.5.4.1.2 | *Aspartame*

The studies included in the assessment encompassed 16 animal studies. Among these, three studies were allocated to tier 1, 10 studies to tier 2 and three studies were allocated to tier 1 or tier 2 depending on the endpoint, following the risk of bias evaluation, see Table 7.

[26]Call for technical and toxicological data on sweeteners authorised as food additives in the EU - Extended deadline for submitting data: 30/6/2018. https://www.efsa.europa.eu/en/data/call/170621.

TABLE 7 Animal studies included in the assessment.

Authors, year	Study type[a]	Exposure duration	Species/strain	N of animals	Dose level	Dose level in mg/kg bw per day	RoB tier[b]
Documentation as provided to EFSA n. 15[c]	Chronic; Carcinogenicity	104 weeks	Rats, (Charles River, albino, caesarian-derived)	60 animals/sex/control group & 40 animals/sex/treated groups	0, 1, 2, 4, 8 g/kg bw per day asp in the diet ad libitum	0, 1000, 2000, 4000, 8000	1
Shibui et al. (2019)	Chronic; Carcinogenicity	104 weeks	Rats, Slc Wistar	60 animals/sex/group	0, 1, 2, 4 g/kg bw per day asp in the diet ad libitum	0, 1000, 2000, 4000	2
Ishii (1981)[d]	Chronic; Carcinogenicity	104 weeks	Rats, Wistar	60 animals/sex/group (examined at 104 weeks), 10 animals/sex/group (examined at 26 weeks) and 16 animals/sex/group (examined at 52 weeks)	0, 1, 2, 4 g/kg bw per day asp in the diet ad libitum	0, 1000, 2000, 4000	2
Ishii et al. (1981)[d]	Chronic; Carcinogenicity	104 weeks	Rats, Wistar	60 animals/sex/group (examined at 104 weeks), 10 animals/sex/group (examined at 26 weeks) and 16 animals/sex/group (examined at 52 weeks)	0, 1, 2, 4 g/kg bw per day asp in the diet ad libitum	0, 1000, 2000, 4000	2
Documentation as provided to EFSA n. 14[e]	Chronic; Carcinogenicity	104 weeks	Rats, (Charles River, albino, caesarian-derived)	60 animals/sex/control group & 40 animals/sex/treated groups	0, 1, 2, 4, 8 g/kg bw per day asp in the diet ad libitum	0, 1000, 2000, 4000, 8000	1 and 2
Documentation as provided to EFSA n. 16[f]	Chronic; Carcinogenicity	104 weeks	Mice, ICR Swiss Mice	72 animals/sex/control group, 36 animals/sex/1 g/kg bw group, 37 males & 35 females/2 & 4 g/kg bw groups	0, 1, 2, 4 g/kg bw per day asp in the diet ad libitum	0, 1000, 2000, 4000	1 and 2
Documentation as provided to EFSA n. 17[g]	Chronic; Maternal and developmental toxicity/ Carcinogenicity	104 weeks post-weaning (F1A weanlings from parental animals which had been treated with the compound at same doses for 60 day prior to mating and then throughout gestation and nursing periods).	Rats, (Charles River, albino, caesarean-derived)	60 animals/sex/control group & 40 animals/sex/treated groups	0, 2, 4 g/kg bw per day asp in the diet ad libitum	0, 2000, 4000	2
Choudhary and Sheela Devi (2015)	Sub-chronic	90 days	Rats, Wistar (albino)	6 males/group	0 (saline), 40 mg/kg bw per day asp in saline via gavage	0, 40	2
Collison et al. (2016)	Sub-chronic	F1 in utero and then 140 days (F0 females treated for 3 weeks prior to mating)	Mice, C57Bl/6J	F0 7–12 females/group; F1 18 males/group	0 (distilled water), 46 mg/kg bw asp daily in drinking water ad libitum (Sigma Aldrich)	0, 46	2

(Continues)

TABLE 7 (Continued)

Authors, year	Study type[a]	Exposure duration	Species/strain	N of animals	Dose level	Dose level in mg/kg bw per day	RoB tier[b]
Abhilash et al. (2014)	Sub-chronic	180 days	Rats, Wistar	6 males/group	0 (normal drinking water), 50, 500, 1000 mg/kg bw asp daily in drinking water (asp 98%)	0, 50, 500, 1000	2
Jones et al. (2023)	Sub-chronic	16 weeks for males (females only exposed 1–5 days during mating)	Mice, C57BL/6	F0 & F1: 8 males/group for breeding, and for F0 & F1 behavioural studies, 1–2 males and 1–2 females per litter (x4).	For F0 males only: 0% (plain drinking water), 0.015% and 0.03% asp in drinking water ad libitum. Equal to approx. 43.2 and 86.4 mg/kg bw asp per day.	0, 43, 86	2 (F0 only)
Jones et al. (2022)	Sub-chronic	12 weeks for behavioural tests, for producing multiple generations and RNA sequencing; 18 weeks for serum metabolic biomarkers analyses	Mice, C57BL/6	8/sex/group for producing multiple generations (exposed males mated with non-exposed females); 8/sex/group for behavioural analyses; 6 F0 males/group and 4 F1 and F2 males/group for serum metabolic biomarkers; 4 males/group for RNA sequencing; 6 animals/sex/lineage for gene expression.	0% (plain drinking water), 0.0075%, 0.015% and 0.03% asp in drinking water ad libitum. Equal to approx. 21.6 mg/kg bw per day, 43.2 mg/kg bw per day and 86.4 mg/kg bw per day asp for males and approx. 23.9 mg/kg bw per day, 47.8 mg/kg bw per day and 95.5 mg/kg bw per day asp for females.	0, 22/24, 43/48, 86/95 (M/F)	2 (F0 only)
Rathaus et al. (2024)	Sub-chronic	20 weeks	Mice, C57BL/6	10 males/group	0, 411.75 mg/L asp in drinking water, daily. Equivalent to 61.8 mg/kg bw per day	0, 61.8	1 and 2
Ediga et al. (2023)	Sub-acute	30 days	Rats, Wistar	6 males/group	0 (0.9% NaCl solution), 50 mg/kg bw per day asp (in 0.9% NaCl solution), via gavage (purity 98%)	0, 50	1
Ma et al. (2024)	Sub-acute	4 weeks	Mice, C57Bl/6	10 males/group	0 (no saline, no gavage); 0 (saline gavage at either 10:30 am or 10:30 pm); 80 mg/kg bw per day asp in saline via gavage at either 10:30 am or 10:30 pm	0, 80	1

TABLE 7 (Continued)

Authors, year	Study type[a]	Exposure duration	Species/strain	N of animals	Dose level	Dose level in mg/kg bw per day	RoB tier[b]
Finamor et al. (2014)	Sub-acute	6 weeks	Rats, Wistar	10 males/group	0 (154 mmol/L NaCl solution via gavage daily, and from week 5, i.p. injections of 154mmol/L NaCl solution daily for 2 weeks), 40 mg/kg/bw per day asp via gavage in 154 mmol/L NaCl solution and from week 5 i.p. injections of 154 mmol/L NaCl solution daily for 2 weeks	0, 40	2

Abbreviations: asp, aspartame; i.p., intraperitoneal.

[a]Sub-acute: < 8 weeks; Sub-chronic: ≥ 8 weeks to < 1 year; Chronic: ≥ 1 year.

[b]A single study might be assigned to more than one tier, as each endpoint is evaluated independently.

[c]The EFSA's 2013 scientific opinion on aspartame reports this as study ID E87.

[d]Data deriving from the same study.

[e]The EFSA's 2013 scientific opinion on aspartame reports this as study ID E33-34.

[f]The EFSA's 2013 scientific opinion on aspartame reports this as study ID E75.

[g]The EFSA's 2013 scientific opinion on aspartame reports this as study ID E70.

The WoE assessment of the 18 animal studies (Table 7) is presented in detail in Annex E1. Table 9 shows the WoE rating according to predefined downgrading and upgrading elements for each health outcome category (HOC). Each HOC consists of groups of endpoints (see Table 8), each endpoint being addressed in one or more of the included animal studies.

TABLE 8 Health outcome categories (HOCs) and related endpoints of the appraised animal studies subjected to WoE evaluation (see Annex E1). HOCs and endpoints are listed in alphabetical order.

Health outcome categories (HOCs)	Endpoints
Additional clinical chemistry	Acid/base balance; immunotox/inflammation; serum calcium; serum chloride; serum magnesium; serum potassium; serum sodium
Carcinogenicity	Adipose tissue; adrenal gland; aorta; bone; bone marrow; brain; epididymides; esophagous; eye; forestomach; gall bladder; glandular stomach; harderian glands; heart; histiocytic sarcomas incidence; hystiocitic sarcoma; intestine histopathology; intracranial neoplasms; kidney; lacrimal glands; large intestine; liver; lung; lymph nodes; lymphocytic leukaemias incidence; lymphomas; mammary gland; nerve; no. animals bearing haemolymphoreticular tumours; ovary; pancreas; parathyroid; peritoneum; pituitary; pleura; prostate; salivary glands; seminal vescicle; skeletal muscle; skin; small intestine; spinal cord; spleen; stomach; sub-cutaneous mass tissue; testis; thymus; thyroid; trachea; tumour type incidence; urinary bladder; uterus; vagina
General toxicity	Body composition; clinical signs and pathology; growth and body weight; intake
Glucose Insulin Homeostasis	Glucose and glucose tolerance (fasted); glucose and glucose tolerance (not fasted); insulin and insulin tolerance (fasted); insulin and insulin tolerance (non-fasting); ketones (urine)
Haematotoxicity	Coagulation; histopathology; leukocytes (differential); leukocytes (total); red cell (haematocrit); red cell (haemoglobin); red cell (indices); red cell (total)
Liver toxicity	Liver histopathology; liver relative weights; liver triglycerides; liver weights; serum albumin; serum albumin/globulin ratio; serum alp; serum alt; serum ast; serum birilubin; serum cholesterol; serum hdl cholesterol; serum ldl cholesterol; serum total proteins; serum triglycerides; serum urea
Nephrotoxicity	Bun; kidney histopatholoy; kidney relative weight; kidney weight; serum creatinine; serum urea; serum uric acid; urinalysis - calcium; urinalysis - chloride; urinalysis - magnesium; urinalysis - microscopic examination; urinalysis - ph; urinalysis - potassium; urinalysis - sodium; urinalysis - specific gravity; urinalysis - total proteins; urinary bladder histopathology
Neurotoxicity	Anxiety-like behaviour; behaviour (cage-side); behaviour (depressive-like); behaviour (novelty); brain tissue cytokines; brain/cns histopathology; organ weight; oxidative status (brain); phenylalanine status; pns histopathology; spatial learning
Other organs toxicity	Adrenal gland; aorta; bone; eye; gastrointestinal tract/digestive system; heart; lung; lymph nodes; pancreas; parathyroid glands; peritoneum; pituitary gland; pleura; coagulating gland and seminal vesicle; mammary gland; ovary; prostate; testes and epididymes; uterus; vagina; salivary glands; skeletal muscle/peripheral nerve; skin; spleen; thymus; thyroid gland; trachea; unspecified organs/multisystem

In addition to the apical and related endpoints reported in Table 8, other endpoints were reported in the studies considered but they were not included in the WoE evaluation as they were not considered relevant for the derivation of a possible HBGV. However, in some instances non-apical endpoints were evaluated as supporting evidence for the apical endpoints in the WoE assessment. Based on the included animal data, the Panel evaluated the confidence in the body of evidence for the identified HOCs; see Table 9.

TABLE 9 Summary table of rating confidence in the body of evidence for each health outcome category: Animal studies. For a detailed assessment, see Annex E1.

Health outcome categories (HOCs) investigated	Initial rating (No. of studies)[a]	Elements for Downgrading[b] Concern for: Risk of bias, unexplained inconsistency, relevance of studies,[c] imprecision	Elements for Upgrading[b]: Magnitude of effect, dose–response, consistency across study population/study design	Final rating of confidence	Effect observed (yes/no)
Additional clinical chemistry	High (6)	Downgrading (risk of bias and/or unexplained inconsistencies)	No upgrading	Moderate	No
Carcinogenicity	High (7)	No downgrading	No upgrading	High	No
General toxicity	High (10)	Downgrading (risk of bias)	No upgrading	Moderate	No
Glucose/insulin homeostasis	High (7)	Downgrading (risk of bias)	No upgrading	Moderate	No
Haematotoxicity	High (5)	No downgrading	No upgrading	High	No
Liver toxicity	High (7)	Downgrading (risk of bias and/or unexplained inconsistencies)	No upgrading	Moderate	No
Nephrotoxicity	High (5)	Downgrading (risk of bias and/or unexplained inconsistencies)	No upgrading	Moderate	No
Neurotoxicity	High (8)	Downgrading (risk of bias and/or relevance)	No upgrading	Low	Yes
Other organs toxicity	High (6)	Downgrading (risk of bias)	Upgrading (consistency)	High	No

[a]The total number of studies assessed was 16. The number in parenthesis refers to studies considered under the specific HOCs.

[b]Please refer to Appendix A and Annex E1 and to the protocol (EFSA FAF Panel, 2023) for further explanations on what is assessed under each element.

[c]Refers to directness, applicability and relevance of the study for addressing the topic of the evaluation (NTP-OHAT, 2019).

Additional clinical chemistry

Changes in clinical chemistry parameters were evaluated in six studies of different duration and design, one in mice (Ma et al., 2024) and five in rats (Abhilash et al., 2014; Choudhary & Sheela Devi, 2015; Ishii et al., 1981; Documentation provided to EFSA n. 14 and 17).

In the studies evaluated, the number of animals per group and sex ranged between 6 and 60. The doses comprised 0, 40, 46, 50, 80, 500, 1000 mg/kg bw per day in the repeated dose studies (28–180 days) and 0, 1000, 2000, 4000 and 8000 mg/kg bw per day in the long-term assays (104 weeks). The Panel noted that a dose of 8000 mg/kg bw per day markedly exceeds current limit doses recommended in OECD test guidelines.

No consistent effects were seen on serum/blood levels of Cl^{-}, Mg^{2+} or K^{+} (Abhilash et al., 2014; Ishii et al., 1981; Documentation provided to EFSA n. 14 and 17). There was a single observation of increased Ca^{2+} in the serum of males at 8000 mg/kg bw per day at week 104 (Documentation provided to EFSA n. 14), which was not evident in females of the same dose group. Serum sodium concentrations were increased at week 104 in the males at 2000 and 4000 mg/kg bw per day (Documentation provided to EFSA n. 17) and at 4000 and 8000 mg/kg bw per day (Documentation provided to EFSA n. 14). However, females showed nearly no alteration with the exception of an increase at 4000mg/kg bw per day at week 52 (Ishii et al., 1981). One sub-chronic study (Abhilash et al., 2014) reported unaltered serum sodium levels.

Serum globulins (Documentation provided to EFSA n. 14 and 17 Ishii et al., 1981) and cytokine levels (interleukins 2, 4, 6 and 10, tumour necrosis factor alpha, interferon gamma) (Choudhary & Sheela Devi, 2015; Ma et al., 2024) were determined in serum/blood and showed some changes with no clear patterns.

Considering the final rating of confidence in the body of evidence for the HOC 'additional clinical chemistry' as 'moderate' (downgrading for unexplained inconsistency across studies) and the absence of any toxicological changes (see Table 9 and Annex E1), the Panel considered that there is moderate confidence in the body of evidence that the exposure to aspartame (E 951) is not associated with adverse changes in clinical chemistry parameters (Table 10).

Carcinogenicity

The overall data on carcinogenicity comprise six long-term studies with five studies in rats (Ishii et al., 1981; Documentation provided to EFSA n. 14, 15 and 17) and one in mice (Documentation provided to EFSA n. 16). Two of these studies are follow-up reports of previously published studies, where the brain was further evaluated histologically (Documentation provided to EFSA n. 15, on the material of Documentation provided to EFSA n. 14 and 17; Ishii, 1981, on the material of Ishii et al., 1981). These studies were published between 1973 and 2010 and were the key studies already included in the latest EFSA opinion of 2013 (EFSA ANS Panel, 2013) (Documentation provided to EFSA n. 14–17; Ishii et al., 1981; and Ishii, 1981). After 2013, one further study was published on the histopathological re-evaluation of specimens of Ishii et al. (1981) (Shibui et al., 2019). Most studies used at least 35 animals per group and sex. The doses comprise 0, 1000, 2000, 4000 and 8000 mg/kg bw per day. The animal studies are well documented and comprehensive covering the histopathological evaluation of all important tissues and gross lesions.

Documentation provided to EFSA n. 15 is a supplementary study on the material of Documentation provided to EFSA n. 14 and 17, applying a more detailed histological analyses of the brain (8 instead of 2 coronal sections of the brain). In the material of Documentation provided to EFSA n. 14 there was a small increase in brain neoplasms: 4 astrocytomas at 1000 mg/kg bw per day; 1 astrocytoma at 2000 mg/kg bw per day; 1 astrocytoma, 3 ependymomas and 1 oligodendroglioma at 4000 mg/kg bw per day; 1 meningeal sarcoma and 1 unclassified glioma at 8000 mg/kg bw per day (40 males and 40 females per treatment group). This increase was considered to be due to an unexpected zero incidence of intracranial tumours in 60 males and 60 females of the concurrent control group. For comparison, in Documentation provided to EFSA n. 17, four intracranial tumours were reported in 120 control animals.

Overall, in seven long-term studies (104 weeks) in two rodent species (rats and mice) across a large dose range up to 8000 mg/kg bw per day there was no evidence for treatment-related increases in benign and/or malignant tumours of any type. No differences in tumour numbers, frequency and incidences between control and treated animals were reported in chronic and two-generation studies.

Considering the final rating of confidence in the body of evidence for the HOC 'carcinogenicity' as 'high' (no downgrading) and the absence of any toxicological changes (see Table 9 and Annex E1), the Panel considered that there is high confidence in the body of evidence that exposure to aspartame (E 951) is not associated with carcinogenicity (Table 10).

General toxicity

Changes in parameters related with general toxicity were evaluated in 11 studies of different duration and design, six in mice (Collison et al., 2016; Jones et al., 2022; Ma et al., 2024; Rathaus et al., 2024; Documentation provided to EFSA n. 16) and five in rats (Finamor et al., 2014; Ediga et al., 2023; Ishii et al., 1981; Documentation provided to EFSA n. 14 and 17).

Reduction in survival was reported in female rats at an aspartame dose of 8000 mg/kg bw per day in diet compared with control, in a chronic (104 week) study (Documentation provided to EFSA n. 14), but not at doses of 1000, 2000 and 4000 mg/kg bw per day. After 52 weeks at the highest dose (8000 mg/kg bw per day), body weight gain was reduced 17% and 23% compared to controls in male and female rats, respectively, terminal weights of male and female rats were reduced (14% and 17%, respectively). Weight gain and terminal weights in groups that received lower doses were reduced less than

10% compared to control animals. At 52 weeks, reduced weight gain and terminal weights in this study correlated with a decrease in food consumption of 5% at 8000 mg/kg bw per day in males and up to 17% in females at 4000 and 8000 mg/kg bw per day. The reduced food consumption was likely due to reduced palatability of the highest dose. Water intake was not measured in this study. In another chronic study (104 week) (Ishii et al., 1981), in which 4000 mg/kg bw per day given in diet was the highest dose, reduction in food consumption was about 10% in both males and females after 52 weeks with weight loss not exceeding 10%. Reduced food consumption of 13% was also reported in male rats (Ediga et al., 2023) in a study lasting 30 days at a single dose of 50 mg/kg bw per day given by gavage; however, in this study, terminal weight increased while water consumption decreased. Reduction in food consumption, which was not dose-related, was reported in male animals and did not correspond with reduced body weight gain after 52 weeks in two chronic (104 weeks) studies (Documentation provided to EFSA n. 16 and 17) in rats and mice at aspartame doses in the range 1000–4000 mg/kg bw per day given in diet. There were no changes in water consumption relative to controls in two studies in mice of which one lasted 20 weeks (62 mg/kg bw per day, Rathaus et al. (2024)) and the other lasted 20 weeks following in utero dosing (46 mg/kg bw per day; F0 treated for 3 weeks prior to mating) (Collison et al., 2016). None of the studies demonstrated differences at macroscopic (Ishii et al., 1981; Documentation provided to EFSA n. 16 and 17) or ophthalmoscopy examinations (Documentation provided to EFSA n. 16) between exposed and control mice or rats of either sex up to 104 weeks of test substance intake. There was no change in body composition in a single study of male mice dosed 62 mg/kg bw per day for 20 weeks (Rathaus et al., 2024). Furthermore, the incidence of necrosis in adipose tissue was not dose-related in rats dosed 1000–4000 mg/kg bw per day for 104 weeks (Ishii et al., 1981).

Considering the final rating of confidence in the body of evidence for the HOC 'general toxicity' as 'moderate' (downgrading for risk of bias) and the absence of any toxicological changes (see Table 9 and Annex E1), the Panel considered that there is moderate confidence in the body of evidence that the exposure to aspartame (E 951) is not associated with adverse changes in general toxicity parameters (Table 10).

Glucose/insulin homeostasis

Changes in glucose/insulin homeostasis were evaluated in seven studies, two in mice (Collison et al., 2016; Rathaus et al., 2024) and five in rats (Finamor et al., 2014; Abhilash et al., 2014; Ishii et al., 1981; Documentation provided to EFSA n. 14 and 17).

Neither glucose nor insulin homeostasis was adversely disturbed in rats or mice of either sex following intake of aspartame in drink or feed in doses from 40 mg to 8000 mg/kg bw per day in studies lasting from 6 to 104 weeks including one-generation studies with in utero exposure. Prior to GTT or ITT, animals were fasted in Rathaus et al. (2024) and non-fasted in Collison et al. (2016). Occasional increases or decreases in glucose or insulin levels compared with controls were within normal levels for the species or strain in question, such as about 20% decrease in blood glucose level in both sexes of rats after 52 weeks at 8000 mg/kg bw per day (Documentation provided to EFSA n. 14) and 10% increase in blood glucose levels (measured in week 18) in F1 male mice dosed 46 mg/kg bw per day for 20 weeks following in utero dosing. There were no changes in ketone or ketone body levels relative to controls in two chronic studies (Ishii et al., 1981; Documentation provided to EFSA n. 17) in rats dosed 1000–4000 mg/kg bw per day.

Considering the final rating of confidence in the body of evidence for the HOC 'glucose and insulin homeostasis' as 'moderate' (downgrading for risk of bias) and the absence of any toxicological changes (see Table 9 and Annex E1), the Panel considered that there is moderate confidence in the body of evidence that the exposure to aspartame (E 951) is not associated with impairment of glucose and insulin homeostasis (Table 10).

Haematotoxicity

Changes on haematological parameters were evaluated in five studies, one in mice (Documentation provided to EFSA n. 16) and four in rats (Shibui et al., 2019; Ishii et al., 1981; Documentation provided to EFSA n. 14 and 17).

There were no adverse changes in haematological endpoints in mice or rats ascribed to aspartame in the dose range of 1000–8000 mg/kg bw per day in studies lasting up to 104 weeks, including a one-generation study (60 days pretreatment of F0). Occasional statistically significant changes were recorded but they were only observed in one sex or not temporally reproducible in the same study, and were within the normal range for the species in question. Histopathological examination of bone marrow did not indicate that the haematogenic activity (haematopoiesis) in rats was affected by aspartame up to doses of 8000 mg/kg bw per day given for 104 weeks (Documentation provided to EFSA n. 14 and Shibui et al. (2019), which is a re-evaluation of Ishii et al., 1981).

Considering the final rating of confidence in the body of evidence for the HOC 'haematotoxicity' as 'high' (no downgrading) and the absence of any toxicological changes (see Table 9 and Annex E1), the Panel considered that there is high confidence in the body of evidence that the exposure to aspartame (E 951) is not associated with adverse changes in haematological parameters (Table 10).

Liver toxicity

Seven studies of different duration and design, two in mice (Documentation provided to EFSA n. 16; Rathaus et al., 2024) and five in rats (Shibui et al., 2019; Abhilash et al., 2014; Ishii et al., 1981; Documentation provided to EFSA n. 14 and 17), measured endpoints relevant for the assessment of effects of aspartame on liver.

No adverse hepatic effects were observed in studies in rats and mice at doses up to 8000 mg/kg bw per day for up to 104 weeks (serum ALP, serum ALT, serum AST, serum albumin, serum total protein, serum total bilirubin, liver histopathology, relative (to body weight) and absolute liver weight), the highest dose tested for these endpoints. For serum cholesterol, serum albumin/globulin ratio and serum triglycerides, no changes associated with adverse effects were seen at doses up to 4000 mg/kg bw per day for up to 104 weeks, the highest dose tested for these additional endpoints. There were no effects on serum urea in rats at doses up to 1000 mg/kg bw per day (the highest dose tested for this endpoint) for 180 days. In mice, liver triglycerides were unaffected after a single dose of 62 mg/kg bw per day for 20 weeks (Annex E1). A non-dose-dependent reduction in serum HDL cholesterol was reported in mice at a dose of 3.75 mg/kg bw per day for 8 weeks, but this was considered inconsistent/incidental since no effects were seen in another mice study at a single dose of 62 mg/kg bw per day for 20 weeks.

Considering the final rating of confidence in the body of evidence for the HOC 'liver toxicity' as 'moderate' (downgrading for risk of bias and/or unexplained inconsistencies) and the absence of any toxicological changes (see Table 9 and Annex E1), the Panel considered that there is moderate confidence in the body of evidence that the exposure to aspartame (E 951) is not associated with adverse effects on liver (Table 10).

Nephrotoxicity

Five studies of different duration and design, one in mice (Documentation provided to EFSA n. 16) and four in rats (Abhilash et al., 2014; Ishii et al., 1981; Documentation provided to EFSA n. 14 and 17), measured endpoints relevant for the assessment of effects of aspartame on kidneys.

No adverse renal effects were observed in studies in rats for several endpoints at doses up to 4000 mg/kg bw per day for up to 104 weeks (urinary Cl^{-}, urinary Mg^{2+}, urinary K^{+}, urinary Na^{+}, urinary microscopic changes, urinary total protein and urinary specific gravity) or in rats at doses up to 1000 mg/kg bw per day for 180 days (serum creatinine, serum urea, serum uric acid), the highest dose tested for these endpoints. However, all these endpoints were determined in a single study. Urinary calcium levels were statistically significantly increased at weeks 26, 52 and 104 in one study in rats in a dose-dependent manner from 2000 or 4000 mg/kg bw per day. Urinary acidification was apparent in females in one study but only at 26 and 52 weeks at a dose of 2000 or 4000 mg/kg bw per day (Annex E1). No changes in kidney weight were seen in mice at doses up to 4000 mg/kg bw per day (the highest dose tested for this endpoint, Annex E1) at 104 weeks.

Several minor histopathological changes (Annex E1) were observed in the kidney and urinary bladder, inconsistently between studies and predominantly confined to high doses (4000 and 8000 mg/kg bw per day for 104 weeks). Given their inconsistency, these changes were considered either incidental or associated with the terminal body weight loss seen at very high doses due to palatability issue, therefore they were not considered further.

Considering the final rating of confidence in the body of evidence for the HOC 'nephrotoxicity' as 'moderate' (downgrading for risk of bias and/or unexplained inconsistencies) and the absence of any toxicological changes (see Table 9 and Annex E1), the Panel considered that there is moderate confidence in the body of evidence that the exposure to aspartame (E 951) is not associated with adverse effects on kidneys (Table 10).

Neurotoxicity

Included in the evaluation were eight original study reports (Finamor et al., 2014; Jones et al., 2022, 2023; Ma et al., 2024; Documentation provided to EFSA n. 14, 16 and 17; Ishii et al., 1981 and Ishii, 1981) and one re-analysis (Shibui et al., 2019) of histological samples described earlier (Ishii et al., 1981 and Ishii, 1981). The included studies measured several neurotoxicity-related endpoints; one measured oxidative stress related adaptive changes (Finamor et al., 2014) and another brain tissue cytokine levels (Ma et al., 2024) (Table 9, Annex E1).

Of the eight included studies, four were chronic high-dose studies in rats and mice (Documentation provided to EFSA n. 14, 16 and 17 Ishii et al., 1981 and Ishii, 1981; Shibui et al., 2019), which reported no treatment-related effects on clinical signs and brain histopathology at doses of 1000–8000 mg/kg bw per day.

Two studies from one laboratory (Jones et al., 2022, 2023) reported behavioural effects (on anxiety and spatial learning) in adult (F0) mice at doses < 100 mg/kg bw per day. Jones et al. (2022) reported increased anxiety-like behaviour in open field test (reduced time in the central area of the field) and in elevated zero maze (reduced time in the open arms, measured at week 12) in F0 mice dosed for 12 weeks with 40–100 mg/kg bw per day. Jones et al. (2023) reported reduced Y-maze spontaneous alternations but no effect on total arm entries after 4–12 weeks dosing, and in Barnes maze, slower spatial learning (switch to spatial strategy) but no effect on memory or reversal learning after 14–15 weeks dosing with 43–86 mg/kg bw per day (without evidence of dose–response) in F0 male mice.

One study (Finamor et al., 2014) reported effects consistent with increased brain oxidative stress in rats dosed 40 mg/kg bw per day for 6 weeks. The study reported increased lipid peroxidation (lipid hydroperoxides LOOH, thiobarbituric reactive substances TBARS, carbonyl protein), decreased activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and decreased non-protein thiols (NPSH) and total reactive antioxidant potential (TRAP) in whole brain homogenate. No behavioural tests or histopathological evaluation were performed.

One study (Ma et al., 2024) reported statistically significant changes in cytokine levels in prefrontal cortex and hippocampus of male mice dosed 80 mg/kg bw per day for 28 days. The level of all pro-inflammatory (TNF-α, IFN-γ and IL-6)

cytokines as well as the anti-inflammatory cytokine IL-4 increased in treated animals while the anti-inflammatory cytokine IL-10 decreased. The cytokine levels in control and treated animals (in ρg/mL) were almost identical between the two brain areas. The cytokine brain tissue levels closely followed the serum concentration patterns for all cytokines. SEMs were almost identical between all groups within the same tissue and cytokine (except for IL-4). Behavioural tests were performed; however, they were not included for WoE assessment due to high risk of bias (Annex D).

In summary, there is limited evidence for anxiety-like and spatial learning effects (Jones et al., 2022, 2023), increases in measurements of brain oxidative stress (Finamor et al., 2014) and differences in brain cytokine levels (Ma et al., 2024) at doses at or below 100 mg/kg bw per day. Without independent replication and further characterisation, the confidence in these studies is low.

Considering the final rating of confidence in the body of evidence for the HOC 'neurotoxicity' as 'low' (downgrading for risk of bias and/or relevance) and the identification of adverse effects (see Table 9 and Annex E1), the Panel considered that there is low confidence in the body of evidence that the exposure to aspartame (E 951) is associated with adverse effects on behaviour and other neurotoxic parameters.

Other organ toxicity

Six long-term studies, one in mice (Documentation provided to EFSA n. 16) and five in rats (Choudhary & Sheela Devi, 2015; Shibui et al., 2019; Documentation provided to EFSA n. 14 and 17 Ishii et al., 1981) measured endpoints relevant for the assessment of effects of aspartame on other organ toxicity (Table 8), i.e. organs and tissues other than kidney, liver and brain which are assessed under other HOCs. A 90-day study in rats (Choudhary & Sheela Devi, 2015) measuring serum cytokines following aspartame dosing of 40 mg/kg bw per day, was assessed together with organs involved in inflammation and immunity.

No remarkable histopathological or organ weight differences between control and treated animals, covering a full list of organs and tissues, were noted across a dose range varying from 1000 to 8000 mg/kg bw per day (given in feed), in rats and mice for 104 weeks in four independent studies.

Considering the final rating of confidence in the body of evidence for the HOC 'other organ toxicity' as 'high' (downgrading for risk of bias and upgrading for consistency across species, study type and tier level) and the absence of any toxicological changes (see Table 9 and Annex E1), the Panel considered that there is high confidence in the body of evidence that the exposure to aspartame (E 951) is not associated with adverse changes in other organ toxicity parameters (Table 10).

TABLE 10 Translation of confidence ratings into level of evidence for conclusions of adverse effects or no adverse effects on health for the included animal studies for each of the HOC considered in the assessment.

HOC	Final rating of confidence	Level of evidence
Additional clinical chemistry	**Moderate (no effect)**	**Moderate** There is moderate confidence in the body of evidence that exposure to aspartame (E 951) is not associated with adverse changes in clinical chemistry parameters.
Carcinogenicity	**High (no effect)**	**High** There is high confidence in the body of evidence that exposure to aspartame (E 951) is not associated with carcinogenicity
General toxicity	**Moderate (no effect)**	**Moderate** There is moderate confidence in the body of evidence that exposure to aspartame (E 951) is not associated with adverse changes in general toxicity parameters.
Glucose/insulin homeostasis	**Moderate (no effect)**	**Moderate** There is moderate confidence in the body of evidence that exposure to aspartame (E 951) is not associated with impairment of glucose and insulin homeostasis.
Haematotoxicity	**High (no effect)**	**High** There is high confidence in the body of evidence that exposure to aspartame (E 951) is not associated with adverse changes in haematological parameters.
Liver toxicity	**Moderate (no effect)**	**Moderate** There is moderate confidence in the body of evidence that exposure to aspartame (E 951) is not associated with adverse effects on liver.
Nephrotoxicity	**Moderate (no effect)**	**Moderate** There is moderate confidence in the body of evidence that exposure to aspartame (E 951) is not associated with adverse effects on kidneys.
Neurotoxicity	**Low (effect)**	**Low** There is low confidence in the body of evidence that exposure to aspartame (E 951) is associated with adverse effects on behaviour and other neurotoxic parameters.
Other organ toxicity	**High (no effect)**	**High** There is high confidence in the body of evidence that exposure to aspartame (E 951) is not associated with adverse changes in other organ toxicity parameters.

3.5.4.1.3 | *5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP)*

No new data were received on DKP in response to the call for biological and toxicological data and no new data were identified from the updated literature search (Appendix A).

3.5.4.1.4 | *Acesulfame*

The WoE assessment for acesulfame K (E 950) was based on the evaluation of approximately 100 endpoints comprising six HOCs extracted from 11 animal studies considered of low (tier 1) or moderate (tier 2) RoB (EFSA FAF Panel, 2025). Overall, the evidence base supported a high (General toxicity, Haematotoxicity, Liver toxicity, Nephrotoxicity, Other organ toxicity) or moderate (Additional clinical chemistry, Glucose/insulin homeostasis) level of evidence of no adverse effect of acesulfame K (E 950) on health.[27]

3.5.4.2 | *Human studies*

3.5.4.2.1 | *The salt of aspartame-acesulfame (E 962)*

No data were received on the salt of aspartame-acesulfame (E 962) in response to the call for biological and toxicological data.[28] No studies were identified from the updated literature search on the salt of aspartame-acesulfame (E 962). One study (Örkü et al., 2023) evaluated a binary mixture of aspartame (E 951) and acesulfame K (E 950) (Appendix A).

3.5.4.2.2 | *Aspartame*

The studies included in the assessment comprised 27 human studies. Among these, 9 were allocated to tier 1, and 18 to tier 2, following the risk of bias evaluation, see Table 11. Annex E2 reports all the human studies evaluated, grouped by outcome within the different HOCs, for which a WoE analysis was performed. The outcomes considered and evaluated in the WoE for the included data are shown in Table 12. The details on the systematic literature search and appraisal are described in Appendix A.

[27]General toxicity (high level of evidence of no adverse effect on health), Additional clinical chemistry (moderate level of evidence of no adverse effect on health), Haematotoxicity (high level of evidence of no adverse effect on health), Liver toxicity (high level of evidence of no adverse effect on health), Nephrotoxicity (high level of evidence of no adverse effect on health), Other organ toxicity (high level of evidence of no adverse effect on health), Glucose/insulin homeostasis (moderate level of evidence of no adverse effect on health).

[28]Available online: https://www.efsa.europa.eu/en/data/call/170621.

TABLE 11 Human studies included in the assessment.

Authors, year (RefID)	Type of study design	Dose[a]	Intervention/exposure	Number of subjects (gender)	Population (mean age in years)	RoB tier
Palomar-Cross et al. (2023)	Case–control (CaCo)	Consumers divided into groups of moderate consumers (< third quartile) and high consumers (≥ third quartile) aspartame-containing products	Study from 2008 to 2013 FFQ questionnaire	8452 participants (4342 M & 4110 F); 3629 controls, Colorectal cancer *N* = 1881, Chronic lymphocyte leukaemia *N* = 109, Prostate *N* = 972, Stomach *N* = 351 Breast *N* = 1510	63.7 ± 11.9 years	1
Debras, Chazelas, Srour, et al. (2022)	Cohort study (Co)	9.35 ± 31.84 mg per day (mean)	Study from 2009 to 2021; median follow-up: 7.8 years (NutriNet-Santé cohort) 3 × 24 h dietary records (non-consecutive days) were randomly assigned over a 2 week period, every 6 months Dietary records were validated against interview and biomarkers	102,865 (22,154 M and 80,711 F); Total cancers *N* = 3358: obesity-related cancers[b] *N* = 2023; Breast *N* = 979, Prostate *N* = 403	42.22 ± 14.5	1
McCullough et al. (2014)	Cohort study (Co)	Intakes quartiles (plus Q1 for the non-consumers): Q1 (0 mg/day), Q5 (145 mg/day)	Consumption during 1999–30/06/2009 (CPS-II Nutrition Cohort) Information collected through 152-item modified Willett FFQs (1999 and 2003), which included specific questions regarding carbonated beverage consumption during previous year.	100,442 participants; 43.2% M & 56.8% F Cases *N* = 998 NHL, myeloma *N* = 198, Diffuse large B-cell lymphoma *N* = 258, Chronic lymphocytic leukaemia N = 267, follicular lymphoma *N* = 142, other B-cell lymphomas *N* = 192, T-cell lymphoma N = 45	69.2 ± 6.1 years	1
Chien et al. (2023)	Cohort study (Co)	Intake: 0.001 mg M & 0.0004 mg F; Intakes tertiles estimated as the proportion of daily intake to ADI, F: T1 (0.09 ± 0.05), T2 (0.32 ± 0.15), T3 (3.94 ± 6.97); M: T1 (0.11 ± 0.05), T2 (0.39 ± 0.21), T3 (8.92 ± 16.94)	Study from 2018 to 2021 (TPLS); average length of the follow-up interval was 3.45 months until the end of puberty. Data collection: 24 h dietary record at baseline; Semiquantitative NNS-FFQ used to estimate NNS consumption level in the 3 months preceding the time of examination	1893 participants (654 M & 1239 F)	Children/adolescents 9.69 ± 1.82 (F) and 11.78 ± 1.93 (M); 10.41 ± 2.11(6–15 years	1
Fulgoni 3rd and Drewnowski (2022)	Cohort study (Co)	Intakes recorded as tertiles only (without mg/day information)	Study from 1988 to 1994 with 10.3 year follow-up (average) (NHANES) 24 h dietary recall	12,438 (5995 M and 6443 F) for aspartame mortality analyses 15,948 total participants (7725 M and 8223 F); *N* = 322 events	44.52 ± 0.48 year (SEM) (for entire study pop.n)	2

(Continues)

TABLE 11 (Continued)

Authors, year (RefID)	Type of study design	Dose[a]	Intervention/exposure	Number of subjects (gender)	Population (mean age in years)	RoB tier
Chen et al. (2025)	Cohort study (Co)	Three groups: No consumption, consumption ≤ median and consumption > median (which accounted for 0.24% of the ADI of aspartame)	Study from September 2018 to December 2022 (TPLS) 24-h dietary records, NNS-FFQ questionnaire	858 F	9.72 ± 1.83 years–10.01 ± 1.79 years	2
Chen, Yeh, et al. (2024)	Cohort study (Co)	Three groups: No consumption, consumption ≤ median and consumption > median	Study from July 2018 to May 2021 Taipei Maternal–Infant Nutrition Cohort (TMINC) NNS-FFQ questionnaire, considering both portion size and frequency	877 pregnant F from	33.28 ± 3.69 years–33.62 ± 4.07 years	2
Lin et al. (2024)	Cohort study (Co)	Three groups: no consumption, ≤ median (0.01% ± 0.0001 ADI M & F) and > median (0.41% ± 0.0084 ADI M & 0.24% ± 0.0052 ADI F)	TPLS 24-h dietary records, NNS-FFQ questionnaire	1257 participants (399 (31.7%) M & 858 (68.3%) F)	10.42 ± 2.05 years (11.80 ± 1.80 yea M & 9.78 ± 1.83 years F)	2
Romanos-Nanclares et al. (2025)	Cohort study (Co)	Intakes quartiles (plus zero intake): 0 mg/day, 12.3 ± 6.5 mg/day (quartile 1), 50.2 ± 17.3 mg/day (quartile 2), 118.9 ± 28.9 mg/day (quartile 3), 357.7 ± 195.3 mg/day (quartile 4)	Data on-going from 1984 (NHS) and 1991 (NHS II) with nearly 30 years of follow-up Semiquantitative FFQ every 2–4 years (participants indicated the consumption for specified amounts of approximately 130 foods over the previous year) 25 year study (CARDIA cohort)	4,377,358 F person-years of follow-up 10,814 total breast cancer cases, basal-like breast cancer = N = 304, Oestrogen receptor–positive breast N = 7363 Oestrogen receptor–negative breast cancer (7363), Luminal A N = 2488, Luminal B N = 1111, HER2 N = 241 3088 young adults (1736 F and 1352 M)	41.4 ± 9.6 year - 49.3 ± 12.2 year	2
Steffen et al. (2023)	Cohort study (Co)	Intake quintiles: Q1 3.0 (0.8–3.9) mg/day; Q2 1; 4.7 (3.9–5.6) mg/day; Q3 6.8 (5.6–8.4) mg/day; Q4 13.9 (8.4–28.5) mg/day; Q5 106.5 (>28.5) mg/day	25 year study (CARDIA cohort) Questionnaire to assess usual dietary intake during the previous month at exam years 0, 7, and 20. Participants provided details about foods and beverages, including brand name information and nutrient modification (e.g., artificially sweetened foods and beverages), frequency, and amounts consumed	3088 young adults (1736 F and 1352 M)	25.2 year	2
Mueller et al. (2015)	Cohort study (Co) prospective	Intakes categorised as 0, < 50 and > 50 mg/day	Followed annually for 10 visits (1986–1997) 3-day food record at baseline (2 weekdays and 1 weekend day)	1988 F participants	10.0 ± 0.6 year	1

TABLE 11 (Continued)

Authors, year (RefID)	Type of study design	Dose[a]	Intervention/exposure	Number of subjects (gender)	Population (mean age in years)	RoB tier
Debras, Chazelas, Sellem, et al. (2022)	Cohort study (Co) prospective	9.13 ± 30.95 mg per day mean	Study from 2009 to 2021; median follow-up: 9 years (NutriNet-Santé cohort) 3 × 24 h dietary records (non-consecutive days) were randomly assigned over a 2 week period, at baseline and every 6 months thereafter. Dietary records were validated against interview and biomarkers	103,388 participants (20,903 M & 82,485 F) cases cardiovascular diseases $N = 1502$, coronary heart disease $n = 730$, cerebrovascular diseases $n = 777$	42.22 ± 14.41 year	1
Debras et al. (2023)	Cohort study (Co) prospective	9.13 ± 30.95 mg per day mean	Study from 2009 to 2022; median follow-up: 8.97 ± 2.33 years (NutriNet-Santé cohort) 3 × 24 h dietary records (non-consecutive days) were randomly assigned over a 2 week period, every 6 months Dietary records were validated against interview and biomarkers	105,588 (21,960 M & 83,628 F)	42.5 ± 14.6 year	1
Kuk & Brown (2016)	Cross-sectional (CrSe)	252.0 ± 11.1 mg intake reported for consumer group (based on 628 aspartame consumers, mean)	Study data from 1988 to 1994 (NHANES III) 24 h dietary recall	2856 (1363 M & 1493 F) both consumers and non-consumers	54.9 ± 0.4 year (non-consumer) and 52.8 ± 0.5 year (aspartame consumers)	2
Hess et al. (2018)	Cross-sectional study (CrSe)	Individuals are considered consumer if dietary intake ≥ 17 mg aspartame	Data collected in 2016 2-week data collection period (3 × 24 h dietary recalls)	125 participants (29 M & 34 F in the non-nutritive sweetener (NNS) consumers; 25 M & 37 F in the NNS non-consumers); 33 consumers of aspartame	38.8 ± 16.9 year for NNS consumers, 34.5 ± 16.0 year for NNS non-consumers	2
Wulaningsih et al. (2017)	Cross-sectional study (CrSe)	56.16 ± 3.3 mg for M ($n = 7151$) and 71.56 ± 3.41 mg for F ($n = 8096$)	NHANES III survey (1988–1994, 1st and 2nd phases) 24 h dietary recall	15,731 participants (7403 M & 8328 F) (1st phase: 7743 participants; 2nd phase: 7988 participants)	40.18 ± 0.58 year - 50.60 ± 0.53 year	2
Duran Agüero et al. (2014)	Cross-sectional study (CrSe)	185.3 ± 140.3 (M, normal weight) 192.2 ± 209.3 (M, overweight) 176.2 ± 138.2 (F, normal weight) 164.1 ± 153.9 (F, overweight)	Survey on weekly food consumption The survey (FFQ) was adapted to include only those foods that contain sweeteners. Serving sizes were described using home measuring elements (e.g. cup, glass, plate, etc.)	571 participants (281 M & 290 F)	13.2 ± 6.3 year	2

(Continues)

TABLE 11 (Continued)

Authors, year (RefID)	Type of study design	Dose[a]	Intervention/exposure	Number of subjects (gender)	Population (mean age in years)	RoB tier
Sylvetsky et al. (2025)	Cross-sectional study (CrSe)	intakes (tertiles): low aspartame consumers < 19.5 mg/day, medium aspartame consumers 19.5–77.7 mg/day, high aspartame consumers > 77.7 mg/day	1 years study (2015–2016) American Cancer Society's Cancer Prevention Study-3 Diet Assessment Substudy (DAS) 2 × FFQs 1 year apart and 6 × 24-h interviewer-administered dietary recalls by telephone in the interim; they were also asked to provide 2 × fasting blood samples and 2 × 24-h urine samples. Blood and urine samples were collected ~6 mo apart, and a 24-h dietary recall was conducted within a week of each fasting blood sample collection, when possible	624 participants (228 M & 396 F)	52.2 ± 9.6 year	2
Ahmad et al. (2020)	Randomised controlled trial (HCT)	0.425 g mixed in 1 L water containing 0.08 g citric acid and 0.037 g pure lemon extract consumed throughout the day, daily.	2 weeks per test beverage Participants were instructed to consume habitual diets and avoid consuming non-nutritive sweeteners; double-blinded, crossover design.	17 (7 M & 10 F)	24 ± 6.8	1
Higgins et al. (2018)	Randomised controlled trial (HCT)	0, 350 or 1050 mg per day via fruit-flavoured beverage and capsules: (corresponding to: 0, 5 or 15 mg aspartame/kg bw per day, based on a 70 kg reference individual)[c]	Daily for 12 weeks	100 total participants; 33–34 per group (55% M & 45% F in 350 mg group and 42% M & 58% F in 0 and 1050 mg groups)	21.8 ± 0.6–22.8 ± 1.0 year in exposed groups; 24.2 ± 1.3 year for non-exposed group	1
Pearson et al. (2021)	Randomised controlled trial (HCT)	20 oz. (about 590 mL) of Diet-Coke (containing aspartame, concentration not specified) with a test meal	Single exposure per test beverage Randomised repeated-measures; test meal + either Coca-Cola, Diet-Coke or water; 1-week washout. Single blinded, crossover design Non-nutritive sweetener specific FFQ at baseline.	8 (100% M)	22 ± 1.79	2
Suez et al. (2022)	Randomised controlled trial (HCT)	0.24 g 3× per day	14 days of exposure and 7 days of follow-up Not blinded	120 total; 20 participants exposed to aspartame (10 M & 10 F)	27.75 (25–29.75)	2
Higgins and Mattes (2019)	Randomised controlled trial (HCT)	0.58 g daily in 1.25–1.75 L (dependent on bw at baseline) of coloured, fruit-flavoured beverage	12 weeks Daily exposure; Single blinded	30 participants exposed to aspartame (15 M & 15 F) 123 total participants in the trial	29.5 ± 12.0 years in aspartame group 25.8 ± 6.9–28.2 ± 9.5 years for groups exposed to other sweeteners	2
Kashima et al. (2019)	Randomised controlled trial (HCT)	180 mg (200 g aspartame solution, 0.09% purity)	Single exposure per test scenario Two scenarios with ingestion of aspartame Randomised crossover design[d]	9 total (2 M & 7 F)	22 ± 3 years	2

TABLE 11 (Continued)

Authors, year (RefID)	Type of study design	Dose[a]	Intervention/exposure	Number of subjects (gender)	Population (mean age in years)	RoB tier
Bryant et al. (2014)	Randomised controlled trial (HCT)	150 mg aspartame and 45 g glucose in 250 mL water	Single exposure 1 day per test (at least 3 days between visits) Crossover design	10 total (6 M and 4 F)	21 ± 2.4 years (18–24 years)	2
Temizkan et al. (2015)	Randomised controlled trial (HCT)	72 mg	Single exposure per test beverage Single blinded	16 total participants (4 M & 4 F in each healthy and newly diagnosed, drug-naive T2D groups)	45.0 ± 4.1 years for healthy; 51.5 ± 9.2 years for T2D patients	2
Finley et al. (2019)	Randomised controlled trial (HCT)	8 g	Single exposure	371 total (133 M & 212 F); 118 in aspartame group (gender NR) Blood glucose data for 321 participants only	18.70 ± 1.80 years	2

Abbreviations: CARDIA, Coronary Artery Risk Development in Young Adults; CPS, Cancer Prevention Study; F, female; FFQ, food frequency questionnaire; M, male; NHANES, National Health and Nutrition Examination Survey; NHS, Nurses' Health Study; NNS, non-nutritive sweetener; PABA, para-amino benzoic acid.

[a]As reported by the authors.

[b]Obesity-related cancers: colorectal, stomach, liver, mouth, pharynx, larynx, oesophageal, breast, ovarian, endometrial and prostate cancers.

[c]350 mg asp and 80 mg PABA in 500 mL fruit-flavoured beverage (plus 2 capsules collectively containing 680 mg dextrose, and 2 empty capsules), or 350 mg asp and 80 mg PABA in 500 mL fruit-flavoured beverage (plus 4 capsules collectively containing 700 mg aspartame and 680 mg dextrose) daily.

[d]Administration protocol as follows: mouth rinsing with either 25 mL of water (scenario 1, control) or 2.5% Gymnema sylvestre solution (scenario 2), followed by 30 s rest and then gargling with pure water (60 s) before ingesting 50 g of 0.09% asp solution. Steps repeated 4 times during the same session. Each session took place in a different day.

The outcomes addressed in the studies were aggregated into different health outcome categories (HOCs). Given the differences in outcomes assessed in the human and animal studies the health outcomes within each category may not always be directly comparable.

TABLE 12 Health outcome categories and related outcomes of the appraised human studies subjected to WoE evaluation. HOCs and endpoints are listed in alphabetical order.

Health outcome categories (HOCs)	Outcome/endpoint
Birth outcomes	General development
Cancer	Breast cancer; cancer mortality; colorectal cancer; haematological cancers; obesity-related cancer; overall cancer; prostate cancer; stomach cancer
Cardiovascular risk factors and disease	Blood pressure; bmi; body composition; body weight; cholesterol; cvd; incident obesity; metabolic syndrome; triglycerides; waist circumference
Development	Reproductive development
Glucose/insulin homeostasis	Adiponectine; blood haemoglobin a1c; c-peptide; diabetes; fructosamine; gestational diabetes; gip; glp-1; glucose; insulin; insulin sensitivity
Inflammation/Immunotoxicity	Crp; il-10; il-6; tnf-α
Liver effects	Alt; ast; γ -glutamyltranspeptidase

Based on the included human data, the Panel considered the confidence in the body of evidence for the identified HOCs; see Table 13.

TABLE 13 Rating of the confidence in the body of evidence for each health outcome category investigated in the human studies. HOCs are listed in alphabetical order.

Health outcome categories (HOCs) investigated	Initial rating (No. of studies)[a,b]	Elements for Downgrading[c] Concern for: Risk of bias, unexplained inconsistency across studies, relevance of studies, imprecision	Elements for Upgrading[c]: Residual confounding; magnitude of effect, dose–response, consistency across study population/study design	Final rating of confidence	Association observed (yes/no)
Birth outcomes	Moderate (1)	Downgrading: relevance (single study)	Upgrading: no	Low	No
Cancer	Moderate (5)	Downgrading (unexplained inconsistencies and/or imprecision)	Upgrading: no	Low	Yes
Cardiovascular risk factors and disease	Moderate (10)	Downgrading (RoB and/or unexplained inconsistences)	Upgrading: no	Low	Yes
Development	Moderate (3)	Downgrading (RoB and/or unexplained inconsistences	Upgrading: no	Low	No
Glucose/insulin homeostasis	High/Moderate (13)	Downgrading: (RoB and/or relevance)	Upgrading: no	Low	No
Inflammation/Immunotoxicity	Low (1)	Downgrading: relevance (single study)	Upgrading: no	Very low	No
Liver effects	High (1)	Downgrading: relevance (single study)	Upgrading: no	Moderate	No

[a]For human studies, it is generally expected that: Human controlled trials: high (++++), cohort studies: moderate (+++), Case–control studies: moderate (+++), Cross-sectional studies: low/moderate (++/+++). Here the overall initial rating of confidence is reported which accounts for all the available studies and their respective initial confidence rating (see Annex E2).

[b]The total number of studies assessed was 27. The number in parentheses refers to studies considered under the specific HOC.

[c]Please refer to Appendix A and Annex E2 and to the protocol (EFSA FAF Panel, 2023) for further explanations on what is assessed under each element.

In this section, the confidence in the body of evidence and the translation of confidence ratings into level of evidence for conclusions of adverse effects on health or no adverse effect on health for each HOC are discussed. Further consideration on the overall conclusion is addressed in Sections 3.5.4.3 and 3.7.

Birth outcomes

One cohort study assessed the association between prenatal exposure to aspartame and birthweight (Chen, Yeh, et al., 2024). The study was rated of moderate risk of bias (tier 2).

Chen, Yeh, et al. (2024) reported on the Taipei Maternal–Infant Nutrition Cohort and observed no association between aspartame intake during pregnancy and birthweight (> median aspartame consumption vs. no consumption; β = –29.87; 95% CI = –107.83, 48.08).

Considering the final rating of the confidence in the body of evidence for the HOC 'birth outcomes' as 'low' (downgrading for relevance considering that the body of evidence at this time consists of a single study) and the absence of an effect, the Panel considered that there is inadequate evidence to assess if exposure to aspartame is associated with adverse effects on birthweight (Table 14).

Cancer

Since the EFSA evaluation in 2013 (EFSA ANS Panel, 2013), a total of five studies that examined the association between aspartame and cancer were identified; one case–control study conducted in Spain (Palomar-Cross et al., 2023) and 4 cohort studies conducted in USA (McCullough et al., 2014; Romanos-Nanclares et al., 2025; Fulgoni 3rd & Drewnowski, 2022) and in France (Debras, Chazelas, Srour, et al., 2022). Following RoB evaluation, three studies out of five were rated as tier 1 (low risk of bias) and two studies tier 2 (moderate risk of bias) (Table 11, Annex E2).

A single cohort study conducted in France (Debras, Chazelas, Srour, et al., 2022) reported an association between intake of aspartame and overall cancer (high intake, > 14.45 mg/day in men, > 15.39 mg/day in women versus no intake, HR: 1.15, 95% CI: 1.03–1.28, p-trend = 0.002) and obesity-related cancers (Table 11; HR: 1.15, 95% CI: 1.01–1.32, p-trend = 0.026). Three studies investigated the association between aspartame and breast cancer: two cohorts (Debras, Chazelas, Srour, et al., 2022; Romanos-Nanclares et al., 2025) and one case–control study (Palomar-Cross et al., 2023). The same cohort study by Debras, Chazelas, Srour, et al. (2022) found an association with a dose–response between aspartame (aspartame in all foods) and breast cancer (HR: 1.22, 95% CI: 1.01–1.48, p-trend = 0.036), while the case–control study (Palomar-Cross et al., 2023) found an inverse association with foods containing aspartame and breast cancer (OR: 0.28, 95% CI: 0.08–0.83) and the cohort study conducted in USA (Romanos-Nanclares et al., 2025) found no association between aspartame intake (aspartame only estimated from beverages and table-top sweeteners) (Q4, 297.4 mg/day vs. no intake, HR: 1.01 0.95–1.08) and breast cancer.

One case–control (Palomar-Cross et al., 2023) and one cohort study (McCullough et al., 2014) investigated the association between aspartame and haematopoietic cancers. No statistically significant associations were found between intake of aspartame and chronic lymphocytic leukaemia. The association between aspartame and myeloma and other NHL types (Follicular lymphoma, Diffuse large B-cell lymphoma and other B-cell lymphoma) were examined only in one of the studies (McCullough et al., 2014). No association was found between aspartame and non-Hodgkin lymphoma. No association was found for myeloma and/or Follicular lymphoma and other B-cell lymphoma, except for Diffuse large B-cell lymphoma, in which an association was observed for moderate levels of intake (Q3 vs. Q1, RR: 1.62, 95% CI: 1.07, 2.45; Q2 vs. Q1, RR: 1.82, 95% CI: 1.22, 2.72); but not for higher intake of aspartame (> 17.5 mg/day for men and > 19.6 mg/day for women).

One cohort study (Debras, Chazelas, Srour, et al., 2022) and one case–control study (Palomar-Cross et al., 2023) examined the association between aspartame and prostate cancer. No statistically significant associations were found. One single case–control study (Palomar-Cross et al., 2023) investigated the association between aspartame and gastrointestinal cancers (colorectal and stomach cancer) and found no association. No association was found between aspartame intake and cancer mortality (Fulgoni 3rd & Drewnowski, 2022).

Overall, statistically significant associations pointing to an increased cancer risk were reported only in one cohort study (Debras, Chazelas, Srour, et al., 2022). The Panel noted that consumption of aspartame is likely linked to certain lifestyle and dietary habits which, despite adjustments for diet made in this study, may still leave room for confounding. The Panel noted that in all the epidemiological studies on associations between dietary intake of aspartame and cancer, information bias and residual confounding could still be present to some degree limiting conclusions.

Considering the final rating of confidence in the body of evidence for the HOC 'cancer' as 'low' (downgrading for unexplained inconsistencies between studies and/or imprecision in risk estimates as well as the limited number of studies by cancer type), the Panel considered that there is low confidence in the body of evidence that exposure to aspartame is associated with cancer.

Cardiovascular risk factors and disease

Twelve studies (four cohort studies, five trials and three cross-sectional studies) conducted mainly in USA but also in Taiwan, France, Turkey and Chile, investigated the role of aspartame on cardiovascular diseases and risk factors (metabolic syndrome, blood pressure, cholesterol levels, triglycerides, waist circumference, body mass index/body weight and

body composition, obesity). Most studies were rated as of moderate risk of bias (tier 2) except for a cohort study (Debras, Chazelas, Sellem, et al., 2022) and a trial (Higgins et al., 2018) that were rated as tier 1 (low risk of bias). (Table 11, Annex E2). A single large cohort study (Debras, Chazelas, Sellem, et al., 2022) investigated the association between aspartame from all dietary sources, including table-top sweeteners and the risk of cardiovascular diseases. No association was found between aspartame intake, modelled as a continuous variable (HR: 1.03, 95% CI: 0.94–1.14) and cardiovascular diseases overall. However, when the outcomes were analysed separately, an association was observed for cerebrovascular diseases (HR: 1.17, 95% CI: 1.03–1.33) but not for coronary heart disease (HR: 0.91, 95% CI: 0.78–1.06).

Among 11 studies examining the role of aspartame on cardiovascular risk factors, only a single cohort study reported an association between high aspartame intake (> 104.1 mg daily) and obesity (HR: 1.64, 95% CI 1.39–1.93), as well as increases in body weight, waist circumference and body fat (Steffen et al., 2023). On the other hand, in a cohort study conducted in children and adolescents, a decrease in weight gain (β: −0.16, 95% CI: −0.27–0.06) and in body fat (β: −1.21, 95% CI: −2.04–0.38) was observed (Chien et al., 2023). Out of seven studies that examined the relationship between intake of aspartame and body composition in terms of fat and lean mass and abdominal obesity, only two studies reported an association. In one cohort study (Steffen et al., 2023), aspartame was associated with an increase in visceral, sub-cutaneous and intermuscular adipose tissue and in one cross-sectional study, aspartame intake was associated with abdominal obesity (Wulaningsih et al., 2017).

Of the four studies that investigated the effect/association between aspartame and triglycerides levels (Higgins et al., 2018; Higgins & Mattes, 2019; Pearson et al., 2021, Hess et al., 2018) only a cross-sectional study reported a statistically significant association (Hess et al., 2018). Two clinical trials (Higgins et al., 2018; Higgins & Mattes, 2019) found no effect of oral aspartame on cholesterol levels (total, LDL and HDL). No association was found between aspartame intake and blood pressure (Higgins et al., 2018) and/or metabolic syndrome (Hess et al., 2018).

Considering the final rating of confidence in the body of evidence for the HOC 'cardiovascular risk factors and disease' as 'low' (downgrading for unexplained inconsistencies and/or risk of bias), the Panel considered that there is low confidence in the body of evidence that exposure to aspartame is associated with cardiovascular risk factors and disease (Table 14).

Development

Three publications representing two cohort studies assessed the association between postnatal aspartame exposure and reproductive developmental effects. Two studies rated as of moderate risk of bias (Tier 2, Chen et al., 2025; Lin et al., 2024), and the other was rated as low risk of bias (Tier 1, Mueller et al., 2015) (Table 11, Annex E2).

An Asian population was assessed in two studies (Chen et al., 2025; Lin et al., 2024), while the other study was conducted in the USA (Mueller et al., 2015). Between the two studies evaluated (Annex E2), statistically significant associations were observed between aspartame and early menarche and precocious puberty but with opposite effect direction. Aspartame intake was associated with early menarche in Mueller et al. (2015) (incident menarche before 11 years of age; RR per 1-SD increase: 1.14; 95% CI, 1.03–1.26). In the Taiwan Puberty Longitudinal Study, Lin et al. (2024) observed an opposite-direction association between higher-than-median aspartame consumption (B2 vs. no intake) and precocious puberty (OR: 0.67, 95% CI 0.48–0.94) in girls and boys; when stratified by gender the association remained statistically significant only among girls (OR: 0.63, 95% CI: 0.42–0.96). In the same study population, Chen et al. (2025) did not find statistically significant associations for puberty-onset timing (age at Tanner stage 2 in years; beta, −0.07; 95% CI, −0.25, 0.1), LH or FSH in girls.

Considering the final rating of confidence in the body of evidence for the HOC 'development' as 'low' (downgrading for risk of bias and/or inconsistency) and the overall absence of effects, the Panel considered that there is inadequate evidence to assess if exposure to aspartame is associated with developmental effects (Table 14).

Glucose/insulin homeostasis

Thirteen studies, including nine randomised controlled trials, one cohort studies and three cross-sectional studies, with a sample size ranging from 8 to 105,588 participants assessed endpoints relevant to glucose and insulin homeostasis (Table 11, Annex E2).

Nine studies (71%) (Pearson et al., 2021; Ahmad et al., 2020; Suez et al., 2022; Higgins & Mattes, 2019; Higgins et al., 2018; Kashima et al., 2019; Bryant et al., 2014; Temizkan et al., 2015; Finley et al., 2019) were randomised controlled trials with a sample size ranging from 8 to 371 participants (median sample size, 29.5). Most randomised studies included healthy young adults, with the mean age of the participants being below 30 years in 9 out of 10 studies and a mean BMI lower than 25 kg/m^2 in 8 out of 10 studies. The aspartame intervention daily dose ranged from 1 to 114 mg/kg bw per day (standard 70 kg adult weight) and it was administered for 2 ($n = 2$) or 12 ($n = 2$) weeks, while in five studies, the intervention was administered only once. Four studies implemented a cross-over design (Pearson et al., 2021; Ahmad et al., 2020; Kashima et al., 2019; Bryant et al., 2014).

One cohort study (Debras et al., 2023) and three cross-sectional studies (Kuk & Brown, 2016; Hess et al., 2018; Sylvetsky et al., 2025) were performed with a sample size ranging from 125 to 105,588 participants.

Across all studies assessed, statistically significant associations were observed for type-2 diabetes in one study (Debras et al., 2023). Debras et al. (2023) reporting on the Prospective NutriNet-Santé Cohort in France evaluated the association between aspartame consumption and type-2 diabetes and found a statistically significant association in an appropriately adjusted model (HR: 1.63; 95% CI: 1.38–1.9, p-trend < 0.001). Statistically significant associations were also observed for

blood glucose (fasting or OGTT values) in three studies (Pearson et al., 2021; Hess et al., 2018; Finley et al., 2019); two of these findings (Pearson et al., 2021; Finley et al., 2019) were of low relevance as they came from interventional studies that assessed single aspartame administrations and the third signal came from a small cross-sectional study.

The Panel noted that, as with many sweeteners, many of these studies were not designed to assess any potential adverse effect of aspartame or its salts on glucose homeostasis. Instead, aspartame was used as placebo or reference when assessing the effects of carbohydrate-rich food on blood glucose (and associated measures). Therefore, the exposure in most of the studies was lower than the ADI (Table 11). Furthermore, many of the studies were only assessing effects on post prandial glucose response or effects on glucose homeostasis in the short-term, while few studies assessed changes in glucose homeostasis over few weeks (Ahmad et al., 2020; Hess et al., 2018; Suez et al., 2022). Overall, no consistent effects related to intake of aspartame were observed on measures of glucose and insulin homeostasis in these studies.

Considering the final rating of confidence in the body of evidence for the HOC 'glucose and insulin homeostasis' as 'low' (downgrading for relevance and risk of bias) and the absence of effects (see Table 13 and Annex E2), the Panel considered that there is inadequate level of evidence available to assess if the exposure to aspartame does not adversely affect glucose and insulin homeostasis (Table 14).

Inflammation/immunotoxicity

A single cross-sectional study conducted in USA studied the associations between aspartame intake (around 180 mg/day) and inflammatory markers C-reactive protein (CRP), interleukin-10 (IL-10), interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and showed no association in any of the parameters evaluated (Sylvetsky et al., 2025).

Considering the final rating of confidence in the body of evidence for the HOC 'inflammation/immunotoxicity' as 'very low' (downgrading for relevance considering that the body of evidence at this time consists of a single study), the Panel considered that there is inadequate evidence to assess if exposure to aspartame is associated with adverse effects on the immune system (Table 14).

Liver effects

Higgins et al. (2018) conducted in USA a 12-weeks randomised controlled parallel trial to assess the effect of aspartame (350 or 1050 mg vs. 0 mg) on liver parameters (ALT, alanine transaminase; AST, aspartate transaminase; GGTP, γ-glutamyltranspeptidase) and found no effects in any of the parameters evaluated.

Considering the final rating of confidence in the body of evidence for the HOC 'liver effects' as 'moderate' (downgrading for relevance considering that the body of evidence at this time consists of a single study), the Panel considered that there is moderate confidence in the body of evidence that exposure to aspartame is not associated with adverse effect on the liver (Table 14).

TABLE 14 Translation of confidence ratings into level of evidence for conclusions of adverse effects or no adverse effects on health for the included human studies for each of the HOC considered in the assessment. HOCs are listed in alphabetical order.

HOC	Final rating of confidence	Level of evidence
Birth outcomes	**Low (no effect)**	**Inadequate** There is insufficient evidence to assess if exposure to aspartame is associated with adverse effects on birthweight
Cancer	**Low (Effect)**	**Low** There is low confidence in the body of evidence that exposure to aspartame is associated with cancer.
Cardiovascular risk factors and disease	**Low (Effect)**	**Low** There is low confidence in the body of evidence that exposure to aspartame is associated with adverse effects on cardiovascular risk factors and disease.
Development	**Low (No effect)**	**Inadequate** There is insufficient evidence to assess if exposure to aspartame is associated with developmental effects.
Glucose and insulin homeostasis	**Low (No effect)**	**Inadequate** There is insufficient evidence available to assess if the exposure to aspartame does not adversely affect glucose and insulin homeostasis.
Inflammation/ Immunotoxicity	**Very low (No effect)**	**Inadequate** There is insufficient evidence to assess if exposure to aspartame is associated with adverse effects on the immune system.
Liver effects	**Moderate (No effect)**	**Moderate** There is moderate confidence in the body of evidence that exposure to aspartame is not associated with adverse effects on the liver.

3.5.4.2.3 | *5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP)*

Since the adoption of the EFSA opinion on aspartame (EFSA ANS Panel, 2013), no new data were received on DKP in response to the call for biological and toxicological data. No new relevant studies were identified from the updated literature search (Appendix A).

3.5.4.2.4 | *Acesulfame*

The WoE assessment for acesulfame K (E950) was based on the evaluation of more than 20 endpoints comprising five HOCs extracted from 13 human studies considered of low (tier 1) or moderate (tier 2) RoB (EFSA FAF Panel, 2025). Overall, the data supported (i) a moderate level of evidence for no effect on glucose/insulin homeostasis, (ii) a low level of evidence for an association with cancer, cardiovascular risk factors and disease, and development and (iii) an inadequate level of evidence for birth outcomes.

3.5.4.3 | *Integration of the evidence from animal and human studies*

3.5.4.3.1 | *The salt of aspartame-acesulfame (E 962)*

Due to the paucity of data on the salt of aspartame-acesulfame (E 962), no WoE assessment was performed for this food additive. The FAF Panel, however, performed the WoE assessment of its two components, aspartame and acesulfame.

3.5.4.3.2 | *Aspartame*

Human and animal evidence streams were integrated for similar HOCs (Tables 15 and 16), in accordance with Appendix A and with definition given in the Protocol (EFSA FAF Panel, 2023) and taking into considerations the assessment Sections 3.5.4.1 and 3.5.4.2. The conclusions from the integration of evidence, shown in Table 16, were expressed in terms of likelihood of an association between the intake of aspartame and an adverse effect on human health. In the case of the HOCs with data available only from human studies (i.e. birth outcomes, development) or animal studies (i.e. haematotoxicity, general toxicity, nephrotoxicity, neurotoxicity, other organ toxicity), the integration of evidence was done considering the option 'missing data' from Figure A.2, Appendix A. 'Missing data' pertains to data missing in the body of evidence in the current assessment of aspartame in accordance with the revised protocol (EFSA FAF Panel, 2023). Consideration was given to the outcome of the WoE assessments which includes data between January 2013 and June 2025, studies previously evaluated by EFSA in 2013 and considered for setting an ADI for aspartame (E 951) (EFSA ANS Panel, 2013), and when deemed relevant, other evidence from the previous re-evaluation of aspartame (E 951) (EFSA ANS Panel, 2013).

TABLE 15 Overview of the HOCs for which human and animal evidence streams were integrated.

HOC human studies	HOC animal studies (endpoints extracted)
Cancer	Carcinogenicity
Cardiovascular risk factors and diseases	General toxicity (body weight and body fat composition)
	Liver toxicity (serum triglycerides and total cholesterol)
Glucose/insulin homeostasis	Glucose/insulin homeostasis
Inflammation/immunotoxicity	Additional clinical chemistry (serum cytokines)
Liver effects	Liver toxicity

Abbreviation: HOC, health outcome category(ies).

Birth outcomes

The HOC 'birth outcomes' was evaluated in a single human study which included prenatal exposure to aspartame. No animal data were available for the integration. The Panel considered that based on the data from the updated literature search, there was insufficient evidence to assess whether exposure to aspartame is associated with developmental effects concerning birthweight. Additional consideration was given to evidence and the conclusions reported in the previous evaluation (EFSA ANS Panel, 2013) on development studies. In the former assessment (EFSA ANS Panel, 2013), it was noted that in developmental studies in animals, reduced pup body weight at birth was observed at the dose of 4000 mg aspartame/kg bw per day. The ANS Panel considered that these findings could be attributed to a combination of malnutrition and nutritional imbalance due to excessive exposure to phenylalanine derived from aspartame. In support of this hypothesis, the ANS Panel noted that administration of a dose of L-phenylalanine equimolar to aspartame led to a similar decrease in maternal and pup body weight, as observed in a concurrent aspartame group. The ANS Panel concluded that high phenylalanine blood levels are associated with developmental toxicity in several species.

Taking these findings into account, the ADI of 40 mg/kg bw per day of aspartame was derived by concentration-dose modelling. In agreement with the ANS Panel (EFSA ANS Panel, 2013), the FAF Panel concluded that it is unlikely that exposure to aspartame at or below the ADI of 40 mg/kg bw per day causes an adverse increase of phenylalanine in the blood, which is associated with reduced birth weight or other adverse birth outcomes in humans.

Cancer/carcinogenicity

The HOC 'cancer' from human studies was integrated with the HOC 'carcinogenicity' in animal studies. The Panel considered the level of evidence in human data to be low for the association between aspartame and cancer. The level of evidence in the included animal studies for the absence of carcinogenic effects was 'high', and the Panel considered that there is a high confidence in the body of evidence that exposure to aspartame is not associated with carcinogenicity. Integrating the evidence from human and animal studies, the Panel concluded that it is unlikely that exposure to aspartame (E 951) is associated with cancer in humans. The Panel further noted that no concern for genotoxicity was identified for aspartame (see Section 3.5.3.2).

Cardiovascular risk factors and disease/General toxicity and liver toxicity

The Panel considered it appropriate to integrate the endpoints bodyweight, body fat composition from 'general toxicity' and serum triglycerides and total cholesterol from 'liver toxicity' from animal studies with the HOC 'cardiovascular risk factors and diseases' addressed in human studies. The Panel considered that there is low confidence in the body of evidence for an association between exposure to aspartame and cardiovascular risk factors and disease as findings in human studies were inconclusive. The level of evidence for absence of effects for the endpoints body weight and body fat composition under HOC 'general toxicity' and triglyceride and total cholesterol under HOC 'liver toxicity' combined was evaluated as moderate/high. Therefore, the Panel concluded that it is unlikely that exposure to aspartame (E 951) is associated with cardiovascular risk factors and disease in humans.

Development

The HOC 'development' (onset of puberty) was evaluated in human studies which included postnatal exposure to aspartame. The Panel considered that there was insufficient evidence to assess whether exposure to aspartame is associated with developmental effects concerning puberty. No data from animal studies addressing these endpoints were available. Therefore, the Panel considered that it is not possible to conclude on the likelihood that exposure to aspartame (E 951) is associated with developmental effects concerning onset of puberty.

Glucose and insulin homeostasis

The HOC 'glucose and insulin' was evaluated in human and animal studies. The level of evidence was considered as inadequate and moderate for absence of adverse effects in human and animal studies, respectively. The Panel considered that overall, both the human and animal studies reviewed provided consistent evidence, yet with limitations in the human studies; therefore, more weight was given to the animal studies. No adverse disturbances of glucose and insulin homeostasis was reported in the previous evaluation (EFSA ANS Panel, 2013). Therefore, the Panel concluded that it is 'unlikely' that exposure to aspartame (E 951) is associated with disturbances of glucose/insulin homeostasis in humans.

Inflammation/immunotoxicity and Additional clinical chemistry

The Panel considered it appropriate to integrate the endpoint 'serum cytokines' from the HOC 'additional clinical chemistry' from animal studies with the HOC 'Inflammation/immunotoxicity' addressed in a single human study. The level of evidence for no effect was rated as inadequate for the human study and as moderate for the animal studies. Considering that only few endpoints were assessed in the animal studies and the inconclusive pattern of the observed changes, the Panel concluded that it is not possible to conclude whether the exposure to aspartame (E 951) is associated with disturbances of the immune system in humans.

Liver effects/Liver toxicity

The HOC 'liver effects' and 'liver toxicity' were evaluated in human and animal studies. A single human study reported no effect in a limited number of serum liver enzymes. The level of evidence was rated as moderate for the absence of effects on biomarkers of liver impairment in humans. In the seven assessed animal studies, no adverse aspartame-related effects were observed for several liver-related endpoints. The level of evidence was evaluated as moderate for the absence of liver toxicity. Therefore, the Panel concluded that it is unlikely that exposure to aspartame (E 951) is associated with adverse effects on liver in humans.

General toxicity

There was moderate confidence in the body of evidence that exposure to aspartame (E 951) is not associated with adverse changes in general toxicity parameters in animal studies. General toxicity was not assessed in human studies. The Panel considered that the animal studies reviewed provided moderate level of confidence in the evidence for no effects of aspartame exposure on changes in general toxicity parameters (except at doses which markedly exceeded current limit doses recommended in OECD test guidelines). The Panel concluded that it is unlikely that exposure to aspartame (E 951) is associated with general toxicity in humans.

Haematotoxicity

The level of evidence for absence of an effect was evaluated as high in animal studies. Haematological parameters were not reported in human studies. The Panel concluded that it is unlikely that exposure to aspartame (E 951) is associated with haematological effects in humans.

Nephrotoxicity

The level of evidence for absence of effects was evaluated as moderate in animal studies. Nephrotoxicity parameters were not investigated in human studies. The Panel considered that the animal studies reviewed provided moderate level of confidence in the evidence for no effects of aspartame exposure on changes in the kidney. The Panel concluded that it is unlikely that aspartame (E 951) is associated with nephrotoxicity in humans.

Neurotoxicity

Effects were reported both for some behavioural endpoints and for non-apical endpoints in animal studies, such as brain tissue cytokine levels and oxidative status. The level of evidence was evaluated as low for an association between exposure to aspartame and neurotoxicity. Evidence from human studies was not available. Based on the WoE assessment in the present opinion, the Panel considered that it is not possible to conclude on the likelihood that exposure to aspartame (E 951) is associated with neurotoxicity in humans. However, additional consideration was given to evidence on potentially neurotoxic metabolites of aspartame reported in the previous evaluation (EFSA ANS Panel, 2013), to assess whether the new data support them. As reported in the former assessment (EFSA ANS Panel, 2013), data in humans showed no increase of blood methanol above basal levels (about 20 mg/L) up to a dose of 150 mg/kg bw (Stegink, 1984), the blood concentration of methanol for which toxic effects are to be expected is 200 mg/L (Kostic and Dart, 2003). Also for formate, the neurotoxic metabolite of methanol, no increase in blood concentration was observed up to a dose of 150 mg/kg bw (Stegink, 1984). Given that the level of evidence was considered low for an association between exposure to aspartame and neurotoxic effects in animals, and in the absence of mechanistic explanation, the Panel concluded that it is unlikely that exposure to aspartame (E 951) at or below the ADI of 40 mg/kg bw per day is associated with neurotoxicity in humans.

Other organ toxicity (not carcinogenicity)

No aspartame-related histopathological findings or adverse effect on organ weight in any organ or tissue (lung, kidney and brain were assessed under separate HOCs) were observed in the chronic toxicity animal studies, testing a wide dose range. The level of evidence for absence of effects was evaluated as high. Biomarkers of adverse effects on organs or tissues were not investigated in the human studies. The Panel concluded that it is unlikely that exposure to aspartame (E 951) is associated with toxicity in any organ or tissue in humans.

3.5.4.3.3 | *5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP)*

No new data were received on DKP in response to the call for biological and toxicological data and no new data were identified from the updated literature search (Appendix A). Therefore, no WoE assessment was performed for DKP.

3.5.4.3.4 | *Acesulfame*

In the 2025 re-evaluation of acesulfame K (E 950), based on animal and human evidence, the EFSA FAF Panel concluded that it is unlikely that intake of acesulfame K (E 950) is associated with cancer, disturbances of glucose or insulin homeostasis, or effects on cardiovascular risk factors and disease. Based on human evidence only, the Panel considered that it was not possible to conclude that intake of acesulfame K is associated with birth outcomes (preterm delivery) or the onset of puberty in girls. Based on animal data only, the FAF Panel concluded that it is unlikely that acesulfame K is associated with general toxicity, liver toxicity, nephrotoxicity, haematotoxicity or other organ toxicity (excluding carcinogenicity) in humans (EFSA FAF Panel, 2025).

TABLE 16 Overview of the conclusions on the level of likelihood of an association or absence of association between the exposure to aspartame (E 951) and an adverse effect on human health for each HOC or combination of HOCs. HOCs are listed in alphabetical order.

Evidence stream	HOC (endpoint)	Effect or association observed (yes/no)	Level of evidence	Integration of the evidence (likelihood)[a]
Human	Birth outcomes	No	Inadequate	Unlikely[b]
Animal	–	–	Missing data	It is unlikely that exposure to aspartame is associated with reduced birth weight in humans
Human	Cancer	Yes	Low	Unlikely
Animal	Carcinogenicity	No	High	It is unlikely that exposure to aspartame (E 951) is associated with cancer in humans.
Human	Cardiovascular risk factors and disease	Yes	Low	Unlikely
Animal	Endpoints extracted from: General toxicity (body weight, body composition); Liver toxicity (total cholesterol, serum triglycerides)	No	Moderate/High	It is unlikely that exposure to aspartame (E 951) is associated with cardiovascular risk factors and disease in humans.
Human	Development	No	Inadequate	Not possible to conclude
Animal	–	–	Missing data	It is not possible to conclude whether exposure to aspartame (E 951) is associated with developmental effects concerning onset of puberty in humans.
Human	Glucose/insulin homeostasis	No	Inadequate	Unlikely[c]
Animal	Glucose/insulin homeostasis	No	Moderate	It is unlikely that exposure to aspartame (E 951) is associated with disturbances on Glucose/insulin homeostasis in humans
Human	Inflammation/Immunotoxicity	No	Inadequate	Not possible to conclude[d]
Animal	Endpoints extracted from: Additional clinical chemistry (serum cytokines)	No	Moderate	It is not possible to conclude whether exposure to aspartame (E 951) is associated with disturbances of the immune system in humans
Human	Liver effects	No	Moderate	Unlikely
Animal	Liver toxicity	No	Moderate	It is unlikely that exposure to aspartame (E 951) is associated with adverse effects on liver in humans.
Human	–	–	Not applicable	Unlikely[e]
Animal	General toxicity	No	Moderate	It is unlikely that exposure to aspartame (E 951) is associated with general toxicity in humans.
Human	–	–	Missing data	Unlikely
Animal	Haematotoxicity	No	High	It is unlikely that exposure to aspartame (E 951) is associated with haematotoxicity in humans.
Human	–	–	Missing data	Unlikely[f]
Animal	Nephrotoxicity	No	Moderate	It is unlikely that exposure to aspartame (E 951) is associated with nephrotoxicity in humans.
Human	–	–	Missing data	Unlikely[g]
Animal	Neurotoxicity	Yes	Low	It is unlikely that exposure to aspartame (E 951) is associated with neurotoxicity in humans.

TABLE 16 (Continued)

Evidence stream	HOC (endpoint)	Effect or association observed (yes/no)	Level of evidence	Integration of the evidence (likelihood)[a]
Human	–	–	Missing data	Unlikely
Animal	Other organ toxicity	No	High	It is unlikely that exposure to aspartame (E 951) is associated with toxicity in any organ or tissue in humans.

Abbreviation: HOC, Health Outcome Category(ies).

[a]The integration of evidence was complemented by additional considerations from the Panel on the biological plausibility of each effect or mode of action (MoA). When deemed relevant by expert judgement, consideration also was given to the evidence and conclusions of the previous evaluation (EFSA ANS Panel, 2013) to assess whether the new data support them. Additional footnotes indicate HOCs for which additional considerations were applied.

[b]The integration of the evidence based on the data presented in Sections 3.5.4.1.2 and 3.5.4.2.2 was 'inadequate'. For the conclusion on the likelihood of an effect, additional considerations were given to evidence and the conclusions reported in the previous evaluation (EFSA ANS Panel, 2013).

[c]The integration of the evidence based on the data presented in Sections 3.5.4.1.2 and 3.5.4.2.2 was as likely as not. For the conclusion on the likelihood of an effect, additional considerations were given to evidence and the conclusions reported in the previous evaluation (EFSA ANS Panel, 2013).

[d]The integration of the evidence based on the data presented in Sections 3.5.4.1.2 and 3.5.4.2.2 was 'as likely as not'. For the conclusion on the likelihood of an effect, additional consideration was given to the biological plausibility and expert judgement.

[e]The integration of the evidence based on the data presented in Sections 3.5.4.1.2 and 3.5.4.2.2 was 'as likely as not'. For the conclusion on the likelihood of an effect, additional consideration was given to on the biological plausibility and expert judgement.

[f]The integration of the evidence based on the data presented in Sections 3.5.4.1.2 and 3.5.4.2.2 was 'as likely as not'. For the conclusion on the likelihood of an effect, additional consideration was given to on the biological plausibility and expert judgement.

[g]The integration of the evidence based on the data presented in Sections 3.5.4.1.2 and 3.5.4.2.2 was 'as likely as not'. For the conclusion on the likelihood of an effect, additional considerations were given to evidence and the conclusions reported in the previous evaluation (EFSA ANS Panel, 2013).

3.5.5 | Hazard characterisation and identification of a reference point

The SCF considered that the salt of aspartame-acesulfame (E 962) represents a source of aspartame and acesulfame and that the potential exposure is the same with an equivalent blend of aspartame and acesulfame K (SCF, 2000b). The FAF Panel agreed with this and considered that the respective ADIs of aspartame and acesulfame would apply.

3.5.5.1 | *Aspartame*

For the selection of the reference point (RP) for aspartame, considering the outcome of the WoE assessment, the Panel noted that there are no new eligible studies relevant for identification of a RP (no integrated evidence conclusions were 'likely' or 'very likely'; Table 16). The Panel concluded that the integrated available evidence indicated no reason to change the previously established ADI for aspartame and therefore confirmed the ADI of 40 mg/kg bw per day for aspartame.

3.5.5.2 | *5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP)*

No new data were received on DKP in response to the call for biological and toxicological data, and no new data were identified from the updated literature search (Appendix A).

The toxicity of DKP was previously evaluated at the time of the re-evaluation of aspartame (EFSA ANS Panel, 2013). The ANS Panel noted that in the toxicological studies on aspartame, including long-term and carcinogenicity studies, the content of DKP in the aspartame tested ranged from 0.1% up to 4% (EFSA ANS Panel, 2013). In agreement with the JECFA monograph (JECFA, 1980) and the SCF evaluation (1989), the ANS Panel identified a NOAEL for DKP of 750 mg DKP/kg bw per day (ESFA ANS Panel, 2013). Since no new evidence is available, in line with the ANS Panel, the FAF Panel considered the previously established ADI for DKP of 7.5 mg kg bw per day (SCF, 1989) for the current assessment.

3.5.5.3 | *Acesulfame*

In the re-evaluation of acesulfame K (EFSA FAF Panel, 2025), the FAF Panel established an ADI for acesulfame K (E 950) of 15 mg/kg bw per day based on application of the default 100-fold uncertainty factor to the NOAEL of 1500 mg/kg bw per day (the highest dose tested) in a 2- year study in the rat.

3.5.5.4 | *Salt of aspartame-acesulfame (E 962)*

The Panel considered that upon ingestion, the salt of aspartame-acesulfame (E 962) will be fully dissociated into its two components, aspartame and acesulfame, and therefore the ADIs of 40 and 15 mg per kg bw per day for aspartame and acesulfame K, respectively, should not be exceeded.

3.6 | Environmental considerations

3.6.1 | The salt of aspartame-acesulfame (E 962)

The applicable EU legislation on food additives establishes that the approval of food additives should consider, among other factors, also the protection of the environment. In the framework of the re-evaluation of salt of aspartame-acesulfame (E 962) under Regulation (EU) No. 257/2010, EFSA has not received any information from IBOs or any other interested party concerning any environment risks of E 962. Studies addressing the environmental impact of the salt of aspartame-acesulfame (E 962) were not available in the literature however, data and information on aspartame and acesulfame were available and are considered in this section. Specifically, the studies considered in this section encompasses the studies which were identified from a systematic review conducted by the Agriculture and Environment Research Unit (AERU), University of Hertfordshire (2021) and from the updated literature search (Appendix A).

3.6.2 | Aspartame

As reported in Section 3.5.1, after oral ingestion, aspartame is metabolised in humans into aspartic acid, phenylalanine and methanol. These metabolites are then absorbed and enter normal endogenous metabolic pathways. It is, therefore, not expected that unchanged aspartame, when consumed as food additive, would reach the environment via wastewater.

Notwithstanding the above, several studies retrieved in the literature described the development of methods for the removal of aspartame from (waste) water (see Annex F). Subedi and Kannan (2014), Sun et al. (2014), Chen, Zeng, et al. (2024), Yue et al. (2023) and Shen et al. (2023) investigated the fate of various artificial sweeteners in wastewater treatment plants and reported for aspartame removal efficiencies ranging from 50% to 97%. Guo et al. (2021) reported an overview of the removal efficiency of aspartame through wastewater from different studies (ranging from 15.4% to 100%).

Regarding the fate of aspartame in the environment, AERU (2021) reported some estimates of the abiotic degradation of aspartame and indicated that aspartame may be considered persistent in the environment under some conditions (pH > 5). In Gatidou et al. (2020) a ready biodegradability test was performed (OECD 301F) and aspartame resulted as readily biodegradable. Jahani et al. (2020) and Tran et al. (2014) reported that aspartame is readily biodegradable and that it can be biochemically removed by activated sludge processes.

Several studies retrieved in AERU (2021) and in the updated literature search reported analytical results of the measurement of aspartame in environmental compartments (see Annex F). In these studies, aspartame was not consistently detected in waste, marine and surface water (not detected or detected at levels in the order of ug/L). It was not detected in ground water. Aspartame was not consistently detected in surface water sediment (highest detected concentration 568 ng/g). The Panel noted that the available data on the environmental concentrations of aspartame are based on isolated monitoring studies and are not part of systematic monitoring programmes. The available studies included data from both EU and non-EU countries and may not be fully representative of the European situation. The international platform of chemical monitoring (IPCHEM)[29] includes data on the concentration of aspartame in different environmental compartments from EU and non-EU countries from EMPODAT.[30] In this database aspartame was reported as not detected in waste, surface and ground water and sediment.

Some studies retrieved in AERU (2021) and in the updated literature search assessed the ecotoxicological effects of aspartame (see Annex F). The available studies covered different taxa of aquatic organisms, but with few exceptions, did not fully address the chronic toxicity of aspartame. No studies on sediment organisms were available. Some studies assessing effects on terrestrial organisms were also available. When the chronic studies are considered, a developmental toxicity study on aspartame (and saccharin) in medaka (*Oryzias latipes*) was available reporting increased heart rate (7.8%), slightly suppressed head growth and behavioural effects following the exposure to 60 or 300 mg/L (Lee & Wang, 2015). It is noted that this test was performed at concentrations higher than those recommended by the Fish, Early-life Stage Toxicity Test, OECD TG 210. Su et al. (2025) performed a 60 day-feeding study on largemouth bass (*Micropterus salmoides*), however, the interpretation of the results of this study is difficult considering that feeding (dietary) studies are not generally recommended as standard fish toxicity tests and that the ecotoxicological relevance of some of the endpoints addressed in this study is unclear. Similarly, the relevance of the results of the fish dietary study from Kim et al. (2011) is unclear. Wu et al. (2024) investigated the potential effects of aspartame (tested concentrations 0.49, 49, 490 ug/L) on the development of various tissues and organs in zebrafish (*Danio rerio*) embryos. No effects on survival, heart rate, coiling tail were observed. No concentration/related changes in the hatching rate were observed. A slight delay in pigmentation and an increase in body length was observed in all groups. The ecotoxicological relevance of these changes is unclear. Studies on the toxicity on aquatic invertebrates of appropriate duration were not available. In Nykyforov et al. (2022) a decrease in fecundity of *Daphnia magna* was observed but the study was performed at very high concentrations (600 mg/L) and lasted for only 4 days. Kobeticova et al. (2016, 2018) performed standard ecotoxicity studies on green algae (*Desmodesmus subspicatus*) and duckweed (*Lemna minor* L.). No effects (growth rate) were observed in algae at 100 mg/L. The lowest established NOEC for duckweed was 6.25 mg/L, based on frond number. Kobeticova et al. (2018) investigated also the effects on terrestrial plants and soil invertebrates (*Enchytraeus crypticus*) in two limit tests at 100 mg/kg of aspartame. No effects were observed in plants but a reduction in potworm' juveniles was observed. A study on the potential effects on ants (*Polyrhachis vicina* Roger) was also available; the relevance of this study testing doses at and above the ADI in a non-standard setting is unclear.

Regarding the metabolites and degradation products of aspartame, aspartic acid and phenylalanine are naturally occurring amino acids and methanol is reported to be readily biodegradable.[31] As reported in Section 3.1.4, DKP is the major degradation product of aspartame. DKP is not well absorbed from the gut and was recovered in the faeces, primarily as unchanged DKP. Following oral administration of DKP, metabolism to phenylacetic acid occurs in the gastrointestinal tract, phenylacetic acid can then be absorbed and is rapidly excreted in the urine as phenylacetic acid and, following conjugation with glutamate, to phenylacetylglutamine. Consequently DKP, phenylacetylglutamine and phenylacetic acid have the potential to reach the environment via wastewater. Phenylacetylglutamine is a naturally occurring compound that is also generated during the metabolism of phenylalanine. Limited data on environment were available in the literature concerning DKP. In Berset and Ochsenbein (2012), DKP was analysed in wastewater, tap water, surface water and ground water and reported as not detected in all cases except for wastewater (effluent) in which DKP was detected in 4% of the analysed samples ($n = 28$) up to 200 ng/L. Overall, considering the principles of EFSA FAF Panel guidance (2026a; Section 6) and that (i) aspartame is metabolised in humans to naturally occurring or readily degradable compounds and (ii) that it is reported as readily biodegradable, a concern for the environment is not anticipated. It is, however, noted that whilst not expected, aspartame is reported to be detected in the environment in some instances at concentrations in the ug/L range; possible sources of this contamination are unknown. Additionally, limited information was available on the environmental impact of DKP.

3.6.3 | Acesulfame

Regarding acesulfame, EFSA FAF Panel (2025) already reviewed the literature available for acesulfame K and reported that '*the concentrations measured in surface water (highest concentration 122 μg/L; Montes et al., 2019) are lower than the available*

[29] https://ipchem.jrc.ec.europa.eu/#discovery.

[30] https://ipchem.jrc.ec.europa.eu/#databaseConsole/EMPODAT/aspartame/22839-47-0/none/none.

[31] Methanol 100.000.599 | cda9f272-bca1-4de5-b894-80783369405c - ECHA CHEM.

ecotoxicological effect concentrations. However, the Panel noted that the available data on the environmental concentrations of acesulfame are based on isolated monitoring studies and are not part of systematic monitoring programmes. The available studies included data from both EU and non-EU countries and may not be fully representative of the European situation. These data therefore give only a rough indication of the concentration of acesulfame and may not have captured the worst-case exposure for aquatic organisms'.

Considering the principles of the EFSA FAF Panel guidance (2026; Section 6) and considering (i) that acesulfame is not extensively metabolised in human, (ii) that it may not be fully degraded in a sewage treatment plant, (iii) that is not readily degradable, (iv) that there is evidence of the presence of acesulfame in the environment and (v) that there is some uncertainty in relation to the formation of transformation products from acesulfame during waste treatment and in the environment, further considerations may be needed to address the possible impact of acesulfame to the environment.

3.7 | Discussion

The present opinion deals with the re-evaluation of the salt of aspartame-acesulfame (E 962), which is authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008.

According to the EU specifications, the salt of aspartame-acesulfame (E 962) is produced by heating an approximately 2:1 ratio (w/w) of aspartame and acesulfame K in aqueous solution under acidic conditions, followed by crystallisation. However, the Panel noted that the specifications do not explicitly require the aspartame and acesulfame K used in the manufacture of E 962 to comply with the respective EU specifications for E 951 and E 950.

No technical information, including analytical data, or details on the manufacturing process of the salt of aspartame-acesulfame (E 962) were submitted to EFSA. Nonetheless, it was noted that the existing purity criteria (Commission Regulation (EU) No 231/2012) for E 962 do not align with the listed impurities and limits defined in the specifications for aspartame (E 951), nor with the recommendation for the amendment of the specifications for acesulfame K (E 950) as proposed by the Panel in its 2025 re-evaluation (EFSA FAF Panel, 2025).

In the absence of analytical data on the salt of aspartame-acesulfame (E 962) and information on its manufacturing process, the Panel considers that the EU specifications for E 962 should be revised to ensure its safety can be adequately assessed. In particular the definition of the salt of aspartame-acesulfame (E 962) in the EU specifications should be modified and required that the food additive E 962 is prepared by the reaction of an approximately 2:1 ratio (w/w) of aspartame and acesulfame K, which should meet the specifications laid down for aspartame (E 951) in Commission Regulation (EU) No 231/2012, and for acesulfame K (E 950) according to the proposed revision for the specifications as recommended in the Panel's re-evaluation (EFSA FAF Panel, 2025), until such revisions are formally incorporated into Commission Regulation (EU) No. 231/2012. These revisions include, in particular, the introduction of limits for the organic impurities 5-chloro-acesulfame and acetoacetamide.

Furthermore, during the re-evaluation of acesulfame K (E 950), the Panel recommended lowering the limits for lead and mercury in the EU specifications based on the analytical data available. In contrast, no analytical data on the actual occurrence of arsenic and lead in aspartame (E 951) were available to the ANS Panel at the time of its re-evaluation in 2013 and no information was available on the lowest technologically achievable levels for lead and arsenic in E 951 (EFSA ANS Panel, 2013). The current EU specifications for E951 establish limits of 3 mg/kg for arsenic and 1 mg/kg for lead. Considering the exposure to aspartame (7.6 and 26.7 mg/kg bw per day for the highest mean and 95th percentile, respectively) and considering that for example arsenic could be present in E951 at the current limit in the EU specifications, the MOE would be 3 and < 1 respectively, indicating that there would be a potential concern. The Panel considers that these limits for toxic elements in E 951 should also be reviewed, in line with the approach adopted for other sweeteners and actual levels in the food additive.

No information on the stability of the salt of aspartame-acesulfame (E 962) has been submitted to EFSA following calls for data; no information was retrieved through the literature search. The Panel considered that wherever the salt of aspartame-acesulfame (E 962) is dissolved in food, it is expected to be dissociated into its individual components, aspartame and acesulfame. The stability of acesulfame K (E 950) was assessed in its re-evaluation (EFSA FAF Panel, 2025). Screening of publications on the stability of aspartame (E 951), identified in the literature search conducted for this assessment, corroborated what was already concluded at the time of its re-evaluation (EFSA FAF Panel, 2013) that aspartame undergoes several degradation reactions in foods depending on pH, temperature and storage conditions. The major degradation products formed via hydrolysis are L-phenylalanine, L-aspartic acid and methanol. Cyclisation of aspartame yields DKP, particularly under heat or neutral to alkaline pH. Additional products arise from rearrangement and cleavage reactions, including α-aspartylphenylalanine (L-aspartyl-L-phenylalanine), β-aspartame and β-aspartylphenylalanine. Minor products include phenylalanine–aspartic acid dipeptides and related intermediates formed through interconversion pathways. The relative proportions of these degradation products vary according to the food matrix and processing conditions, with DKP representing the principal degradation product.

In the re-evaluation of aspartame (E 951) (EFSA ANS Panel, 2013), it was considered that up to 24% of DKP can be formed under specific processing conditions. Based on the highest 95th percentile exposure to aspartame in toddlers (26.7 mg/kg bw per day), the resulting exposure to DKP would be 6.4 mg/kg bw per day, which is below the ADI for DKP of 7.5 mg/kg bw per day (see Section 3.5.5).

The only new evidence obtained on the stability of E 951 was a study by Trawiński and Skibiński (2023) that identified three possible new photodegradation products of aspartame (ortho-, meta- and para-hydroxyaspartame) in some commercial

soft drinks exposed to simulated sunlight, alongside the known degradation products DKP, α-aspartylphenylalanine and phenylalanine methyl ester. Their formation appeared formulation-dependent and based on the reported mass spectrometry response data they occurred at much lower concentrations than the known degradation products. No toxicity data were identified. In silico QSAR analysis performed by the Panel did not indicate any genotoxicity alerts for the proposed phenolic derivatives.

No biological or toxicological data specifically addressing the salt of aspartame-acesulfame (E 962) were received in response to the call for data, and no relevant studies investigating E 962 were identified from the literature. Therefore, in line with previous assessments and considering that, upon ingestion, the salt of aspartame-acesulfame (E 962) dissociates into its individual components, the Panel evaluated its safety based on the available information on aspartame (E 951) and acesulfame K (E 950).

For acesulfame K (E 950), the Panel considered that the EFSA opinion published in 2025, in which an ADI of 15 mg/kg bw per day was derived, was sufficiently recent to be used in the present assessment. For aspartame (E 951), an update of the exposure and toxicological assessment of the EFSA opinion of 2013 was performed, taking into account newly available data. Both the EFSA 2025 assessment of acesulfame K and the present assessment of aspartame were performed following the structured protocols for sweeteners on hazard identification and characterisation (EFSA FAF Panel, 2023) and exposure assessment (EFSA FAF Panel, 2024a, 2024b).

Regarding ADME, aspartame is hydrolysed in the gastrointestinal tract into aspartic acid, phenylalanine and methanol, which are absorbed and enter endogenous metabolic pathways (EFSA ANS Panel, 2013). Acesulfame is almost fully absorbed, not metabolised and primarily excreted unchanged in urine (EFSA FAF Panel, 2025).

No concern for genotoxicity was identified for aspartame, including its main degradation product DKP and for acesulfame K (E 950) (EFSA FAF Panel, 2025). The Panel considered that the available data do not raise a concern for genotoxicity for the salt of aspartame-acesulfame (E 962) based on the individual assessments of the two components (aspartame and acesulfame).

The biological and toxicological data for acesulfame K and aspartame were assessed by the FAF Panel in 2025 (EFSA FAF Panel, 2025) and in the present opinion, respectively. Based on the integration of the human and animal evidence streams, along with additional considerations from the Panel on the biological plausibility of each effect or mode of action (MoA) or evidence and conclusions of the previous evaluation (EFSA ANS Panel, 2013) the Panel considered that it is unlikely that the exposure to aspartame is associated with: cancer, cardiovascular risk factors and disease, disturbances on glucose/insulin homeostasis, developmental effects concerning birthweight, adverse effects on liver, general toxicity, haematotoxicity, nephrotoxicity, neurotoxicity or with toxicity in any organ or tissue, in humans. In accordance with the matrix of integration of evidence, it was not possible to conclude whether the exposure to aspartame was associated with developmental effects concerning onset of puberty or disturbances of the immune system, in humans due to low confidence in the body of evidence (e.g. inconclusive patterns of observed changes, missing data and/or single studies). Nonetheless, the Panel considers there is no indication that would raise a concern in these respects.

Overall, for aspartame, the Panel noted that no treatment-related adverse effects were identified that would require the identification of a new reference point (RP); therefore the Panel confirmed the previously established ADI of 40 mg/kg bw per day for aspartame (E 951) (EFSA ANS Panel, 2013).

The Panel noted that the salt of aspartame-acesulfame (E 962) contributes to the overall dietary exposure to both aspartame and acesulfame and that the respective ADIs for aspartame (E 951) and acesulfame K (E 950), being 40 and 15 mg per kg body weight per day, respectively, should not be exceeded. In the present opinion, exposure to aspartame from both E 951 and E 962 was assessed, while exposure to acesulfame from E 950 and E 962 was evaluated in the EFSA opinion on acesulfame K (EFSA FAF Panel, 2025).

Dietary exposure to aspartame from the use of the salt of aspartame-acesulfame (E 962) and aspartame (E 951) was estimated according to different exposure scenarios based on consumers only as described in Section 3.4. Currently, the use of the salt of aspartame-acesulfame (E 962) and aspartame (E 951) is authorised in the EU in 34 food categories, and concentration data were available for 29 categories as described in Section 3.3.1. The Panel noted that the exposure to aspartame from the use of the salt of aspartame-acesulfame (E 962) and aspartame (E 951) as food additives according to Annex II to Regulation (EC) No. 1333/2008 was overestimated in all scenarios (see Section 3.4.2). The Panel considered the *refined brand-loyal exposure assessment scenario* the most appropriate exposure scenario for the risk assessment. In this scenario, among consumers only of at least one food category containing aspartame from the use of the salt of aspartame-acesulfame (E 962) and aspartame (E 951), mean exposure to aspartame ranged from 0.1 mg/kg bw per day in infants and adolescents to 7.7 mg/kg bw per day in infants. The 95th percentile of exposure ranged from 0.7 mg/kg bw per day in the elderly to 26.7 mg/kg bw per day in toddlers. The Panel noted that, in this scenario, none of the exposure estimates (mean and P95 in any population groups) to aspartame resulting from the use of both E 951 and E 962, exceeded the ADI of 40 mg/kg bw per day for aspartame.

The Panel considered that the highest estimate of exposure to acesulfame K (E 950) from E 950 and E 962 was generally below the established ADI of 15 mg/kg bw per day in all population groups (EFSA FAF Panel, 2025).

The ANS Panel reported that the assessment of phenylalanine plasma levels from a dose of aspartame at the ADI of 40 mg/kg bw per day was not applicable to PKU patients and that these individuals needed total control of dietary phenylalanine intake, requiring that products containing aspartame indicated on the labelling the source of phenylalanine (EFSA ANS Panel, 2013). The FAF Panel agreed with this conclusion.

4 | UNCERTAINTY

The uncertainties, and the direction of the uncertainty, related to the exposure assessments for aspartame and the salt of aspartame-acesulfame are summarised in Table 4 of Section 3.4.2.

The uncertainties concerning the animal and human studies on aspartame were addressed in the WoE assessment or explained in the respective sections above (Sections 3.5.4, 3.5.5 and 3.7).

The Panel considered that the uncertainty regarding 5-chloro-acesulfame, for which in silico QSAR analysis indicated genotoxicity alerts (EFSA FAF Panel, 2025), as a possible impurity in acesulfame K (E 950) remains applicable to the salt of aspartame-acesulfame (E 962), since the salt is produced using acesulfame K.

Overall, the uncertainties addressed were considered not to affect the conclusions on the safety of aspartame, acesulfame K and therefore on the salt of aspartame-acesulfame (E 962).

5 | CONCLUSIONS

The Panel considered that upon ingestion, the salt of aspartame-acesulfame (E 962) dissociates into aspartame and acesulfame ions which correspond to those of the authorised sweeteners aspartame (E 951) and acesulfame K (E 950). The Panel therefore assessed the safety of E 962 by separate assessments of E 950 and E 951.

For acesulfame K, the FAF Panel established an ADI of 15 mg/kg bw per day (EFSA FAF Panel, 2025). Exposure to acesulfame resulting from the use of both E 950 and E 962 was generally below the ADI in all population groups.

For aspartame, the Panel confirmed the ADI of 40 mg/kg bw per day previously established by the EFSA ANS Panel (EFSA ANS Panel, 2013). Exposure to aspartame resulting from the use of both E 951 and E 962 was below the ADI in all population groups.

The Panel concluded that there is no safety concern at the reported uses and use levels for E950, E 951 and E 962.

6 | RECOMMENDATIONS

The Panel recommended the European Commission to consider:

- revising the definition in the EU specifications to require that the salt (E 962) is prepared from aspartame and acesulfame K which should meet the specifications laid down for E 951 in Commission Regulation (EU) No. 231/2012, and for E 950 according to the proposed revision for the specifications as recommended in the Panel's re-evaluation (EFSA FAF Panel, 2025), until such revisions are formally incorporated into Commission Regulation (EU) No 231/2012.
- reviewing the limits for arsenic and lead in the EU specifications for aspartame (E 951) in line with the approach adopted for other sweeteners re-evaluated under Commission Regulation (EU) No. 257/2010.

7 | DOCUMENTATION AS PROVIDED TO EFSA

1. International Chewing Gum Association (ICGA Europe). Submission of data on use levels of salt of aspartame – acesulfame (E 962) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 02 October 2018.
2. Food Drink Europe (FDE). Submission of data on use levels of salt of aspartame - acesulfame (E 962) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 27 September 2018.
3. European Fruit Juice Association (AIJN). Submission of data on use levels of salt of aspartame - acesulfame (E 962) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 24 November 2018.
4. Specialised Nutrition Europe (SNE). Submission of data on use levels of aspartame (E 951) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 29 January 2021.
5. Food Drink Europe (FDE). Submission of data on use levels of aspartame (E 951) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 18 November 2020.
6. Chocolat Frey. Submission of data on use levels of aspartame (E 951) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 30 November 2020.
7. Food Supplement Europe (FSE). Submission of data on use levels of aspartame (E 951) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 17 November 2020.

8. European Dairy Association (EDA). Submission of data on use levels of aspartame (E 951) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 17 November 2020.
9. International Chewing Gum Association (ICGA Europe). Submission of data on use levels of aspartame (E 951) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 16 November 2020.
10. International Sweetener Association (ISA). Submission of data on use levels of aspartame (E 951) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 17 November 2020.
11. Italian Association of Confectionary and Pasta Industries (AIDEPI). Submission of data on use levels of aspartame (E 951) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 19 March 2021.
12. Active Nutrition International. Submission of data on use levels of aspartame (E 951) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 30 November 2020.
13. MOWI. Submission of data on use levels of aspartame (E 951) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 30 November 2020.
14. Submission to the call for scientific data on aspartame (E 951). Data submitted in 2011. Study report previously evaluated in the 2013 EFSA opinion (Study ID E33-34). See Annex B.
15. Submission to the call for scientific data on aspartame (E 951). Data submitted in 2011. Study report previously evaluated in the 2013 EFSA opinion (Study ID E87). See Annex B.
16. Submission to the call for scientific data on aspartame (E 951). Data submitted in 2011. Study report previously evaluated in the 2013 EFSA opinion (Study ID E75). See Annex B.
17. Submission to the call for scientific data on aspartame (E 951). Data submitted in 2011. Study report previously evaluated in the 2013 EFSA opinion (Study ID E70). See Annex B.

ABBREVIATIONS

ABC	ATP-binding cassette
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
AP	aspartylphenylalanine
AST	aspartate aminotransferase
BCRP	breast cancer resistance protein
BMI	body mass index
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
CNS	central nervous system
CRP	C-reactive protein
CVD	cardiovascular disease
DKP	5-benzyl-3,6-dioxo-2-piperazineacetic acid
EDA	European Dairy Association
FAF	EFSA Panel on Food Additives and Flavourings
FC(s)	Food Category(ies)
FDE	Food Drink Europe
FFQ	Food Frequency Questionnaire
FSE	Food Supplement Europe
GIP	glucose-dependent insulinotropic polypeptide
GLP	Good Laboratory Practice
GLP-1	glucagon-like peptide-1
GNPD	Global New Products Database
GPx	glutathione peroxidase
GR	glutathione reductase
GST	glutathione-S-transferase
HbA1c	glycated haemoglobin
HBGV	health-based guidance value
HCT	human controlled trial
HDL	high-density lipoprotein cholesterol
HOC(s)	health outcome category(ies)

HR	hazard ratio
IARC	International Agency for Research on Cancer
IBO	interested business operator
ICGA	International Chewing Gum Association
IL	interleukin
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LDL	low-density lipoprotein cholesterol
LOD	limit of detection
LOOH	lipid hydroperoxides
LOQ	limit of quantification
MoA	mode of action
MPL(s)	maximum permitted level(s)
MRP2	multidrug resistance-associated protein 2
NNS	non-nutritive sweetener
NOAEL	no observed adverse effect level
NPSH	non-protein thiols
NTP	National Toxicology Program
NTP-OHAT	National Toxicology Program Office of Health Assessment and Translation
OR	Odds ratio
PABA	para-aminobenzoic acid
P-gp	P-glycoprotein
PKU	phenylketonuria
PNS	peripheral nervous system
QS	quantum satis
RefID	reference identifier
RoB	risk of bias
RR	relative risk
SCF	Scientific Committee on Food
SEM	Standard error of the mean
SNE	Specialised Nutrition Europe
SOD	superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TMINC	Taipei Maternal–Infant Nutrition Cohort
TNF-α	tumour necrosis factor alpha
TPLS	Taichung Pu-Lun Study
TRAP	total reactive antioxidant potential
UDS	unscheduled DNA synthesis
UV–Vis	ultraviolet–visible spectroscopy
WoE	weight of evidence

ACKNOWLEDGEMENTS

The Panel wishes to thank the following for the support provided to this scientific output: Simona Aprile, Gabriele Gagliardi, Joanne Gartlon, Christina Kirkou, Antonio Rivas Cornejo, Lieve Herman, Pauline Le Galle and Concepcion Medrano-Padial. The Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2011-00727

PANEL MEMBERS

Laurence Castle, Monica Andreassen, Gabriele Aquilina, Maria Lourdes Bastos, Polly Boon, Biagio Fallico, Rex FitzGerald, Maria Jose Frutos Fernandez, Bettina Grasl-Kraupp, Ursula Gundert-Remy, Rainer Gürtler, Eric Houdeau, Marcin Kurek, Henriqueta Louro, Patricia Morales, and Sabina Passamonti.

MAP DISCLAIMER

The designations employed and the presentation of material on any maps included in this scientific output do not imply the expression of any opinion whatsoever on the part of the European Food Safety Authority concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

REFERENCES

Abhilash, M., Varghese, M. V., Paul, M. V. S., Alex, M., & Nair, R. H. (2014). Effect of long-term intake of aspartame on serum biochemical parameters and erythrocyte oxidative stress biomarkers in rats. *Comparative Clinical Pathology*, *24*(4), 927–933. https://doi.org/10.1007/s00580-014-2013-8

AERU (Agriculture and Environment Research Unit, University of Hertfordshire), Lewis, K. A., & Tzilivakis, J. (2021). Review and synthesis of data on the potential environmental impact of artificial sweeteners. *EFSA Supporting Publications*, *18*(10), EN-6918. https://doi.org/10.2903/sp.efsa.2021.EN-6918

Ahmad, S. Y., Friel, J., & MacKay, D. S. (2020). The effect of the artificial sweeteners on glucose metabolism in healthy adults: A randomized double-blinded crossover clinical trial. *Applied Physiology Nutrition and Metabolism*, *45*(6), 606–612. https://doi.org/10.1139/apnm-2019-0359

Ayoub-Charette, S., McGlynn, N. D., Lee, D., Khan, T. A., Blanco Mejia, S., Chiavaroli, L., Kavanagh, M. E., Seider, M., Taibi, A., Chen, C. T., & Sievenpiper, J. L. (2023). Rationale, design and participants baseline characteristics of a crossover randomized controlled trial of the effect of replacing SSBs with NSBs versus water on glucose tolerance, gut microbiome and cardiometabolic risk in overweight or obese adult SSB consumer: Strategies to oppose SUGARS with non-nutritive sweeteners or water (STOP Sugars NOW) trial and ectopic fat sub-study. *Nutrients*, *15*(5), 1238. https://doi.org/10.3390/nu15051238

Basílio, M., Silva, L. J. G., Pereira, A. M. P. T., Pena, A., & Lino, C. M. (2020). Artificial sweeteners in non-alcoholic beverages: Occurrence and exposure estimation of the Portuguese population. *Food Additives & Contaminants: Part A*, *37*(12), 2040–2050. https://doi.org/10.1080/19440049.2020.1812734

Berset, J.-D., & Ochsenbein, N. (2012). Stability considerations of aspartame in the direct analysis ofartificial sweeteners in water samples using high-performance liquid chromatography–tandem massspectrometry (HPLC–MS/MS). *Chemosphere*, *88*(5), 563–569. https://doi.org/10.1016/j.chemosphere.2012.03.030

Bryant, C. E., Wasse, L. K., Astbury, N., Nandra, G., & McLaughlin, J. T. (2014). Non-nutritive sweeteners: no class effect on the glycaemic or appetite responses to ingested glucose. *European Journal of Clinical Nutrition*, *68*(5), 629–631. https://doi.org/10.1038/ejcn.2014.19

Buffini, M., Goscinny, S., Van Loco, J., Nugent, A. P., Walton, J., Flynn, A., Gibney, M. J., & McNulty, B. A. (2018). Dietary intakes of six intense sweeteners by Irish adults. *Food Additives & Contaminants: Part A*, *35*(3), 425–438. https://doi.org/10.1080/19440049.2017.1411619

Carvalho, C., Correia, D., Severo, M., Magalhães, V., Casal, S., Ramos, E., Lopes, C., & Torres, D. (2022). Dietary exposure to artificial sweeteners and associated factors in the Portuguese population. *Food Additives & Contaminants: Part A*. https://doi.org/10.1080/19440049.2022.2075039

Chazelas, E., Druesne-Pecollo, N., Esseddik, Y., Szabo de Edelenyi, F., Agaësse, C., De Sa, A., Lutchia, R., Rebouillat, P., Srour, B., Debras, C., Wendeu-Foyet, G., Huybrechts, I., Pierre, F., Coumoul, X., Julia, C., Kesse-Guyot, E., Allès, B., Galan, P., Hercberg, S., … Touvier, M. (2021). Exposure to food additive mixtures in 106,000 French adults from the NutriNet-santé cohort. *Scientific Reports*, *11*, 19680. https://doi.org/10.1038/s41598-021-98496-6

Chen, T., Zeng, Q., Cao, M., Zhang, L., Adyari, B., Ma, C., Wang, K., Gao, D., Hu, A., Sun, Q., & Yu, C.-P. (2024). Fate of contaminants of emerging concern in two wastewater treatment plants after retrofitting tertiary treatment for reduction of nitrogen discharge. *Environmental Research*, *249*, Article 118344. https://doi.org/10.1016/j.envres.2024.118344

Chen, Y. C., Wang, Z. L., Lin, Y. F., Lin, C. Y., Hsu, S. Y., Nacis, J. S., & Hsieh, R. H. (2025). Aspartame consumption linked to delayed puberty and mitochondrial dysfunction: Evidence from human and animal studies. *The Journal of Nutritional Biochemistry*, *140*, 109889. https://doi.org/10.1016/j.jnutbio.2025.109889

Chen, Y. C., Yeh, Y. C., Lin, Y. F., Hsu, S. Y., Nacis, J. S., Hsu, J. W., & Hsieh, R. H. (2024). Aspartame intake during pregnancy induces placental dysfunction through impaired mitochondrial function and biogenesis modulation. *Placenta*, *158*, 285–292. https://doi.org/10.1016/j.placenta.2024.11.003

Chien, Y. H., Lin, C. Y., Hsu, S. Y., Chen, Y. H., Wu, H. T., Huang, S. W., & Chen, Y. C. (2023). Effects of nonnutritive sweeteners on body composition changes during pubertal growth. *Nutrients*, *15*(10). https://doi.org/10.3390/nu15102319

Choudhary, A. K., & Sheela Devi, R. (2015). Longer period of oral administration of aspartame on cytokine response in Wistar albino rats. *Endocrinología y Nutrición (English Edition)*, *62*(3), 114–122. https://doi.org/10.1016/j.endoen.2015.02.010

Collison, K. S., Inglis, A., Shibin, S., Andres, B., Ubungen, R., Thiam, J., Mata, P., & Al-Mohanna, F. A. (2016). Differential effects of early-life NMDA receptor antagonism on aspartame-impaired insulin tolerance and behavior. *Physiology & Behavior*, *167*, 209–221. https://doi.org/10.1016/j.physbeh.2016.09.011

de Dios, R., Proctor, C. R., Maslova, E., Dzalbe, S., Rudolph, C. J., & McCarthy, R. R. (2023). Artificial sweeteners inhibit multidrug-resistant pathogen growth and potentiate antibiotic activity. *EMBO Molecular Medicine*, *15*(1), e16397. https://doi.org/10.15252/emmm.202216397

de Souza Lopes, A., Elisabete Costa Antunes, A., Idelça Aires Machado, K., & da Silva, M. C. (2023). The impact of antimicrobial food additives and sweeteners on the growth and metabolite production of gut bacteria. *Folia Microbiologica*, *68*(5), 813–821. https://doi.org/10.1007/s12223-023-01076-6

De Winter, L., Casteels, K., Corthouts, K., Van de Kerckhove, K., Van der Vaerent, K., Vanmeerbeeck, K., & Matthys, C. (2015). Dietary intake of non-nutritive sweeteners in children with type 1 diabetes mellitus. *Food Additives & Contaminants: Part A*, *33*(1), 19–26. https://doi.org/10.1080/19440049.2015.1112039

Debras, C., Chazelas, E., Sellem, L., Porcher, R., Druesne-Pecollo, N., Esseddik, Y., de Edelenyi, F. S., Agaesse, C., De Sa, A., Lutchia, R., Fezeu, L. K., Julia, C., Kesse-Guyot, E., Alles, B., Galan, P., Hercberg, S., Deschasaux-Tanguy, M., Huybrechts, I., Srour, B., & Touvier, M. (2022). Artificial sweeteners and risk of cardiovascular diseases: Results from the prospective NutriNet-Sante cohort. *BMJ*, *378*, e071204. https://doi.org/10.1136/bmj-2022-071204

Debras, C., Chazelas, E., Srour, B., Druesne-Pecollo, N., Esseddik, Y., Szabo de Edelenyi, F., Agaesse, C., De Sa, A., Lutchia, R., Gigandet, S., Huybrechts, I., Julia, C., Kesse-Guyot, E., Alles, B., Andreeva, V. A., Galan, P., Hercberg, S., Deschasaux-Tanguy, M., & Touvier, M. (2022). Artificial sweeteners and cancer risk: Results from the NutriNet-Sante population-based cohort study. *PLoS Medicine*, *19*(3), e1003950. https://doi.org/10.1371/journal.pmed.1003950

Debras, C., Deschasaux-Tanguy, M., Chazelas, E., Sellem, L., Druesne-Pecollo, N., Esseddik, Y., Szabo de Edelenyi, F., Agaesse, C., De Sa, A., Lutchia, R., Julia, C., Kesse-Guyot, E., Alles, B., Galan, P., Hercberg, S., Huybrechts, I., Cosson, E., Tatulashvili, S., Srour, B., & Touvier, M. (2023). Artificial sweeteners and risk of type 2 diabetes in the prospective NutriNet-Sante cohort. *Diabetes Care*, *46*(9), 1681–1690. https://doi.org/10.2337/dc23-0206

Diogo, J. S. G., Silva, L. S. O., Pena, A., & Lino, C. M. (2013). Risk assessment of additives through soft drinks and nectars consumption on Portuguese population: A 2010 survey. *Food and Chemical Toxicology*, *62*, 548–553. https://doi.org/10.1016/j.fct.2013.09.006

Duran Agüero, S., Oñate, G., & Haro Rivera, P. (2014). Consumption of non-nutritive sweeteners and nutritional status in 10-16 year old students. *Arch Argent Pediatr*, *112*, 207–214. https://doi.org/10.5546/aap.2014.eng.207

Ediga, M. G., Annapureddy, S., Salikineedy, K., & Nimgampalle, M. (2023). Aspartame consumption causes cognitive impairment in streptozotocin-induced diabetic Wistar rats. *Biologia*, *78*(9), 2393–2407. https://doi.org/10.1007/s11756-023-01363-0

EFSA (European Food Safety Authority). (2006). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to a new long-term carcinogenicity study on aspartame. *EFSA Journal*, *4*(5), 356. https://doi.org/10.2903/j.efsa.2006.356

EFSA (European Food Safety Authority). (2007). Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. *EFSA Journal*, *5*(1), 438. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority). (2009a). Opinion on a request from the European Commission related to the 2nd ERF carcinogenicity study on aspartame. *EFSA Journal*, *7*(4), 945. https://doi.org/10.2903/j.efsa.2009.945

EFSA (European Food Safety Authority). (2009b). Updated opinion on a request from the European Commission related to the 2nd ERF carcinogenicity study on aspartame, taking into consideration study data submitted by the Ramazzini Foundation in February 2009. *EFSA Journal*, *7*(4), 1015. https://doi.org/10.2903/j.efsa.2009.1015

EFSA (European Food Safety Authority). (2011). EFSA statement on the scientific evaluation of two studies related to the safety of artificial sweeteners. *EFSA Journal*, *9*(2), 2089. https://doi.org/10.2903/j.efsa.2011.2089

EFSA (European Food Safety Authority). (2015a). Principles and process for dealing with data and evidence in scientific assessments. *EFSA Journal*, *13*(5), 4121. https://doi.org/10.2903/j.efsa.2015.4121

EFSA (European Food Safety Authority). (2015b). The food classification and description system FoodEx2 (revision 2). *EFSA Supporting Publications*, EN-804. https://doi.org/10.2903/sp.efsa.2015.EN-804

EFSA (European Food Safety Authority). (2020a). Outcome of the public consultation on a draft protocol for the assessment of hazard identification and characterisation of sweeteners. *EFSA Supporting Publications*, *17*(2), EN-1803. https://doi.org/10.2903/sp.efsa.2020.EN-1803

EFSA (European Food Safety Authority). (2020b). Outcome of the public consultation on a draft protocol for assessing exposure to sweeteners as part of their safety assessment under the food additives re-evaluation programme. *EFSA Supporting Publications*, *17*(8), EN-1913. https://doi.org/10.2903/sp.efsa.2020.EN-1913

EFSA (European Food Safety Authority). (2023). Harmonised approach for reporting reliability and relevance of genotoxicity studies. *EFSA Supporting Publications*, *20*(9), EN-8270. https://doi.org/10.2903/sp.efsa.2023.EN-8270

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources). (2011). Statement on two recent scientific articles on the safety of artificial sweeteners. *EFSA Journal*, *9*(2), 1996. https://doi.org/10.2903/j.efsa.2011.1996

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). (2013). Scientific opinion on the re-evaluation of aspartame (E 951) as a food additive. *EFSA Journal*, *11*(12), 3496. https://doi.org/10.2903/j.efsa.2013.3496

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings). (2023). Revised protocol on Hazard identification and characterisation of sweeteners. *EFSA Journal*. https://doi.org/10.5281/ZENODO.7788969

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Arcella, D., Boon, P., Cascio, C., Castle, L., Dujardin, B., Gergelova, P., Horvath, Z., Leblanc, J.-C., Lindtner, O., Riolo, F., Shah, R., & Tard, A. (2024a). Revised protocol for assessing exposure to sweeteners as part of their safety assessment under the food additives re-evaluation programme. *Zenodo* https://zenodo.org/records/13912094

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., Batke, M., Bruzell, E., … Younes, M. (2024b). Re-evaluation of saccharin and its sodium, potassium and calcium salts (E 954) as food additives. *EFSA Journal*, *22*(11), e9044. https://doi.org/10.2903/j.efsa.2024.9044

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., Batke, M., Bruzell, E., … Lodi, F. (2025). Re-evaluation of acesulfame K (E 950) as food additive. *EFSA Journal*, *23*(4), e9317. https://doi.org/10.2903/j.efsa.2025.9317

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., Barmaz, S., Carfì, M., … Zakidou, P. (2026a). Guidance on the scientific data requirements for an application for authorisation of a food additive submitted under regulation (EC) No 1331/2008. *EFSA Journal*, *24*(1), e9778. https://doi.org/10.2903/j.efsa.2026.9778

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., Fernandez, M. J. F., Grasl-Kraupp, B., Gundert-Remy, U., Gürtler, R., Houdeau, E., Kurek, M., Louro, H., Morales, P., Passamonti, S., Batke, M., Douros, A., … Lodi, F. (2026b). Re-evaluation of sucralose (E 955) as a food additive and evaluation of a new application on extension of use of sucralose (E 955) in fine bakery wares. *EFSA Journal*, *24*(2), e9854. https://doi.org/10.2903/j.efsa.2026.9854

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. (2012). Guidance on selected default values to be used by the EFSA scientific committee, scientific panels and units in the absence of actual measured data. *EFSA Journal*, *10*(3), 2579. https://doi.org/10.2903/j.efsa.2012.2579

Finamor, I. A., Ourique, G. M., Pes, T. S., Saccol, E. M., Bressan, C. A., Scheid, T., Baldisserotto, B., Llesuy, S. F., Partata, W. A., & Pavanato, M. A. (2014). The protective effect of N-acetylcysteine on oxidative stress in the brain caused by the long-term intake of aspartame by rats. *Neurochemical Research*, *39*(9), 1681–1690. https://doi.org/10.1007/s11064-014-1360-9

Finley, A. J., Tang, D., & Schmeichel, B. J. (2019). Sweet nothings. *Social Psychology*, *50*(5–6), 322–331. https://doi.org/10.1027/1864-9335/a000376

Frankenfeld, C. L., Sikaroodi, M., Lamb, E., Shoemaker, S., & Gillevet, P. M. (2015). High-intensity sweetener consumption and gut microbiome content and predicted gene function in a cross-sectional study of adults in the United States. *Annals of Epidemiology*, *25*(10), 736–742.e4. https://doi.org/10.1016/j.annepidem.2015.06.083

Fry, J. C., Meyers, B. I., & Mayhew, D. A. (2012). Aspartame-acesulfame. In L. O'Brien Nabors (Ed.), *Alternative sweeteners* (4th ed., pp. 77–91). CRC Press. https://doi.org/10.1201/b11242

Fulgoni, V. L., 3rd, & Drewnowski, A. (2022). No association between low-calorie sweetener (LCS) use and overall cancer risk in the nationally representative database in the US: Analyses of NHANES 1988-2018 data and 2019 public-use linked mortality files. *Nutrients*, *14*(23). https://doi.org/10.3390/nu14234957

Gatidou, G., Vazaiou, N., Thomaidis, N. S., & Stasinakis, A. S. (2020). Biodegradability assessment of food additives using OECD 301F respirometric test. *Chemosphere*, *241*, 125071. https://doi.org/10.1016/j.chemosphere.2019.125071

Guo, W., Li, J., Liu, Q., Shi, J., & Gao, Y. (2021). Tracking the fate of artificial sweeteners within the coastal waters of Shenzhen city, China: From wastewater treatment plants to sea. *Journal of Hazardous Materials*, *414*, 125498. https://doi.org/10.1016/j.jhazmat.2021.125498

Hess, E. L., Myers, E. A., Swithers, S. E., & Hedrick, V. E. (2018). Associations between nonnutritive sweetener intake and metabolic syndrome in adults. *Journal of the American College of Nutrition*, *37*(6), 487–493. https://doi.org/10.1080/07315724.2018.1440658

Higgins, K. A., Considine, R. V., & Mattes, R. D. (2018). Aspartame consumption for 12 weeks does not affect Glycemia, appetite, or body weight of healthy, lean adults in a randomized controlled trial. *The Journal of Nutrition*, *148*(4), 650–657. https://doi.org/10.1093/jn/nxy021

Higgins, K. A., & Mattes, R. D. (2019). A randomized controlled trial contrasting the effects of 4 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. *The American Journal of Clinical Nutrition*, *109*(5), 1288–1301. https://doi.org/10.1093/ajcn/nqy381

Hosseini, A., Barlow, G. M., Leite, G., Rashid, M., Parodi, G., Wang, J., Morales, W., Weitsman, S., Rezaie, A., Pimentel, M., & Mathur, R. (2023). Consuming artificial sweeteners may alter the structure and function of duodenal microbial communities. *iScience*, *26*(12), 108530. https://doi.org/10.1016/j.isci.2023.108530

IARC (IARC Working Group on the Identification of Carcinogenic Hazards to Humans). (2024). *Aspartame, methyleugenol, and isoeugenol (IARC monographs on the identification of carcinogenic hazards to humans, No. 134)*. International Agency for Research on Cancer. https://publications.iarc.who.int/627

International Agency for Research on Cancer (IARC). (2023). Summary of findings of the evaluation of aspartame at the IARC Monographs Programme's 134th meeting. https://www.iarc.who.int/wp-content/uploads/2023/07/Summary_of_findings_Aspartame.pdf

Ishii, H. (1981). Incidence of brain tumors in rats fed aspartame. *Toxicology Letters*, *7*, 433–437. https://doi.org/10.1016/0378-4274(81)90089-8

Ishii, H., Koshimizu, T., Usami, S., & Fujimoto, T. (1981). Toxicity of aspartame and its diketopiperazinefor Wistar rats by dietary administration for 104 weeks. *Toxicology*, *21*, 91–94. https://doi.org/10.1016/0300-483x(81)90119-0

Jahani, R., Dhib, R., & Mehrvar, M. (2020). Photochemical degradation of aqueous artificial sweeteners by UV/H2O2 and their biodegradability studies. *Journal of Chemical Technology & Biotechnology*, *95*(9), 2509–2521. https://doi.org/10.1002/jctb.6432

Janvier, S., Goscinny, S., Le Donne, C., & Van Loco, J. (2015). Low-calorie sweeteners in food and food supplements on the Italian market. *Food Additives & Contaminants: Part B*, *8*(4), 298–308. https://doi.org/10.1080/19393210.2015.1094829

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (1975). *Aspartame (WHO food additives series, No. 15)*. World Health Organization. https://www.inchem.org/documents/jecfa/jecmono/v15je03.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (1980). Toxicological evaluation of certain food additives (WHO Food Additives Series No. 15). https://inchem.org/documents/jecfa/jecmono/v15je01.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (1981). *Aspartame (WHO food additives series, No. 16)*. World Health Organization. https://www.inchem.org/documents/jecfa/jecmono/v16je03.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (1983). *Evaluation of certain food additives and contaminants. Twenty-seventh report of the Join FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 696* https://iris.who.int/bitstream/handle/10665/39165/WHO_TRS_696.pdf?sequence=1

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (1991). Evaluation of certain food additives and contaminants. Thirty-seventh report of the Join FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 806. https://iris.who.int/bitstream/handle/10665/40288/WHO_TRS_806.pdf?sequence=1.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2000). Evaluation of certain food additives and contaminants: Fifty-fifth report of the joint FAO/WHO expert committee on food additives. WHO Technical Report Series, 901, 107. https://iris.who.int/server/api/core/bitstreams/9bd6e609-0990-47cb-8357-997c75d65fe5/content

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2005). *Aspartame-acesulfame salt. In combined compendium of food additive specifications (FAO food and nutrition paper 52, addendum 13)*. Food and Agriculture Organization of the United Nations. https://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-047.pdf

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2023). *Evaluation of certain food additives: Ninety-sixth report of the joint FAO/WHO expert committee on food additives (WHO technical report series, No. 1050)*. World Health Organization. https://www.who.int/publications/i/item/9789240083059

Jones, S. K., McCarthy, D. M., Stanwood, G. D., Schatschneider, C., & Bhide, P. G. (2023). Learning and memory deficits produced by aspartame are heritable via the paternal lineage. *Scientific Reports*, *13*(1), 14326. https://doi.org/10.1038/s41598-023-41213-2

Jones, S. K., McCarthy, D. M., Vied, C., Stanwood, G. D., Schatschneider, C., & Bhide, P. G. (2022). Transgenerational transmission of aspartame-induced anxiety and changes in glutamate-GABA signaling and gene expression in the amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(49), e2213120119. https://doi.org/10.1073/pnas.2213120119

Kashima, H., Taniyama, K., Sugimura, K., Endo, M. Y., Kobayashi, T., & Fukuba, Y. (2019). Suppression of sweet sensing with glucose, but not aspartame, delays gastric emptying and glycemic response. *Nutrition Research*, *68*, 62–69. https://doi.org/10.1016/j.nutres.2019.06.005

Kim, J. Y., Seo, J., & Cho, K. H. (2011). Aspartame-fed zebrafish exhibit acute deaths with swimming defectsand saccharin-fed zebrafish have elevation of cholesteryl ester transfer protein activity inhypercholesterolemia. *Food and Chemical Toxicology*, *49*(11), 2899–2905. https://doi.org/10.1016/j.fct.2011.08.001

Knezovic, Z., Jurcevic Zidar, B., Pribisalic, A., Luetic, S., Jurcic, K., Knezovic, N., & Sutlovic, D. (2025). Artificial sweeteners in food products: Concentration analysis, label practices, and cumulative intake assessment in Croatia. *Nutrients*, *17*(7), 1110. https://doi.org/10.3390/nu17071110

Kobeticova, K., Mocova, K. A., Mrhalkova, L., Frycova, Z., & Koci, V. (2016). Artificial sweeteners and the environment. *Czech Journal of Food Sciences*, *34*(2), 149–153. https://www.webofscience.com/wos/woscc/full-record/WOS:000375411400008

Kobeticova, K., Mocova, K. A., Mrhalkova, L., & Petrova, G. (2018). Effects of artificial sweeteners on Lemna minor. *Czech Journal of Food Sciences*, *36*(5), 386–391. https://www.webofscience.com/wos/woscc/full-record/WOS:000451238600005

Kostic, M. A., & Dart, R. C. (2003). Rethinking the toxic methanol level. *Journal of Toxicology. Clinical Toxicology*, *41*(6), 793–800. https://doi.org/10.1081/clt-120025344

Kuk, J. L., & Brown, R. E. (2016). Aspartame intake is associated with greater glucose intolerance in individuals with obesity. *Applied Physiology, Nutrition, and Metabolism*, *41*(7), 795–798. https://doi.org/10.1139/apnm-2015-0675

Le Donne, C., Mistura, L., Goscinny, S., Janvier, S., Cuypers, K., D'Addezio, L., Sette, S., Catasta, G., Ferrari, M., Piccinelli, R., Van Loco, J., & Turrini, A. (2017). Assessment of dietary intake of 10 intense sweeteners by the Italian population. *Food and Chemical Toxicology*, *102*, 186–197. https://doi.org/10.1016/j.fct.2017.02.014

Lee, W., & Wang, Y. C. (2015). Assessing developmental toxicity of caffeine and sweeteners in medaka (Oryzias latipes). *Springerplus*, *4*, 486. https://doi.org/10.1186/s40064-015-1284-0

Lin, C.-Y., Nguyen, N. N., Tsai, W.-L., Hsieh, R.-H., Wu, H.-T., & Chen, Y.-C. (2024). Aspartame intake delayed puberty onset in female offspring rats and girls. *Molecular Nutrition & Food Research*, *68*(5), 2300270. https://doi.org/10.1002/mnfr.202300270

Loayza, J. J. J., Kang, S., Schooth, L., Teh, J. J., de Klerk, A., Noon, E. K., & Morrison, M. (2023). Effect of food additives on key bacterial taxa and the mucosa-associated microbiota in Crohn's disease: The ENIGMA study. *Gut Microbes*, *15*(1), 2172670. https://doi.org/10.1080/19490976.2023.2172670

Ma, H., Deng, J., Liu, J., Jin, X., & Yang, J. (2024). Daytime aspartame intake results in larger influences on body weight, serum corticosterone level, serum/cerebral cytokines levels and depressive-like behaviors in mice than nighttime intake. *Neurotoxicology*, *102*, 37–47. https://doi.org/10.1016/j.neuro.2024.03.004

Mahmud, R., Shehreen, S., Shahriar, S., Rahman, M. S., Akhteruzzaman, S., & Sajib, A. A. (2019). Non-caloric artificial sweeteners modulate the expression of key metabolic genes in the omnipresent gut microbe *Escherichia coli*. *Journal of Molecular Microbiology and Biotechnology*, *29*(1–6), 43–56. https://doi.org/10.1159/000504511

Mancini, F. R., Paul, D., Gauvreau, J., Volatier, J. L., Vin, K., & Hulin, M. (2015). Dietary exposure to benzoates (E210–E213), parabens (E214–E219), nitrites (E249–E250), nitrates (E251–E252), BHA (E320), BHT (E321) and aspartame (E951) in children less than 3 years old in France. *Food Additives & Contaminants: Part A*, *32*(3), 293–306. https://doi.org/10.1080/19440049.2015.1007535

Martyn, D. M., Nugent, A. P., McNulty, B. A., O'Reilly, E., Tlustos, C., Walton, J., Flynn, A., & Gibney, M. J. (2016). Dietary intake of four artificial sweeteners by Irish pre-school children. *Food Additives & Contaminants: Part A*, *33*(4), 592–602. https://doi.org/10.1080/19440049.2016.1152880

McCullough, M. L., Teras, L. R., Shah, R., Diver, W. R., Gaudet, M. M., & Gapstur, S. M. (2014). Artificially and sugar-sweetened carbonated beverage consumption is not associated with risk of lymphoid neoplasms in older men and women. *The Journal of Nutrition*, *144*(12), 2041–2049. https://doi.org/10.3945/jn.114.197475

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & The PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta analyses: The PRISMA statement. *PLoS Medicine*, *6*(6), e1000097. https://doi.org/10.1371/journal.pmed1000097

Montes, R., Rodil, R., Cela, R., & Quintana, J. B. (2019). Determination of Persistent and Mobile Organic Contaminants (PMOCs) in water by mixed-mode liquid chromatography–tandem mass spectrometry. *Analytical Chemistry*, *91*(8), 5176–5183. https://doi.org/10.1021/acs.analchem.8b05792

Mueller, N. T., Jacobs, D. R., Jr., MacLehose, R. F., Demerath, E. W., Kelly, S. P., Dreyfus, J. G., & Pereira, M. A. (2015). Consumption of caffeinated and artificially sweetened soft drinks is associated with risk of early menarche. *The American Journal of Clinical Nutrition*, *102*(3), 648–654. https://doi.org/10.3945/ajcn.114.100958

NTP-OHAT (National Toxicology Program, Office of Health Assessment and Translation). (2019). *Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration*. National Institute of Environmental Health Sciences. https://ntp.niehs.nih.gov/research/assessments/noncancer/handbook

Nykyforov, V., Novokhatko, O., Digtiar, S., Sakun, O., & Tikhonova, A. (2022). Toxicological assessment of food additives by bioassay. *Journal of Food Science and Technology-Ukraine*, *16*(2), 87–93. https://www.webofscience.com/wos/woscc/full-record/WOS:000930840200011

Onaolapo, A. Y., Abdusalam, S. Z., & Onaolapo, O. J. (2017). Silymarin attenuates aspartame-induced variation in mouse behaviour, cerebrocortical morphology and oxidative stress markers. *Pathophysiology*, *24*(2), 51–62. https://doi.org/10.1016/j.pathophys.2017.01.002

Onaolapo, A. Y., Onaolapo, O. J., & Nwoha, P. U. (2016). Alterations in behaviour, cerebral cortical morphology and cerebral oxidative stress markers following aspartame ingestion. *Journal of Chemical Neuroanatomy*, *78*, 42–56. https://doi.org/10.1016/j.jchemneu.2016.08.006

Onaolapo, A. Y., Onaolapo, O. J., & Nwoha, P. U. (2017). Methyl aspartylphenylalanine, the pons and cerebellum in mice: An evaluation of motor, morphological, biochemical, immunohistochemical and apoptotic effects. *Journal of Chemical Neuroanatomy*, *86*, 67–77. https://doi.org/10.1016/j.jchemneu.2017.09.001

Örkü, S. E., Süyen, G., & Baş, M. (2023). The effect of regular consumption of four low- or no-calorie sweeteners on glycemic response in healthy women: A randomized controlled trial. *Nutrition*, *106*, 111885. https://doi.org/10.1016/j.nut.2022.111885

Oroian, M., Amariei, S., Leahu, A., & Gutt, G. (2013). Acesulfame K, aspartame and saccharin occurrence in carbonated drinks from the Romanian market. In *Recent researches in medicine, biology and bioscience* (pp. 12–15). WSEAS Press. https://www.wseas.us/e-library/conferences/2013/Chania/BIOMED/BIOMED-02.pdf

Otabe, A., Ohta, F., Takumi, A., & Lynch, B. (2019). Mutagenicity and genotoxicity studies of aspartame. *Regulatory Toxicology and Pharmacology*, *103*, 345–351. https://doi.org/10.1016/j.yrtph.2018.01.023

Palomar-Cros, A., Straif, K., Romaguera, D., Aragonés, N., Castaño-Vinyals, G., Martin, V., Moreno, V., Gómez-Acebo, I., Guevara, M., Aizpurua, A., Molina-Barceló, A., Jiménez-Moleón, J.-J., Tardón, A., Contreras-Llanes, M., Marcos-Gragera, R., Huerta, J. M., Pérez-Gómez, B., Espinosa, A., Hernández-Segura, N., … Lassale, C. (2023). Consumption of aspartame and other artificial sweeteners and risk of cancer in the Spanish multicase–control study (MCC-Spain). *International Journal of Cancer*, *153*(5), 979–993. https://doi.org/10.1002/ijc.34577

Pearson, R. C., Green, E. S., Olenick, A. A., & Jenkins, N. T. (2021). Comparison of aspartame- and sugar-sweetened soft drinks on postprandial metabolism. *Nutrition and Health*, *29*(1), 115–128. https://doi.org/10.1177/02601060211057415

Pradeep, P., Judson, R., DeMarini, D., Keshava, N., Martin, T., Dean, J., Gibbons, C., Simha, A., Warren, S., Gwinn, M., & Patlewicz, G. (2021). Evaluation of existing QSAR models and structural alerts and development of new ensemble models for Genotoxicity using a newly compiled experimental dataset. *Computational Toxicology*, *18*(10). https://doi.org/10.1016/j.comtox.2021.100167

Rathaus, M., Azem, L., Livne, R., Ron, S., Ron, I., Hadar, R., Efroni, G., Amir, A., Braun, T., Haberman, Y., & Tirosh, A. (2024). Long-term metabolic effects of non-nutritive sweeteners. *Molecular Metabolism*, *88*, 101985. https://doi.org/10.1016/j.molmet.2024.101985

Recoules, C., Touvier, M., Pierre, F., & Audebert, M. (2025). Evaluation of the toxic effects of food additives, alone or in mixture, in four human cell models. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, *196*, 115198. https://doi.org/10.1016/j.fct.2024.115198

Romanos-Nanclares, A., Schernhammer, E., Willett, W. C., Holmes, M. D., Chen, W. Y., & Eliassen, A. H. (2025). Consumption of aspartame and risk of breast cancer in the Nurses' Health Studies. *JNCI: Journal of the National Cancer Institute*, *117*(4), 795–800. https://doi.org/10.1093/jnci/djae259

Sasaki, Y. F., Kawaguchi, S., Kamaya, A., Ohshita, M., Kabasawa, K., Iwama, K., Taniguchi, K., & Tsuda, S. (2002). The comet assay with 8 mouse organs: Results with 39 currently used food additives. *Mutation Research*, *519*, 103–119. https://doi.org/10.1016/s1383-5718(02)00128-6

SCF (Scientific Committee for Food). (1985a). Report of the Scientific Committee for Food on aspartame. Report of the Scientific Committee for Food Sixteenth series. https://aei.pitt.edu/40825/1/16th_food.pdf

SCF (Scientific Committee for Food). (1985b). Report of the Scientific Committee for Food on acesulfame potassium. Report of the Scientific Committee for Food Sixteenth series. https://food.ec.europa.eu/document/download/8a8fb992-235b-4654-98d3-e4b4d66d3077_en?filename=sci-com_scf_reports_16.Pdf

SCF (Scientific Committee for Food). (1989). Report of the Scientific Committee for Food on aspartame. Report of the Scientific Committee for Twenty-first series. https://aei.pitt.edu/40830/1/21st_food.pdf

SCF (Scientific Committee for Food). (1997). Minutes of the 107th Meeting of the Scientific Committee for Food held on 12–13 June 1997 in Brussels. Section 12.5. https://ec.europa.eu/food/fs/sc/oldcomm7/out13_en.html

SCF (Scientific Committee for Food). (2000a). Minutes of the 120 th Meeting of the Scientific Committee on Food held on 8–9 March 2000 in Brussels. Section 9.4. https://ec.europa.eu/food/fs/sc/scf/out57_en.html

SCF (Scientific Committee for Food). (2000b). Opinion Re-evaluation of acesulfame K with reference to the previous SCF opinion of 1991. https://ec.europa.eu/food/fs/sc/scf/out52_en.pdf

SCF (Scientific Committee for Food). (2002). Opinion of the Scientific Committee on Food Update on the Safety of Aspartame. https://ec.europa.eu/food/fs/sc/scf/out155_en.pdf

SCF (Scientific Committee on Food). (2001). *Guidance on submissions for food additive evaluations by the scientific committee on food*. European Commission. https://ec.europa.eu/food/fs/sc/scf/out98_en.pdf

Shen, G., Lei, S., Li, H., Yu, Q., Wu, G., Shi, Y., Xu, K., Ren, H., & Geng, J. (2023). Occurrence and removal of four artificial sweeteners in wastewater treatment plants of China. *Environmental Science: Processes & Impacts*, *25*(1), 75–84. https://doi.org/10.1039/D2EM00351A

Shibui, Y., Fujitani, S., Iwata, H., Lynch, B., & Roberts, A. (2019). Histological analyses of the Ishii (1981) rat carcinogenicity study of aspartame and comparison with the Ramazzini institute studies. *Regulatory Toxicology and Pharmacology*, *102*, 23–29. https://doi.org/10.1016/j.yrtph.2018.12.010

Silva, P. D., Cruz, R., & Casal, S. (2021). Sugars and artificial sweeteners in soft drinks: A decade of evolution in Portugal. *Food Control*, *120*, 107481. https://doi.org/10.1016/j.foodcont.2020.107481

Sjostedt, N., Deng, F., Rauvala, O., Tepponen, T., & Kidron, H. (2017). Interaction of food additives with intestinal efflux transporters. *Molecular Pharmaceutics*, *14*(11), 3824–3833. https://doi.org/10.1021/acs.molpharmaceut.7b00563

Sprong, C., Vrijenhoek, N., te Biesebeek, J. D., Niekerk, M., Koopman, N., Sam, M., & Milder, I. (2025). Refined intake assessment of seven sweeteners via foods and beverages by the Dutch population using branded data. *Food Additives & Contaminants: Part A*, *42*(5), 539–561. https://doi.org/10.1080/19440049.2025.2479865

Steffen, B. T., Jacobs, D. R., Yi, S. Y., et al. (2023). Long-term aspartame and saccharin intakes are related to greater volumes of visceral, intermuscular, and subcutaneous adipose tissue: the CARDIA study. *Int J Obes*, *47*, 939–947. https://doi.org/10.1038/s41366-023-01336-y

Stegink, L. D. (1984). Aspartame metabolism in humans: Acute dosing studies. In L. D. Stegink & L. J. Filer, Jr. (Eds.), *Aspartame. Physiology and biochemistry* (pp. 509–553). Marcel Dekker Inc. https://www.taylorfrancis.com/chapters/edit/10.1201/9781003065289-31/aspartame-metabolism-humans-acute-dosing-studies-lewis-stegink

Su, Q. W., Yang, J. F., Yang, Z. X., Kong, Q., Xiao, G. H., Liu, D., & Tang, H. J. (2025). Toxicology of aspartame to largemouth bass (Micropterus salmoides) on the basis of antioxidant capacity, liver histology and the intestinal microbiota. *Animal Feed Science and Technology*, *320* https://www.webofscience.com/wos/woscc/full-record/WOS:001402074700001

Subedi, B., & Kannan, K. (2014). Fate of artificial sweeteners in wastewater treatment plants in New York state, U.S.A. *Environmental Science & Technology*, *48*(23), 13668–13674. https://doi.org/10.1021/es504769c

Suez, J., Cohen, Y., Valdes-Mas, R., Mor, U., Dori-Bachash, M., Federici, S., Zmora, N., Leshem, A., Heinemann, M., Linevsky, R., Zur, M., Ben-Zeev Brik, R., Bukimer, A., Eliyahu-Miller, S., Metz, A., Fischbein, R., Sharov, O., Malitsky, S., Itkin, M., … Elinav, E. (2022). Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. *Cell*, *185*(18), 3307–3328.e3319. https://doi.org/10.1016/j.cell.2022.07.016

Sun, Q., Lv, M., Hu, A., Yang, X., & Yu, C.-P. (2014). Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in a wastewater treatment plant in Xiamen, China. *Journal of Hazardous Materials*, *277*, 69–75. https://doi.org/10.1016/j.jhazmat.2013.11.056

Sylvetsky, A. C., Gardner, A. L., Bauman, V., Blau, J. E., Garraffo, H. M., Walter, P. J., & Rother, K. I. (2015). Nonnutritive sweeteners in breast Milk. *Journal of Toxicology and Environmental Health. Part A*, *78*(16), 1029–1032. https://doi.org/10.1080/15287394.2015.1053646

Sylvetsky, A. C., Wang, Y., Reddy, A. G., Um, C. Y., Hodge, R. A., Lichtman, C., Mitchell, D., Nanavati, A., Pollak, M., Wang, Y., Patel, A. V., & McCullough, M. L. (2025). Nonnutritive Sweetener Consumption, Metabolic Risk Factors, and Inflammatory Biomarkers Among Adults in the Cancer Prevention Study-3 Diet Assessment Sub-Study. *The Journal of Nutrition*, *155*(6), 1952–1961. https://doi.org/10.1016/j.tjnut.2025.03.020

Tang, Z.-Z., Chen, G., Hong, Q., Huang, S., Smith, H. M., Shah, R. D., Scholz, M., & Ferguson, J. F. (2019). Multi-omic analysis of the microbiome and metabolome in healthy subjects reveals microbiome-dependent relationships between diet and metabolites. *Frontiers in Genetics*, *10*, 454. https://doi.org/10.3389/fgene.2019.00454

Temizkan, S., Deyneli, O., Yasar, M., Arpa, M., Gunes, M., Yazici, D., Sirikci, O., Haklar, G., Imeryuz, N., & Yavuz, D. G. (2015). Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in patients with type 2 diabetes. *European Journal of Clinical Nutrition*, *69*(2), 162–166. https://doi.org/10.1038/ejcn.2014.208

Tran, N. H., Nguyen, V. T., Urase, T., & Ngo, H. H. (2014). Role of nitrification in the biodegradation of selected artificial sweetening agents in biological wastewater treatment process. *Bioresource Technology*, *161*, 40–46. https://doi.org/10.1016/j.biortech.2014.02.116

Trawiński, J., & Skibiński, R. (2023). Stability of aspartame in the soft drinks: Identification of the novel phototransformation products and their toxicity evaluation. *Food Research International*, *173*(Pt 1), 113365. https://doi.org/10.1016/j.foodres.2023.113365

Van Loco, J., Vandevijvere, S., Cimenci, O., Vinkx, C., & Goscinny, S. (2015). Dietary exposure of the Belgian adult population to 70 food additives with numerical ADI. *Food Control*, *54*, 86–94. https://doi.org/10.1016/j.foodcont.2015.01.029

Van Vliet, K., Melis, E. S., de Blaauw, P., van Dam, E., Maatman, R. G. H. J., Abeln, D., van Spronsen, F. J., & Heiner-Fokkema, M. R. (2020). Aspartame and Phe-containing degradation products in soft drinks across Europe. *Nutrients*, *12*(6), 1887. https://doi.org/10.3390/nu12061887

Vin, K., Connolly, A., McCaffrey, T., McKevitt, A., O'Mahony, C., Prieto, M., Tennant, D., Hearty, A., & Volatier, J. L. (2013). Estimation of the dietary intake of 13 priority additives in France, Italy, the UK and Ireland as part of the FACET project. *Food Additives & Contaminants: Part A*, *30*(12), 2050–2080. https://doi.org/10.1080/19440049.2013.851417

Wu, Y., Lin, Z., Chen, F., Zhang, X., Liu, Y., & Sun, H. (2024). Evaluation of aspartame effects at environmental concentration on early development of zebrafish: Morphology and transcriptome. *Environmental Pollution*, *361*, 124792. https://doi.org/10.1016/j.envpol.2024.124792

Wulaningsih, W., Van Hemelrijck, M., Tsilidis, K. K., Tzoulaki, I., Patel, C., & Rohrmann, S. (2017). Investigating nutrition and lifestyle factors as determinants of abdominal obesity: An environment-wide study. *International Journal of Obesity*, *41*(2), 340–347. https://doi.org/10.1038/ijo.2016.203

Yu, Z., Henderson, I. R., & Guo, J. (2023). Non-caloric artificial sweeteners modulate conjugative transfer of multi-drug resistance plasmid in the gut microbiota. *Gut Microbes*, *15*(1), 2157698. https://doi.org/10.1080/19490976.2022.2157698

Yu, Z., Wang, Y., Bond, P. L., & Guo, J. (2021). Nonnutritive sweeteners can promote the dissemination of antibiotic resistance through conjugative gene transfer. *The ISME Journal*, *15*(7), 2117–2130. https://doi.org/10.1038/s41396-021-00909-x

Yu, Z., Wang, Y., Henderson, I. R., & Guo, J. (2022). Artificial sweeteners stimulate horizontal transfer of extracellular antibiotic resistance genes through natural transformation. *The ISME Journal*, *16*(2), 543–554. https://doi.org/10.1038/s41396-021-01095-6

Yue, J., Guo, W., Li, D., Zhu, Y., Zhao, Q., Wang, A., & Li, J. (2023). Seasonal occurrence, removal and mass loads of artificial sweeteners in the largest water reclamation plant in China. *Science of the Total Environment*, *856*, 159133. https://doi.org/10.1016/j.scitotenv.2022.159133

How to cite this article: 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., Bruzell, E., Fortes, C., … Lodi, F. (2026). Re-evaluation of salt of aspartame-acesulfame (E 962) as food additive. *EFSA Journal*, *24*(9), e10259. https://doi.org/10.2903/j.efsa.2026.10259

APPENDIX A

Tailored protocol for the hazard identification and characterisation of the salt of aspartame-acesulfame (E 962)

The assessment of the salt of aspartame-acesulfame (E 962) followed the protocol on hazard identification and characterisation of sweeteners (EFSA, 2020a; EFSA FAF Panel, 2023). For details on the hazard identification and characterisation, please refer to steps 1.11–1.15.

The decisions specific to the assessment of the salt of aspartame-acesulfame (E 962) are outlined below.

A.1 | EXTENSIVE LITERATURE SEARCH – SALT OF ASPARTAME-ACESULFAME (E 962)

Methodology

For step 1.11, open-ended literature searches were conducted for (i) aspartame-acesulfame salt (E 962), (ii) aspartame (E 951) and (iii) DKP (diketopiperazine) in the three selected databases with the search strings and criteria applied as listed below. Searches included relevant literature published after the latest opinions, specifically EFSA FAF Panel (2013) for aspartame/DKP and SCF (2000a) for aspartame-acesulfame salt, while allowing for a one-year overlap. The last updates of the searches [32] were performed on 4 and 6 June 2025, respectively.

PubMed

Aspartame-acesulfame salt

Set	Search	Results
#1	"Aspartylphenylalanine Methyl Ester"[Title/Abstract] OR "Methyl Aspartylphenylalanine"[Title/Abstract] OR "SC 18862"[Title/Abstract] OR "SC-18862"[Title/Abstract] OR "SC18862"[Title/Abstract] OR "106372-55-8"[Title/Abstract]	11
#2	"E 962"[Title/Abstract] OR "E 962"[Title/Abstract]	2
#3	"aspartame-acesulfame"[Title/Abstract]	62
#4	"aspartame acesulfame salt"[tiab:~5] OR "aspartame acesulfame salts"[tiab:~5] OR "aspartame acesulfame mixture"[tiab:~5] OR "aspartame acesulfame mixtures"[tiab:~5]	9
#5	twinsweet[Title/Abstract]	1
#6	#1 OR #2 OR #3 OR #4 OR #5	79
#7	((#6) AND (("1999/01/01"[Date - Publication]: "3000"[Date - Publication]))) AND (English[Language])	66

Aspartame

Set	Search	Results
1	"Aspartame"[Mesh]	1,142
2	aspartame[Title/Abstract] OR E951[Title/Abstract] OR "E 951"[Title/Abstract] OR "Aspartylphenylalanine methyl ester"[Title/Abstract] OR canderel[Title/Abstract] OR "dipeptide sweetener"[Title/Abstract] OR "Methyl aspartylphenylalanate"[Title/Abstract]	1,746
3	nutrasweet[Title/Abstract] OR Sladex[Title/Abstract] OR "sweet dipeptide*"	24
4	aspartyl[Title/Abstract] AND phenylalanine[Title/Abstract] AND methyl[Title/Abstract] AND ester[Title/Abstract]	115
5	Succinamic[Title/Abstract] AND acid[Title/Abstract] AND "3-amino-N"[Title/Abstract] AND carboxyphenethyl[Title/Abstract] AND ester[Title/Abstract] AND stereoisomer[Title/Abstract]	1
6	"22839-47-0"[Title/Abstract]	2
7	#1 OR #2 OR #3 OR #4 OR #5 OR #6	1,943
8	(#7) AND (("2013/01/01"[Date - Publication]: "3000"[Date - Publication])) AND (English[Language])	808

[32]The establishment of cut-offs dates is a common practice in the domain of systematic reviews methodology to allow for the time needed to perform all the subsequent steps of evidence appraisal, synthesis and weighing.

DKP

Set	Search	Results
14	benzyl[Title/Abstract] AND dioxo[Title/Abstract] AND piperazine[Title/Abstract] AND acetic[Title/Abstract] AND acid[Title/Abstract]	1
15	(((((“5262-10-2”[Title/Abstract] OR DKP[Title/Abstract]) OR (Aspartame[Title/Abstract] AND “impurity A”[Title/Abstract])) OR (aspartame[Title/Abstract] AND “compound A”[Title/Abstract])) OR (Cycloaspartylphenylalanine[Title/Abstract])) OR (“CYCLO L-ASP-L-PHE”[Title/Abstract])) OR (“Cyclo aspartyl-phenylalanyl”[Title/Abstract])	387
16	Benzyl[Title/Abstract] AND dioxo[Title/Abstract] AND piperazineacetic[Title/Abstract] AND acid[Title/Abstract]	2
17	“cyclo aspartyl phenylalanine”[Title/Abstract] OR “cyclo aspartyl phenylalanyl”[Title/Abstract] - Schema: all	0
18	“cyclo aspartyl phenylalanine”[Title/Abstract] OR “cyclo aspartyl phenylalanyl”[Title/Abstract]	0
19	“cycloaspartyl”[Title/Abstract] OR “cyclo aspartyl”[Title/Abstract] - Schema: all	0
20	“cycloaspartyl”[Title/Abstract] OR “cyclo aspartyl”[Title/Abstract]	0
21	Diketopiperazine[Title/Abstract]	1,308
22	Diketopiperazine[Title/Abstract] AND aspartame[Title/Abstract]	25
23	(Dioxopiperazine[Title/Abstract] OR Piperazinedione[Title/Abstract]) OR (Aspartylphenylalanine[Title/Abstract] AND diketopiperazine[Title/Abstract])	277
24	#14 OR #15 OR #16 OR #22 OR #21 OR #23	1,745
25	(#24) AND ((“2013/11/01”[Date - Publication]: “3000”[Date - Publication]))	871

Web of Science (core collection[1])

Aspartame-acesulfame salt

Set	Search	Results
#1	TS=(“Aspartylphenylalanine Methyl Ester” OR “Methyl Aspartylphenylalanine” OR “SC 18862” OR “SC-18862” OR “SC18862” OR “106372-55-8”)	10
#2	TS=(“E 962” OR “E 962”)	9
#3	TS=(“aspartame-acesulfame”)	104
#4	TS=((aspartame NEAR/1 acesulfame) NEAR/2 (salt OR mixture OR mix))	17
#5	TS=(twinsweet)	2
#6	#5 OR #4 OR #3 OR #2 OR #1	133
#7	((#6) AND PY=(1999-2100)) AND LA=(English)	107

Aspartame

Set	Search	Results
#1	aspartame OR E951 OR “E 951” OR “Aspartylphenylalanine methyl ester” OR canderel OR “dipeptide sweetener” OR “Methyl aspartylphenylalanate” (Topic)	3,354
#2	nutrasweet OR Sladex OR “sweet dipeptide*” (Topic) OR aspartyl AND phenylalanine AND methyl AND ester (Topic) OR Succinamic AND acid AND “3-amino-N” AND carboxyphenethyl AND ester AND stereoisomer (Topic) OR “22839-47-0” (Topic)	257
#3	#1 OR #2	3,433
#4	**#1 OR #2 Timespan: 2013-01-01 to 2025-12-31**	1,368

DKP

Set	Search	Results
#10	TS=(benzyl AND dioxo AND piperazine AND acetic AND acid)	1
#11	TS=(“5262-10-2” OR DKP OR (Aspartame AND “impurity A”) OR (aspartame AND “compound A”) OR Cycloaspartylphenylalanine OR “CYCLO L-ASP-L-PHE” OR “Cyclo aspartyl-phenylalanyl”)	1,053
#12	TS=(Benzyl AND dioxo AND piperazineacetic AND acid)	5
#13	TS=(“cyclo aspartyl phenylalanine” OR “cyclo aspartyl phenylalanyl”)	0
#14	TS=(“cyclo aspartyl phenylalanine” OR “cyclo aspartyl phenylalanyl”)	0

SciFinder-N

Aspartame-acesulfame salt

Set	Search	Results
1	106372-55-8-> References Filter by: Document type: journal, review, clinical trial, commentary, preprint, report Language: English Publication year: 1999–2025	7

Aspartame

Set	Search	Results
1	22839-47-0-> References Filter by: Document type: journal, review, clinical trial, commentary, preprint, letter, report Language: English Publication year: 2013–2025	1,970

DKP

Set	Search	Results
1	5262-10-2 -> References Filter by: Document type: journal, review, clinical trial, commentary, preprint, report Language: English Publication year: 2013–2025	17

Results

The final number of references that were screened, after removal of duplicates (based on title, year, author, journal, volume, issue and page numbers) was 333 for the salt of aspartame acesulfame, 2708 for aspartame, 1578 for DKP.

For step 1.11.1 (Screening of the studies for relevance), the general principles reported in the protocol were applied.

The systematic review process for salt of aspartame-acesulfame and for aspartame is illustrated in Figures A.1 and A.2 using the PRISMA flow diagram, adapted from Moher et al. (2009).

For DKP, a total of 7 papers were included at the level of title and abstract screening, whereas 1571 papers were excluded. From the seven papers included for the full-text screening, no studies were considered relevant.

PRISMA 2009 Flow Diagram: salt of aspartame-acesulfame

[1] The final number of references includes primary studies which were identified from systematic reviews
[2] or preliminary categorised into technical data, exposure data, environmental data or toxicological reviews and further screened for confirmation of relevance.
[3] The full list of excluded studies is recorded in Distiller
[4] or preliminary categorised into mode of action and narrative summary

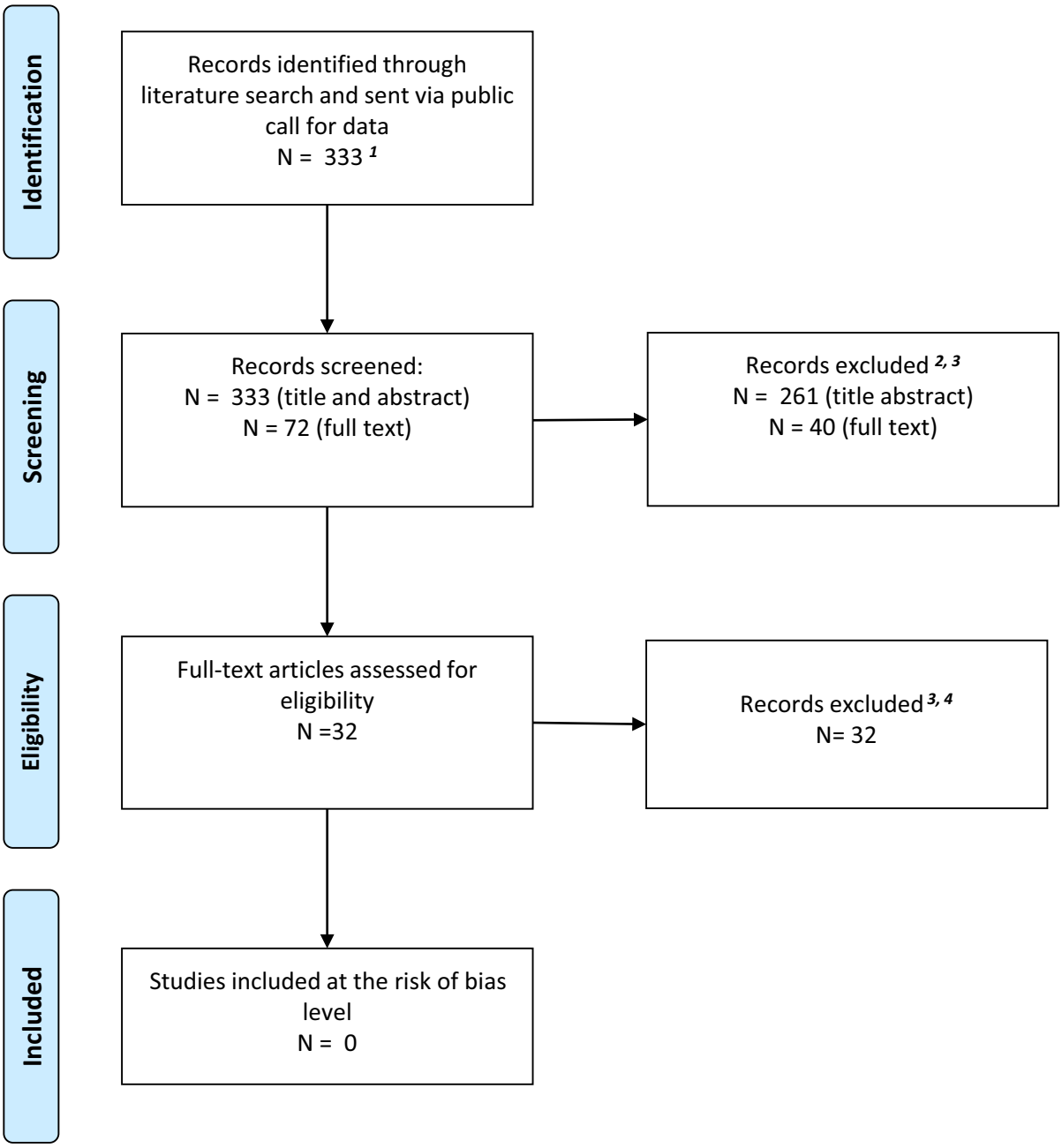


FIGURE A.1 PRISMA flow chart for salt of aspartame-acesulfame (E 962)

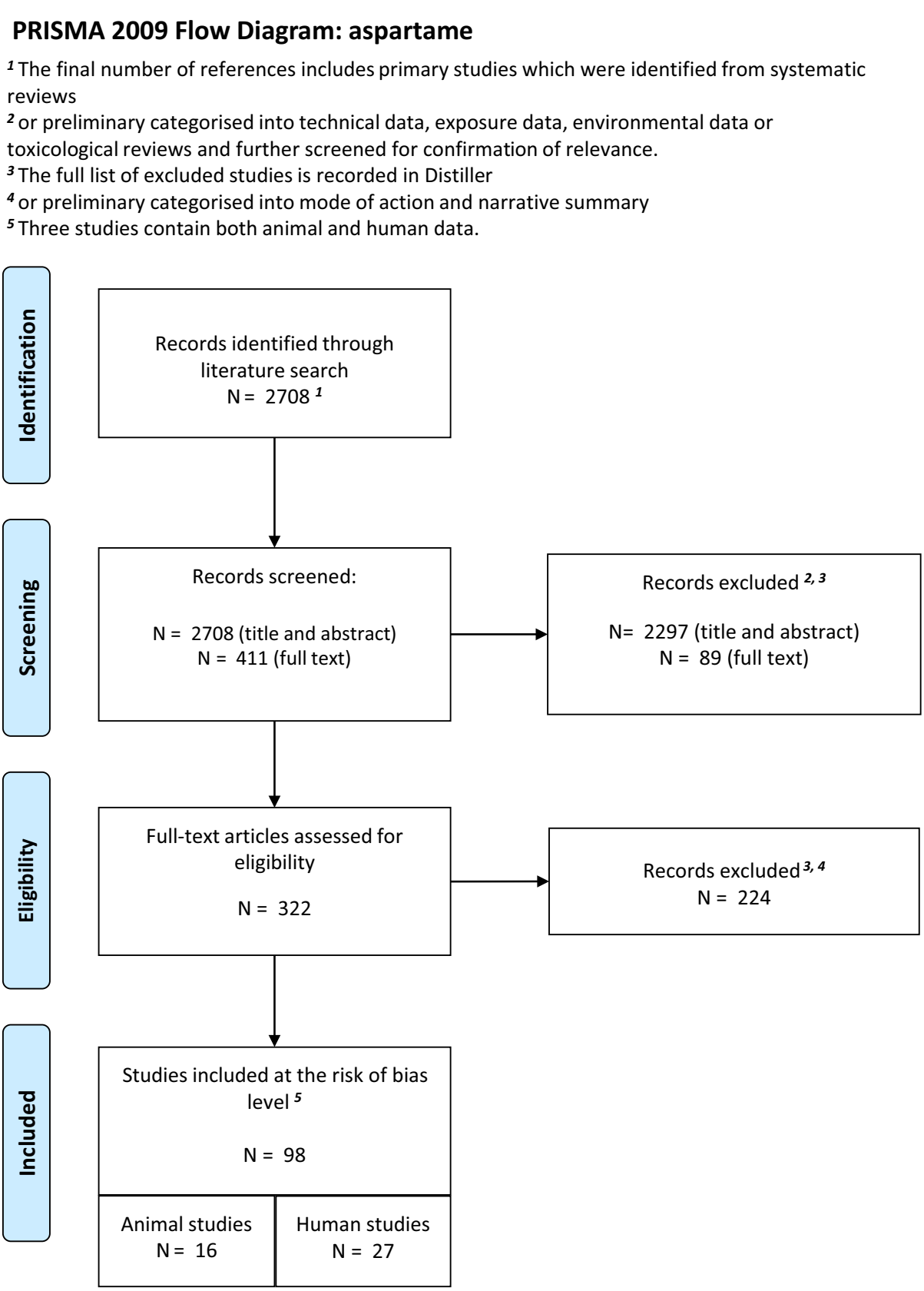


FIGURE A.2 PRISMA flow chart for aspartame (E 951)

A.2 | EVALUATION OF THE RISK OF BIAS (ROB)

Methodology

For step 1.12, the criteria outlined in the revised protocol for the risk of bias evaluation of studies have been applied (EFSA FAF Panel, 2023), including the rules for tier allocation.

The studies evaluated for the RoB were allocated to a tier (from 1 to 3 corresponding to decreasing levels of internal validity) based on the rules as reported in step 1.12 of the revised protocol.

The evaluation of the RoB was conducted in parallel independently by two reviewers and in case a conflict on tier allocation of a study was identified, ad-hoc discussion between the reviewers took place prior to a final agreed tier allocation that was reached by consensus. Any potential conflict between reviewers' assessments is being addressed solely at the tier level, rather than at the level of individual scoring.

The tier was automatically generated by the DistillerSR tool, after input of the ratings for the individual elements of the study considered for the RoB. At the end of the evaluation, the reviewers were requested to express their agreement or disagreement on the tier generated by the tool based on their expert judgement. In case of disagreement, a justification is to be provided.

As reported in the revised protocol (EFSA FAF Panel, 2023), studies on which the derivation of the ADI was based or studies used for identification of a NOAEL/no observed effect level (NOEL) (in case of ADI not specified) were included and evaluated according to the protocol together with any relevant literature published since the previous evaluation by EFSA or SCF, allowing 1 year of overlap, and up to a cut-off date which allowed for the time needed to perform all steps required for the systematic evidence appraisal, synthesis and weighing. If studies that were previously considered as critical were evaluated as having moderate (tier 2) or low risk of bias (tier 1), other non-critical studies from previous assessments, including those received by the interested business operators, would not have to be re-evaluated and subjected to the different steps of the protocol (e.g. RoB, WoE).

Results

For the salt of aspartame-acesulfame (E 962), following the confirmation-of-relevance screening, no studies were subjected to the Risk-of-Bias (RoB) assessment, as none of the studies identified met the criteria for RoB evaluation.

For aspartame (E 951), relevant studies retrieved from the literature were considered and evaluated for the RoB together with the pivotal studies. To facilitate RoB assessment of a very large number of short-term (sub-acute; < 8 weeks) studies (see Annex D), an initial evaluation based on key questions only was performed. Only studies for which none of the key questions resulted in exclusion as ier 3 were subsequently subjected to a full RoB assessment. The results of the RoB assessment are reported in Annex D both for human and animal data. Disagreements with the tier generated by the tool were identified by the reviewers, the final tier level was adjusted based on expert judgement and the reasoning was provided.

A total of 16 animal studies and 27 human studies were evaluated as having moderate (tier 2) or low risk of bias (tier 1) and therefore considered further in the assessment, while those having a high RoB (tier 3) were not considered further. Three studies contain both animal and human data.

A.3 | DATA EXTRACTION

Methodology

For step 1.13 of the revised protocol (EFSA FAF Panel, 2023) information and data from the assessed unpublished animals studies as well as from the included genotoxicity studies are extracted and reported in tabular form; the data extraction forms are outlined in the revised protocol.

Results

The unpublished study reports considered in the present assessment (Documentation provided to EFSA 14-17) were previously assessed and made publicly available by EFSA during the re-evaluation of aspartame (EFSA ANS Panel, 2013) (Annex B). The data extracted from genotoxicity studies are shown in Annex C.

A.4 | WEIGHING THE BODY OF EVIDENCE AND SYNTHESIS OF THE EVIDENCE

Methodology

For step 1.14 (Weighing the body of evidence), a WoE analysis for different health outcome categories, grouped by endpoint as appropriate, was performed on eligible animal and human studies. The WoE analysis was performed using Excel tables. The detailed methodology is reported in the revised protocol (EFSA FAF Panel, 2023).

Results

No weight of evidence (WoE) analysis was conducted for the salt of aspartame-acesulfame (E 962) or DKP, as no studies passed the confirmation of relevance check and no studies were eligible for RoB assessment.

The result of the WoE analysis for aspartame (E 951) is presented in Annex E1 and E2 and summarised in Section 3.5.4 on 'Synthesis of systematically appraised evidence and weight of evidence.

A.5 | INTEGRATION OF ANIMAL AND HUMAN EVIDENCE

Methodology

No integration of data was conducted for the salt of aspartame-acesulfame (E 962) or DKP as no relevant data were available and therefore WoE analysis could not be conducted (see A.4). The integration of animal and human evidence for aspartame (E 951) was performed following the matrix for integration shown in Figure A.3.

The integration of evidence was complemented by additional considerations from the Panel on the biological plausibility of each effect and mode of action (MoA). When deemed relevant by expert judgement, consideration also was given to the evidence and conclusions of the previous evaluation (EFSA ANS Panel, 2013) to assess whether the new data support them. The results of the integration are reported in Section 3.5.4.3.

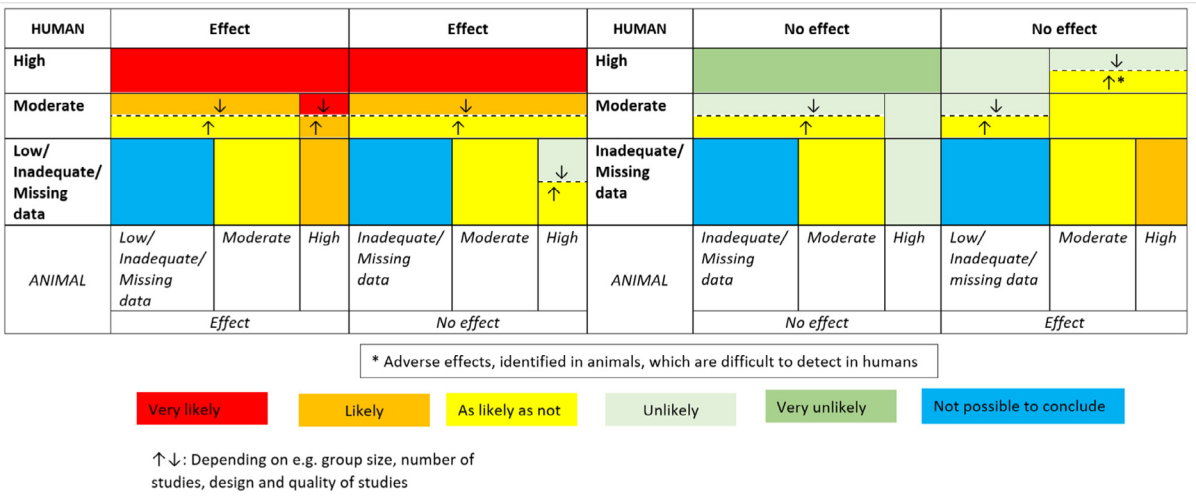


FIGURE A.3 Matrix for integration of animal and human body of evidence based on identification of effect and level of evidence.

APPENDIX B

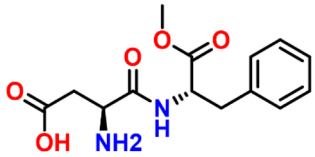
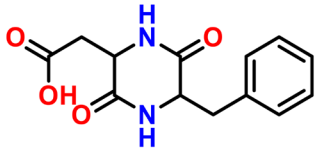
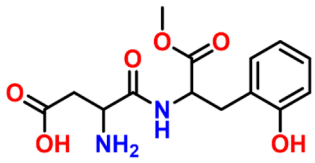
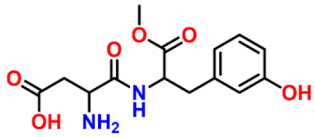
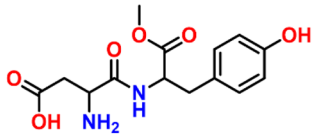
In silico assessment of aspartame degradation products

The genotoxicity and Cramer class of the principal degradation product of aspartame (E 951), 5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP) and of the 'phenolic derivatives of aspartame' (ortho-, meta- and para-hydroxyaspartame) derived from phototransformation experiments Trawiński and Skibiński (2023) were evaluated using in silico tools. Aspartame was evaluated in parallel for comparative purposes. A suite of VEGA statistical models, and the rule-based OECD QSAR Toolbox profilers were applied. For comparative purpose, also aspartame was included in the set of analyzed compounds. In addition, in silico predicted metabolites of DKP were evaluated using OECD QSAR Toolbox profilers.

B.1 | NAME AND CODE OF ANALYZED COMPOUNDS

- Compound 1 – aspartame (CAS 22839-47-0)
- Compound 2 (degradation product resulting from the intramolecular reaction of the primary amine with the methyl ester group of aspartame) - 5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP) (CAS 55102-13-1, stereochemistry not specified; CAS 5262-10-2, 2S,5S configuration)
- Compounds 3-5: proposed structures of the 'phenolic derivatives of aspartame' (ortho-, meta- and para-hydroxyaspartame) derived from phototransformation experiments (Trawiński & Skibiński, 2023).

B.2 | CHEMICAL STRUCTURES

No	Name	CAS	Canonical SMILES	Structure
1	Aspartame	22839-47-0	O=C(O)CC(N)C(=O)NC(C(=O)OC)CC=1C=CC=CC1	
2	5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP)	55102-13-1, stereochemistry not specified	O=C(O)CC1NC(=O)C(NC1=O)CC=2C=CC=CC2	
3	Ortho-hydroxyaspartame	No CAS assigned	COC(=O)Cc1c(O)cccc1NC(=O)NCC(=O)O	
4	Meta-hydroxyaspartame	No CAS assigned	COC(=O)Cc1cc(O)ccc1NC(=O)NCC(=O)O	
5	Para-hydroxyaspartame D-Tyrosine, N-L-a-aspartyl-, 1-methyl ester	72683-62-6	COC(=O)C(Cc1ccc(O)cc1)NC(=O)C(N)CC(O)=O	

B.3 | SUMMARY OF RESULTS

The results obtained using the VEGA predictions models are reported in Table B.1 and the results from the OECD QSAR toolbox are reported in Table B.2.

TABLE B.1 VEGA Models predictions.

Compound n. M/M	1	2	3	4	5
Ames consensus 1.0.4	NM (0.525)	NM (0.5)	NM (0.45)	NM (0.45)	NM (0.45)
Ames CAESAR 2.1.14	*M/L*[1]	NM/M[3,4]	*M/L*[1,2]	*M/L*[1]	*M/L*[1]
Ames ISS 1.0.3	NM/M[2]	*NM/L*[1,2]	NM/M[2]	NM/M[2]	NM/M[2]
Ames SarPy-IRFMN 1.0.8	**NM/G**	NM/M[3,5]	NM/M[5]	NM/M[3]	NM/M[3]
Ames KNN-Read-across 1.0.1	NM/M[2,3]	NM/M[3,5]	NM/M[3,5]	NM/M[3,5]	NM/M[2,3]
CAs CORAL 1.0.1	A/M[1]	**I/G**	A/M[2]	A/M[1]	A/M[1]
In vitro MN IRFMN-VEERMER 1.0.1	*A/L*[1,2,4]	I/M[2,4]	A/M[2,3]	A/M[1,4]	A/M[1,4]
In vivo MN IRFMN 1.0.2	**A/G**	I/M[1]	I/M[2]	I/M[3]	I/M[3]
Cramer TOXTREE 1.0.1[6]	*Class I*	*Class III*	*Class I*	*Class I*	*Class I*

In the table, prediction/reliability codes. In bold the predictions with good reliability; in italics predictions with low reliability.

Code of predictions: NM: non-mutagenic, M: mutagenic, SM: suspect mutagen, I: inactive, A: active, NP: not predicted (not possible to perform an assessment).

Reliability code: G: good reliability (The predicted compound is into the Applicability Domain of the model); M: moderate reliability (The predicted compound could be out of the Applicability Domain of the model); L: low reliability (The predicted compound is outside the Applicability Domain of the model).

[1]Similar molecules found in the training set have experimental values that disagree with the predicted value.

[2]Accuracy of predictions for similar compounds found in the training set is not adequate.

[3]Some similar molecules found in the training set have experimental values that disagree with the predicted value.

[4]Only moderately similar compounds with known experimental value in the training set have been found.

[5]Accuracy of predictions for similar compounds found in the training set is not optimal.

[6]Impossible to perform an assessment.

TABLE B.2 OECD QSAR Toolboox.

Compound	1	2	3	4	5
DNA binding by OASIS	NA	NA	NA	NA	NA
DNA binding by OECD	Michael addition	Michael addition	Michael addition	NA	Michael addition
Carcinog. Genotox/non-genotox by ISS	NA	NA	NA	NA	NA
DNA alerts for Ames, CA, MN vit by OASIS	NA	NA	NA	NA	NA
Ames test alert by ISS	NA	NA	NA	NA	NA
In vivo mutagenicity by ISS (MN)	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor
Protein binding alerts for chromosomal aberrations by OASIS	NA	NA	NA	NA	NA
Toxic hazard classification by Cramer	Class II	Class II	Class III	Class III	Class III

Abbreviation: NA, no alert.

Michael addition: P450 mediated activation to quinones and quinone-type chemicals.

H-acceptor-path3-H-acceptor: non-covalent binding with DNA and/or proteins, such as DNA intercalation or groove-binding.

No alert for carcinogenicity and in vitro genotoxicity was identified by the end-point specific profilers. An alert for in vivo genotoxicity in the micronucleus test (Hacceptor-path3-Hacceptor) was identified in both DKP and aspartame. This alert is related to the capacity of forming non-covalent binding with DNA and/or proteins as the result of the presence of two bonded atoms connecting two hydrogen bond acceptors. However, the analysis of a large genotoxicity dataset (> 9000 substances) indicated the absence of positive predictivity for this alert (PPV, positive predictive value 43%) (Pradeep et al., 2021), which therefore was not taken in further consideration in this, as well as in previous EFSA opinions.

Among general mechanistic profilers, the presence of a benzene ring in the chemical structure was identified by one profiler (DNA binding by OASIS) as an alert for potential DNA binding following the P450-mediated activation to quinone species. However, in the absence of supporting data from end-point specific profilers, the indication from this profiler is considered too unspecific and of insufficient predictivity, as it was also highlighted in the structurally related aspartame which tested negative in genotoxicity assays detecting DNA damage (e.g. Ames test, in vivo come assay).

Overall, the in silico assessment performed did not provide relevant indication of potential genotoxicity for aspartame, DKP or the 'phenolic derivatives of aspartame'. The same conclusions apply to aspartame and DPK metabolites generated by the rat liver metabolism simulator in the OCED QSAR Toolbox.

APPENDIX C

Other endpoints measured in available studies

The Panel noted that additional endpoints were assessed in the available studies which were considered as supportive information for the re-evaluation of aspartame as food additive. These findings are summarised narratively in this section.

Other endpoints on animal's studies

Several studies measured biomarkers of oxidate stress in serum. Two studies in rats, administered with either 40 mg/kg bw per day for 90 days (Choudhary & Sheela Devi, 2015) or up to 1000 mg/kg bw per day for 180 days (Abhilash et al., 2014) focused on parameters related to lipid peroxidation. The findings on levels of LPO products, nitric oxide, vitamin C and GSH (reduced from) in the serum were highly inconsistent with regard to the dose, duration and/or the effects of the treatment. Therefore, these data were not considered further in the WoE assessment.

Several other studies were retrieved which reported neuronal cell degeneration at doses < 100 mg/kg bw per day (Onaolapo et al. (2016); Onaolapo, Abdusalam, and Onaolapo (2017); Onaolapo, Onaolapo, and Nwoha (2017) in mice, Ediga et al. (2023) in rats). However, all of these studies were appraised as tier 3 (high risk of bias) due to shortcomings including lack of blinding for quantitative histopathology, and are thus not considered further in the WoE assessment.

One study reported increases in serum corticosterone at 80 mg/kg bw per day after 4 weeks (Ma et al., 2024). However, increases in serum corticosterone were not observed in Collison et al. (2016) at 46 mg/kg bw per day on day 114. Therefore, this endpoint was not considered further in the present opinion.

Other endpoints on human studies

Örkü et al. (2023) conducted a 4-week randomised controlled trial in 42 healthy adults who consumed water sweetened with saccharin, sucralose or an aspartame-acesulfame mixture at levels reflective of typical dietary intake; no effects were observed on glucose tolerance, insulin sensitivity, GLP-1 secretion, HbA1c, body weight or body composition compared with the water control group.

Effects on human gut microbiota

Several studies investigating the potential impact of sweeteners on the gut microbiota, previously identified in the re-evaluations of acesulfame K, sucralose or saccharin (EFSA FAF Panel, 2024b, 2025, 2026b), also included aspartame, either alone or in combination with other sweeteners (e.g. Ahmad et al., 2020; Ayoub-Charette et al., 2023; Frankenfeld et al., 2015; Hosseini et al., 2023; Loayza et al., 2023; Mahmud et al., 2019; Tang et al., 2019; Yu et al., 2023). These studies examined endpoints related to microbiome composition, antimicrobial activity, bacterial growth, horizontal gene transfer, plasmid persistence and dissemination of antibiotic resistance genes. As discussed in the earlier opinions (EFSA FAF Panel, 2024b, 2025, 2026b), the reported effects were generally observed in in vitro or ex vivo systems, were often of limited magnitude, depended on the bacterial strain and experimental conditions, and their biological relevance remains uncertain. Therefore, the same limitations and considerations apply to the evidence relating to aspartame.

Impact of sweeteners on antimicrobial resistance (AMR)

Several studies investigating the potential effects of sweeteners on antimicrobial resistance (AMR), previously identified in the re-evaluations of acesulfame K, sucralose or saccharin (EFSA FAF Panel, 2024b, 2025, 2026b), also included aspartame, either alone or in combination with other sweetener (e.g. de Dios et al., 2023; de Souza Lopes et al., 2023; Yu et al., 2021, 2022, 2023). These studies examined endpoints related to gut microbial composition, diversity, metabolism and microbiota-related functions. As discussed in the earlier opinions (EFSA FAF Panel, 2024b, 2025, 2026b), the available evidence is heterogeneous in study design and endpoint selection, and there is currently no consensus on when alterations in intestinal microbiota should be considered adverse or on their potential health consequences. Therefore, the same limitations and considerations apply to the evidence relating to aspartame.

ANNEXES

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

ANNEX A

Exposure data and estimates

ANNEX B

Data extraction: toxicological studies

The unpublished study reports considered in this assessment (Documentation provided to EFSA 14-17) were previously assessed and made publicly available by EFSA during of the re-evaluation of aspartame (EFSA ANS Panel, 2013). These studies can be retrieved from https://www.efsa.europa.eu/en/consultations/call/110531. Data relevant for the present assessment are shown in Annex E1 and E2;

ANNEX C

Data extraction genotoxicity studies

ANNEX D

Outcome of the Risk of Bias assessment

ANNEX E1

Weight of Evidence (WoE) tables: animal studies

ANNEX E2

Weight of Evidence (WoE) tables: human studies

ANNEX F

Environmental data