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Re-evaluation of acesulfame K (E 950) as food additive

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

Abstract

The present opinion deals with the re-evaluation of acesulfame K (E 950) as a food additive. Acesulfame K (E 950) is the chemically manufactured compound 6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide potassium salt. It is authorised for use in the European Union (EU) in accordance with Regulation (EC) No 1333/2008. The assessment involved a comprehensive review of existing authorisations, evaluations and new scientific data. Acesulfame K (E 950) was found to be stable under various conditions; at pH lower than 3 with increasing temperatures, it is degraded to a certain amount. Based on the available data, no safety concerns arise for genotoxicity of acesulfame K (E 950) and its degradation products. For the potential impurities, based on in silico data, a concern for genotoxicity was identified for 5-chloro-acesulfame; a maximum limit of 0.1 mg/kg, or alternatively, a request for appropriate genotoxicity data was recommended. Based on the synthesis of systematically appraised evidence of human and animal studies, the Panel concluded that there are no new studies suitable for identification of a reference point (RP) on adverse effects. Consequently, the Panel established an acceptable daily intake (ADI) of 15 mg/kg body weight (bw) per day based on the highest dose tested without adverse effects in a chronic toxicity and carcinogenicity study in rats; a study considered of moderate risk of bias and one of two key studies from the previous evaluations by the Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). This revised ADI replaces the ADI of 9 mg/kg bw per day established by the SCF. The Panel noted that the highest estimate of exposure to acesulfame K (E 950) was generally below the ADI in all population groups. The Panel recommended the European Commission to consider the revision of the EU specifications of acesulfame K (E 950).

KEYWORDS

Acesulfame K, E950, food additive, re-evaluation, sweetener

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CONTENTS

SUMMARY

The present opinion deals with the re-evaluation of acesulfame K (E 950) when used as food additive. Acesulfame K (E 950) is authorised as food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and their specifications defined in the Commission Regulation (EU) No 231/2012.

The European Commission's Scientific Committee on Food (SCF) reviewed Acesulfame K (E 950) in 1985 and set an ADI of 0–9 mg/kg bw per day based on a no observed adverse effect level (NOAEL) from a 2-year dog study (3% acesulfame K in the diet, the highest dietary concentration tested, corresponding to a dose of 900 mg/kg bw per day). The SCF reaffirmed this ADI in 2000, concluding no mutagenic or carcinogenic potential and no toxic effects at high dietary levels in rats or dogs.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated Acesulfame K in 1983 and 1991. Initially setting an ADI of 0–9 mg/kg bw per day from the 2-year dog study, this ADI later was increased to 0–15 mg/kg bw per day based on a longer-term rat study (3% acesulfame K in the diet, the highest dietary concentration tested, corresponding to a dose of 1500 mg/kg bw per day in the rat). JECFA also reviewed toxicological studies on the breakdown products, acetoacetamide and acetoacetamide- N-sulfonic acid; these compounds were found to have a low toxicity and to be not mutagenic.

Acesulfame K (E 950) is the potassium salt of 6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide, produced through chemical synthesis involving several reaction steps and multiple purification processes, including crystallisation, filtration, recrystallisation and centrifugation. The purity of acesulfame K (E950) was reported to be in the range of 99.6%–100.2%. For UV-active organic impurities, the EU specifications set a limit of 20 mg/kg; no specific limits exist for individual organic impurities. The European Pharmacopoeia monograph on acesulfame K (E 950) identifies two organic impurities namely, (i) acetylacetamide, also a degradation product of the food additive and (ii) 5-chloro-acesulfame. The potential exposure to these impurities (at their maximum reported limits of 1 mg/kg for acetylacetamide and 2 mg/kg for 5-chloro-acesulfame) was assessed as a worst-case scenario.

Under acidic conditions, acesulfame K degrades to produce acetylacetamide and acetoacetamide-N-sulfonic acid, both reviewed by JECFA (1991) and found to be of low toxicity and non-mutagenic. No new evidence from a systematic search of published literature on animal toxicological studies or human data indicated otherwise.

To assess the risk associated with the potential presence of acetylacetamide at 1 mg/kg in acesulfame K (E 950), the Panel calculated the margin of exposure (MOE), indicating no safety concern. Also, when considering additional exposure to acetylacetamide as a degradation product of acesulfame K, due to the high MOE (106), no safety concerns are expected to arise based on available data.

For 5-chloro-acesulfame, no experimental toxicity, including genotoxicity data, was retrieved. Quantitative structure activity relationship (QSAR) analysis triggered in silico genotoxicity alerts. Applying a threshold of toxicological concern (TTC) for potentially genotoxic compounds (0.0025 µg/kg bw per day), and assuming a concentration of 5-chloro-acesulfame of 2 mg/kg in acesulfame K (E 950), the potential exposure to this impurity is above the TTC threshold. The Panel therefore recommends that a maximum limit of 0.1 mg/kg for 5-chloro-acesulfame should be included in the specifications for acesulfame K (E 950); alternatively, appropriate genotoxicity data for 5-chloro-acesulfame should be generated.

The Panel found no need for additional limits for arsenic, cadmium or fluoride. For lead and mercury, the Panel recommended lowering specification limits to ensure safety. Microbiological contamination was deemed unlikely; the Panel considered it not necessary to recommend inclusion of microbiological criteria in the EU specifications for acesulfame K (E 950). No concern for small particles or nanoparticles was raised due to acesulfame K's (E 950) complete solubility in water.

The biological and toxicological dataset available to the Panel for the re-evaluation of acesulfame K (E 950) comprised evidence from animal toxicological studies and human data, both published and unpublished, made available to EFSA in response to calls for data and related clarification requests and/or also identified from the published literature. The selection, appraisal and integration of the evidence was performed according to the principles outlined in the revised protocol on hazard identification and characterisation of sweeteners.

Regarding absorption, distribution, metabolism and excretion (ADME), the Panel considered that the data on acesulfame K (E 950) show that it is almost fully absorbed, not metabolised and primarily excreted in urine. It can cross the placenta and enter breast milk. The breast-fed infant is exposed, however to a low extent which accounts for 1.6% of the mother's dose.

The Panel concluded that the newly available studies do not raise a concern for genotoxicity of acesulfame K (E 950), which concurs with the conclusion of the previous SCF opinion (SCF, 2000) based on the database available at that time.

A synthesis of systematically appraised evidence of human and animal studies indicated no exposure-related adverse effects for acesulfame K (E950) at doses spanning more than three orders of magnitude. The systematic appraisal included evaluation of risk of bias (RoB) and weight of evidence (WoE) across relevant health outcomes categories (HOC) based on human and animal studies. The integration of human and animal evidence was expressed in terms of likelihood of an association between the intake of acesulfame K and an adverse effect on health. The level of evidence was high for the absence of adverse effects in animals grouped under the HOC: (i) general toxicity, (ii) haematotoxicity, (iii) nephrotoxicity, (iv) liver toxicity and (v) other organ toxicity. The level of evidence for the absence of adverse effects was moderate for endpoints grouped under (i) glucose/insulin homeostasis and (ii) additional clinical chemistry. For human studies, the level of evidence for an effect related to the HOC (i) cancer and (ii) cardiovascular risk factors and disease and (iii) development was considered low; for (v) birth outcomes, inadequate. The level of evidence for the absence of an effect related to the HOC glucose/insulin homeostasis was moderate.

Following the integration of the evidence from human and animal evidence, the Panel noted that it is unlikely that intake of acesulfame K (E950) is associated with (i) cancer, (ii) disturbances of the glucose or insulin homeostasis, (iii) cardiovascular risk factors and disease, (iv) general toxicity (v) heamatological effects, (vi) nephrotoxicity, (vii) liver toxicity or (viii) toxicity in any organ or tissue. It is not possible to conclude that intake of acesulfame K (E 950) is associated with (i) preterm delivery and (ii) precocious puberty in girls.

Following the integration of evidence from human and animal studies, the Panel concluded that there are no new studies suitable for identification of a reference point (RP) based on adverse effects. Consequently, the Panel established an ADI of 15 mg/kg body weight per day, based on the highest dose tested without adverse effect in a 2-year chronic toxicity and carcinogenicity study in rats (Documentation provided to EFSA No 17), one of two key studies from the previous evaluations by the SCF and JECFA, and considered of moderate RoB (tier 2). The revised ADI replaces the previous ADI of 9 mg/kg bw per day set by the SCF.

The Panel considered dietary exposure to acesulfame K (E 950) estimated according to different exposure scenarios based on consumers only. Currently, acesulfame K (E 950) is authorised as food additive in the EU in 34 food categories; concentration data were available for 29 categories. In all scenarios, the exposure estimates were regarded to overestimate the current exposure to acesulfame K (E 950) in the EU. The Panel considered the refined brand-loyal exposure assessment scenario, the most appropriate exposure scenario for the risk assessment. In this scenario, mean exposure to acesulfame K (E 950) ranged from 0.04 mg/kg bw per day in adolescents to 5.2 mg/kg bw per day in toddlers. The 95th percentile of exposure ranged from 0.2 mg/kg bw per day in children (3–9 years) and adults to 15.7 mg/kg bw per day in toddlers. The main food category contributing to the exposure to acesulfame K (E 950) was FC 14.1.4. 'Flavoured drinks' for all population groups. The Panel concluded that current dietary exposure estimates do not indicate a safety concern for acesulfame K.

Uncertainties identified for exposure assessments and study evaluations were considered to have minimal impact on the overall conclusions regarding the safety of acesulfame K (E 950).

The Panel recommended the European Commission to consider:

- Inserting a maximum limit of 0.1 mg/kg for 5-chloro-acesulfame in the EU specifications or alternatively request appropriate genotoxicity data for 5-chloro-acesulfame.
- Inserting a maximum limit of 1 mg/kg for acetylacetamide in the EU specifications.
- Lowering the limit of lead and mercury in the EU specifications of acesulfame K (E 950).
- Including the CAS number 55589-62-3 in the EU specifications.

1 | INTRODUCTION

The present opinion deals with the re-evaluation of acesulfame K (E950) when used as a food additive. The generic term 'acesulfame K' will be used in the body of this opinion unless more specific information is reported (i.e. E number) in biological and toxicological studies.

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.

1.2 | Information on existing authorisations and evaluations

Acesulfame K (E 950) 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 its specifications are defined in the Commission Regulation (EU) No 231/2012.[3]

The authorised food additive 'salt of aspartame-acesulfame' (E 962), for which the re-evaluation is ongoing,[4] is another source of exposure to acesulfame.

Acesulfame K (E 950) was evaluated by the SCF in 1985 and 2000 (SCF, 1985, 2000); an ADI of 0–9 mg/kg bw was established based on the NOAEL in a 2-year study in the dog (SCF, 1985). In a subsequent evaluation, SCF reconfirmed the ADI of 0–9 mg/kg bw (SCF, 2000). The SCF concluded that acesulfame K is not mutagenic or carcinogenic and that no effects of toxicological significance were observed at dietary levels up to 3% in the rat (equivalent to 1500 mg/kg bw per day) or in the dog (equivalent to 900 mg/kg bw per day). Based on toxicokinetics considerations that the plasma peak concentration and area under the curve (AUC) (24 h) for the dog are several-fold higher than that for the rat, the SCF considered the dog to remain the appropriate species on which to base the ADI.

Acesulfame K was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1983 and 1991 (JECFA, 1983, 1991). The initial evaluation was based on a 2-year study in the dog, and an ADI of 0–9 mg/kg was set

[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.

[4]EFSA-Q-2011-00727.

(JECFA, 1983). In the 1991 evaluation, based on toxicological data obtained from a 2-year study in rats, JECFA increased the ADI to 0–15 mg/kg (JECFA, 1991). This decision was based on the consideration that acesulfame K is not metabolised in any tested species and that the 2-year study in rats represents a greater portion of the animal's lifespan compared to the 2-year study in dogs.

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) (EFSA ANS Panel, 2016) evaluated the extended use of Acesulfame K (E 950) in products falling into category 13.1.5 (Dietary foods for infants and young children for special medical purposes as defined by Directive 1999/21/EC and special formulae for infants). The ANS Panel concluded that the proposed extension of use of acesulfame K (E 950) at the level up to 9 mg/g protein while providing 10 g protein/day to 1- to 3- year-old children would not be of safety concern.

Furthermore, acesulfame K (CAS No 55589-62-3) is permitted as fragrance in cosmetics products (European Commission database-CosIng[5]).

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) and on exposure assessment (EFSA, 2020b; EFSA FAF Panel, 2024) were developed in line with the principles of the EFSA PROMETHEUS project (PROmoting METHods for Evidence Use in Scientific assessments) (EFSA, 2015). 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 (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, 2024).

2.1 | Data

The FAF Panel was not provided with a newly submitted dossier for the re-evaluation of acesulfame K (E 950). In accordance with Regulation (EU) No 257/2010, EFSA launched public calls for data[6,7,8] and contacted interested parties that had replied to the call for data to collect additional clarification or supplemental information (Documentation provided to EFSA n. 1–17).

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature, up to December 2024. 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 acesulfame K, from both acesulfame K (E 950) and salt of aspartame-acesulfame (E 962), were derived from the EFSA Comprehensive European Food Consumption Database[9] (Comprehensive Database). The Mintel's Global New Products Database (GNPD) was checked to identify the uses of 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.

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, 2009a) 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

[5]CosIng - Cosmetics - GROWTH - European Commission.
[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]Available online: https://www.efsa.europa.eu/en/data-report/food-consumption-data.

protocols on hazard identification and characterisation of sweeteners (EFSA, 2020a; EFSA FAF Panel, 2023) and on exposure assessment (EFSA, 2020b; EFSA FAF Panel, 2024).

The FAF Panel assessed the safety of Acesulfame K (E 950) as a food additive in line with the principles laid down in Regulation (EU) 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).

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. 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, 2020a; EFSA FAF Panel, 2023). For the other toxicological endpoints, the methods for hazard identification, including the assessment of internal validity for individual studies (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 to 3. In the current opinion, relevant studies retrieved from the literature with moderate to high RoB were considered and included in the WoE evaluation. In accordance with the revised protocol, studies previously evaluated by the SCF and considered for setting an ADI were also subjected to a RoB evaluation.

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 following terms were used to express the level of confidence in the body of evidence, irrespective of whether an association between exposure to the substance and adverse health outcome(s) were identified: 'high', 'moderate', 'low' and 'very low/no evidence identified'. For each level of confidence in the body of evidence, corresponding expressions for levels of evidence for adverse effects on health were denoted as 'high', 'moderate', 'low' and 'inadequate', respectively. Whereas when no adverse effects on health were identified, expressions for levels of evidence were denoted as 'high', 'moderate' and 'inadequate', respectively. More details on the WoE procedure are outlined in step 1.14 of the revised protocol on hazard identification and characterisation and 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. Hazard identification conclusions i.e. expressions of likelihood of an association between intake of acesulfame k (E 950) and adverse effect on health, were reached on groups of toxicological outcomes following a guidance developed by the FAF Panel (EFSA, 2020a; EFSA FAF Panel, 2023).

Dietary exposure to Acesulfame K (E 950) from its use as a food additive 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 different scenarios (see Section 3.4).

Finally, uncertainties in the hazard identification, characterisation and exposure assessment were identified and discussed.

3 | ASSESSMENT

3.1 | Technical data

3.1.1 | Specifications and identity of E 950

Acesulfame K is 6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide potassium salt.

Specifications for acesulfame K (E 950) have been defined in Commission Regulation (EU) No 231/2012[10] as described in Table 1.

[10]Commission Regulation (EU) No 231/2012: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32012R0231.

TABLE 1 Specifications for acesulfame K (E 950) according to Commission Regulation (EU) No 231/2012 and the JECFA (2006).

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Synonyms	Acesulfame potassium; Potassium salt of 3,4-dihydro-6-methyl- 1,2,3-oxathiazin-4-one-2,2-dioxide	Acesulfame K; INS No. 950
Definition		
CAS No		55589-62-3
Einecs	259-715-3	
Chemical name	6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide potassium salt	Potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide; Potassium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide
Chemical formula	$C_4H_4KNO_4S$	$C_4H_4KNO_4S$
Molecular weight	201.24	201.24
Assay	Content not less than 99% of $C_4H_4KNO_4S$ on the anhydrous basis	Not less than 99% and not more than 101% on the dried basis
Description	Odourless, white, crystalline powder. Approximately 200 times as sweet as sucrose	Odourless, white crystalline powder
Identification		
Solubility	Very soluble in water, very slightly soluble in ethanol	Freely soluble in water, very slightly soluble in ethanol
Spectrophotometry (ultraviolet absorption)	Maximum 227 ± 2 nm for a solution of 10 mg in 1000 mL of water	Dissolve 10 mg of the sample in 1000 mL of water. The solution shows an absorbance maximum at 227 ± 2 nm
Test for potassium	Passes test (test the residue obtained by igniting 2 g of the sample)	Passes test (test the residue obtained by igniting 2 g of the sample)
Precipitation test	Add a few drops of a 10% solution of sodium cobaltinitrite to a solution of 0.2 g of the sample in 2 mL of acetic acid and 2 mL of water. A yellow precipitate is produced	Add a few drops of a 10% solution of sodium cobaltinitrite to a solution of 0.2 g of the sample in 2 mL of acetic acid TS and 2 mL of water. A yellow precipitate is produced
Purity		
Loss on drying	Not more than 1% (105°C, 2 h)	Not more than 1% (105°C, 2 h)
pH		5.5–7.5 (1% solution)
Organic impurities	Passes test for 20 mg/kg of UV-active components	Passes test for 20 mg/kg of UV-active components
Fluoride	Not more than 3 mg/kg	Not more than 3 mg/kg
Lead	Not more than 1 mg/kg	Not more than 1 mg/kg
Mercury	Not more than 1 mg/kg	

An additional identification number for E 950, currently not reported in the Commission Regulation (EU) No 231/2012, is the CAS number 55589-62-3.

The chemical structure of E 950 is given in Figure 1.

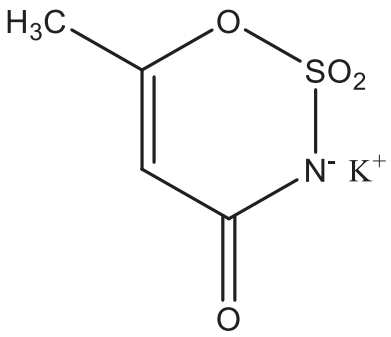


FIGURE 1 Chemical structure for acesulfame K (E 950).

In response to EFSA calls for data,[6,7] one interested business operator (IBO) provided technical data to support the E 950 re-evaluation (Documentation provided to EFSA No 1).

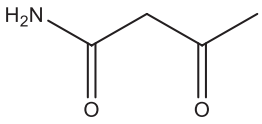
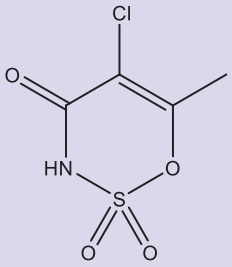
Some commercial samples of E 950 manufactured between 2016 and 2017 were tested against several purity criteria (Documentation provided to EFSA No 1). The purity was analysed in six batches of E 950, being two of the batches analysed by two different laboratories, either by high performance liquid chromatography (HPLC) or according to the Food Chemicals Codex (FCC) monograph on acesulfame K, and found to be in the range of 99.6%–100.2%. The loss on drying was 0.02%–0.03%. The concentration of potassium, determined by ion chromatography (IC), ranged from 19.4% to 19.5%. In six samples, the concentration of sulphate, determined by HPLC, was reported as below a limit of detection (LOD) (not reported) and a maximum limit of 20 mg/kg was indicated. The pH, determined by potentiometric method, was reported as either 6.9 or 7.0, and the pH, determined according to FCC Appendix IIB/C, for two of the samples was reported as either 5.7 or 6.4.

Organic impurities

In the EU specifications, there is a limit of 20 mg/kg for UV-active organic impurities, but there are no limits for specific organic impurities. In the SCF opinion (SCF, 2000), it was mentioned that this limit covers the possible formation of 5-chloro-acesulfame as an impurity in the production procedure.

The EU Pharmacopoeia monograph on acesulfame K includes two organic impurities: impurity A (acetylacetamide) with a limit value of not more than '0.125 per cent' (TLC method) and impurity B (5-chloro-acesulfame) with a limit value of not more than 20 mg/kg (high performance liquid chromatography with ultraviolet detection (HPLC-UV) method) (European pharmacopoeia, 2023). The structures of these two substances are shown in Table 2.

TABLE 2 Chemical structures of acesulfame K impurities included in the EU Pharmacopoeia monograph.

Impurity	Chemical name (IUPAC name)	CAS no.	Structure
A	Acetylacetamide Synonyms: acetoacetamide 3-oxobutanamide	5977-14-0	
B	5-chloro-acesulfame (5-chloro-6-methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide)	72827-08-8	

Acetylacetamide (impurity A, Table 2) is both an impurity and a degradation product of acesulfame K and its formation is described in Section 3.1.4.

5-chloro-acesulfame (impurity B, Table 2) was attributed by Boehshar and Burgard (2003) as an impurity arising during the production process of acesulfame K and further details are presented in Section 3.1.2.

In eight samples (Documentation provided to EFSA No 1), the concentration of 'unspecified impurities' (determined by HPLC-UV) or total organic impurities (determined by HPLC-UV) was reported by the IBO as below the limit of 20 mg/kg.

In the six samples, (Documentation provided to EFSA No 1) impurity A (acetylacetamide) was reported as 'below the LOD' without indicating the LOD of the HPLC method used. A limit value for acetylacetamide was stated to be 0.5–1 mg/kg. The impurity B (5-chloro-acesulfame), determined by HPLC, was reported as below 2 mg/kg equal to the limit value reported in the certificates of analysis.

The potential exposure to the identified organic impurities resulting from the use of E 954, considering that the impurities are present at the reported limit values, i.e. 1 mg/kg for acetylacetamide and 2 mg/kg for 5-chloro-acesulfame, was assessed as a worse-case scenario (Appendix E).

The Panel noted that the MOE for acetylacetamide calculated assuming the presence of this impurity in E 950 at the reported limit of 1 mg/kg, does not raise a safety concern.

The QSAR analysis of 5-chloro-acesulfame K triggered some genotoxicity alerts and therefore a TTC of 0.0025 μg/kg bw per day is applicable when assessing the safety of the presence of this impurity in the food additive (EFSA Scientific Committee, 2019). The Panel noted that the potential exposure to this impurity assuming its presence at the reported limit of 2 mg/kg is above the TTC value. In order to ensure that potential exposure to this impurity resulting from the use of E 950 would not raise a concern, a maximum limit of 0.1 mg/kg for 5-chloro-acesulfame K should be included in the specifications for the food additive (see Appendix E).

Samples of E 950 were analysed by Head space gas chromatography (GC) for the presence of solvents used in the manufacturing process (see Section 3.1.2) (Documentation provided to EFSA No 1). In all samples, the level of the solvents was reported as below the corresponding LOD for each solvent. Considering the different purification steps in the manufacturing process of acesulfame K, the Panel considered the presence of residual solvents in acesulfame K as unlikely.

Inorganic impurities

Regarding toxic elements, the IBO reported the results of the analysis of arsenic, lead, cadmium and mercury in commercial samples of E 950. Additionally, fluoride was also analysed (Documentation provided to EFSA No 1). Details of the data provided are available in Appendix E. The Panel noted that among the potential inorganic impurities tested, only lead, mercury and fluoride have defined limit values in EU specifications. Taking into account the data provided additionally for arsenic and cadmium, and taking also into account that the production process is an organic synthesis with final crystalisation and recrystalisation steps and so systematic contamination by these inorganics is not anticipated, the Panel did not see a need to recommend additional specification limits for arsenic and cadmium.

The Panel calculated the potential exposure to lead and mercury from the use of E 950 considering that: (i) the existing limit in EU specifications; (ii) the reported limit values (0.1 mg/kg for lead and 0.01 for mercury). The exposure calculations to these elements are presented and discussed in Appendix E.

Taking into account the calculations performed by the Panel (Table E.3), the fact that the food additive is not the only potential dietary source of toxic elements and that the maximum limits should be established based on actual levels in the commercial food additive the Panel recommended to lower the specification limit for lead and mercury. If the European Commission decides to revise the current limits in the EU specifications, the estimates of exposure to toxic elements intake as above could be considered.

The Panel also calculated the exposure to fluoride from the use of E 950 considering that it can be present in the food additive at the existing EU limit of 3 mg/kg (Table E.4). The resulting exposure (0.0002 mg/day and 0.0006 mg/day for the mean and 95th percentile in toddlers, respectively) was found to be four orders of magnitude below the tolerable upper intake level of 1.6 mg/day for children (1–3 years of age) proposed by the EFSA Scientific Committee (EFSA Scientific Committee, under public consultation).

Microbiological criteria

Microbiological analyses were performed by the IBO on six samples of E 950 (Documentation provided to EFSA No 1). However, the details on the methodology of analysis were limited (no information on LOD/limit of quantification (LOQ)). In all tested samples, *E. coli, Pseudomonas aeruginosa*, *Salmonella* sp. and *Staphylococcus aureus* were reported as 'negative' (product specification limit, i.e. negative in 1 g), and *Enterobacteriaceae*, total mesophilic counts, yeasts and mould counts were reported as below 10 colony forming unit (CFU)/g (product specification limit).

According to the IBO, any potential viable microorganisms existing in the product would be effectively eradicated throughout the production process, which incorporates a drying step. Furthermore, the IBO claimed that the substantial absence of water (< 1%) in the final product, which is packaged in accordance with hygienic standards, limits microbial growth (Documentation provided to EFSA No 1).

The Panel concurred with the statement of the IBO and considered that no specifications for microbiological criteria are needed to be included into the EU specifications for E 950.

Solubility

The IBO provided results of water solubility tests of E 950 performed according to the European Commission Regulation (EC) No. 440/2008 and OECD TG 105 methods (Documentation provided to EFSA No 2). The water solubility of acesulfame K (E 950) at 20°C was determined to be 237 g/L.

The Panel noted that the ultrafiltration step recommended in the EFSA Guidance particle-TR (EFSA Scientific Committee, 2021) to remove any small particles from the solubilised fraction was not included in this test for solubility from the IBO. Given the nature of this substance, the Panel considered nonetheless that the solubility of E 950 is substantially higher than the value of 33.3 g/L proposed as a criterion to decide whether an additional assessment for the fraction of small particles is needed according to the EFSA Guidance particle-TR (EFSA Scientific Committee, 2021).

Taking into account the water solubility value reported by the IBO for E 950 (i.e. 237 g/L), the Panel noted that E 950 can be considered as fully dissolved when consumed as a food additive. Therefore, the Panel considered that there is no concern with regard to the potential presence of small particles, including nanoparticles and acesulfame K can be assessed following the conventional risk assessment, i.e. EFSA Guidance for submission for food additives evaluations (EFSA ANS Panel, 2012).

Particle size distribution

The IBO provided information on particle size of E 950 determined by laser diffraction (LD) and scanning electron microscopy (SEM) images (Documentation provided to EFSA No 2). Taking into account the high solubility of E 950 in water (see above), the information on particle size was not further considered.

3.1.2 | Manufacturing process

The IBO provided detailed information on the manufacturing process of acesulfame K (E 950).

The production process of E 950 involves the following steps (Documentation provided to EFSA No 1):

1. Acetoacetamide-N-sulfonate triethylammonium salt (amide salt) formation.
2. Amide salt cyclisation and hydrolysis of the resulting sulphur trioxide adduct intermediate yielding acesulfame acid;
3. Neutralisation of the obtained acesulfame acid (using aqueous potassium hydroxide) yielding acesulfame K.
4. The obtained acesulfame K is then subjected to further purification steps, including crystallisation, filtration, recrystallisation, centrifugation and finally drying, sieving and packaging.

Dichloromethane is used as solvent in all the reaction steps.

Boehshar and Burgard (2003) have linked the formation of 5-chloro-acesulfame (impurity B) during the manufacturing of E 950, to the formation of chlorinating agents (e.g. chloromethyl chlorosulfate and methyl bis chlorosulfate) in a side reaction between the sulphur trioxide used and dichloromethane (if used as a solvent).

3.1.3 | Methods of analysis in food

Being a potassium salt that can dissociate (Figure 1), E 950 is normally analysed under chromatographic conditions where it is separated and then detected by UV or by mass spectrometry (MS) as acesulfame acid. Nonetheless, since quantitation invariably employs standards prepared using acesulfame K and since the authorised food additive is defined as acesulfame K, analytical results are correspondingly expressed as acesulfame K.

The IBO provided information on a method of analysis to quantify acesulfame K in foods and beverages using HPLC-UV (Hagenauer-Hener et al., 1990). The food products included lemonades, carbonated drinks, fruit nectars, flavoured milk drinks, delicatessen salads, jams and confectionery. The recovery of acesulfame K in carbonated drinks, flavoured milk drinks and fruit nectars was in the range 97%–110%. The LOD for acesulfame K in all tested food samples ranged from 0.5 to 1 mg/kg.

Many other methods to determine acesulfame K (E 950) in foods and beverages are available in the scientific literature. These deal with the analysis of both solid (e.g. candies, condiments) and liquid (e.g. carbonated soft drinks, beverages) food samples (George et al., 2010; Wasik et al., 2007, Wasik & Ulberth-Buchgraber, 2009; Bergamo et al., 2011; Wu et al., 2012; Vistuba et al., 2015; Yang et al., 2015; Kaufmann et al., 2018; Ozgur & Kasapoglu, 2019; Tighrine et al., 2019; Petrova & Christova-Bagdassarian, 2020).

The methods make use of sample pre-treatments, such as solid-phase extraction (SPE) (Wasik et al., 2007; Wasik & Ulberth-Buchgraber, 2009) and salting out liquid–liquid extraction (SALLE) (Tighrine et al., 2019), and mainly include high performance liquid chromatography (HPLC) or ultra-high performance liquid chromatography (UHPLC), coupled with various detectors, such as UV (George et al., 2010), diode array detection (DAD) (Imanulkhan et al., 2020; Jankulovska et al., 2024; Nour et al., 2007; Sun et al., 2018; Trandafir et al., 2009; Wu et al., 2012), evaporative light scattering detection (ELSD) (Wasik et al., 2007), electrospray ionisation mass spectrometry (ESI/MS) (Yang & Chen, 2009; Zygler et al., 2011) and tandem mass spectrometry (MS/MS) (Wu et al., 2014). Other researchers have used capillary electrophoresis with contactless conductivity detector (CE-C4D) (Stojkovic et al., 2013); UV- vis measurements and partial leas squares (PLS) (Llamas et al., 2008) or FT-Raman spectroscopy (Duarte et al., 2017).

It is beyond the scope of this re-evaluation of E 950 to provide a comprehensive review of all of the analytical methods reported in the literature. It is clear that there are methods widely available for all of the relevant food categories, with acceptable performance characteristics for recovery, selectivity, sensitivity, accuracy and precision.

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

The IBO reported a shelf life for acesulfame K (E 950) of 5 years from the date of manufacture, when properly packed and stored, i.e. stored in the original closed packaging, protected from sunlight, at ambient temperature (max. 30°C) and dry conditions (max. 65% RH) (Documentation provided to EFSA No 1). This statement was supported by experimental data where the long-term stability of E 950 was tested up to 6 years. No significant changes were observed in the monitored parameters (i.e. specifications and purity parameters) throughout the storage period (Documentation provided to EFSA No 1).

In addition, the IBO also provided information from unpublished stability studies of E 950 during processing and storage based on experimental data (Documentation provided to EFSA No 1).

The stability of E 950 was tested in beverages as orange juice (pH 3.2) and tonic (pH 3.7) at different temperatures (from 75°C or 90°C) and time periods (2, 5 or 15 min) (Documentation provided to EFSA No 1). The recovery rates were 97% in the case of orange juice or 102%–110% in tonic. The stability of E 950 was also tested under baking conditions in several foods (simple dough, cookies, cheesecake filling, plain cake, short pastries and apple pie) at different temperatures (from 180°C to 275°C) and time periods (from 5 to 80 min). There was no observed loss of E 950 in any of the tested samples, with recovery rates in the range of 95%–110%.

The stability of E 950 under sterilisation conditions was examined in canned foodstuffs for cherries heated to 103°C for 30 min at different pH-values (2.75–4) and peas heated to 121°C for 10 min at different pH-values (4–6.5) (Documentation provided to EFSA No 1). There was no observed loss of E 950 in any of the tested samples, with recovery rates in the range of 94%–109%.

The stability of acesulfame K was also reported in aqueous ethanol solutions (0%, 5% and 20% ethanol) and at different pH-values (3, 4 and 6) over a 12-months period. No information on the temperature was provided. The recovery rates were in the range of 98%–114% (Documentation provided to EFSA No 1).

The stability of E 950 in foods during processing and storage, based on the results from Lotz et al. (1992) was provided (Documentation provided to EFSA No 1). To simulate non-fermented dairy products subjected to high-temperature processing, heated aqueous buffered solutions (pH 5–7) of acesulfame K (250 mg/L) were heated at different temperatures (101, 111, 121 and 131°C) for various time periods (1, 2, 5, 10, 20 and 30 min). The recovery rates for acesulfame K ranged from 99% to 101%.

The Panel noted that in the JECFA evaluation (JECFA, 1991), acetylacetamide (acetoacetamide) and acetoacetamide-N-sulfonic acid were indicated as degradation products of acesulfame K under specific conditions of pH and temperature.

To assess the stability of acesulfame K under conditions of low pH and prolonged periods of storage, the degradation products acetylacetamide (that is also reported as an impurity) and acetoacetamide-N-sulfonic acid, were monitored in aqueous solutions of acesulfame K (4000 or 20,000 mg/kg) (Documentation provided to EFSA No 1). Samples were adjusted to pH 2.7, 3.0 and 3.3 and stored at 20°C or 30°C over a period of 30 weeks. The analysis for acesulfame K and the 2 degradation products was by high performance liquid chromatograph–diode array detection (HPLC–DAD). At the end of the storage period, the recovery rate of acesulfame K was as follows: at pH 2.7, the recovery rate was 84.6% and 95.1% at 30°C and 20°C, respectively; at pH 3.0, the recovery rate was 92.3% and 97.8% at 30°C and 20°C, respectively; at pH 3.3, the recovery rate was 95.0% and 99.0% at 30°C and 20°C, respectively. At the end of the storage period, the acetylacetamide content (calculated as % of the initial acesulfame K content) was as follows: at pH 2.7, 0.583% and 0.070% at 30°C and 20°C, respectively; at pH 3.0, 0.149% and 0.018% at 30°C and 20°C, respectively; at pH 3.3, 0.041% and 0.003% at 30°C and 20°C, respectively. At the end of the storage period, the acetoacetamide-N-sulfonic acid content (calculated as % of the initial acesulfame K content) was as follows: at pH 2.7, 2.70% and 1.89% at 30°C and 20°C, respectively; at pH 3.0, 1.65% and 1.06% at 30°C and 20°C, respectively; at pH 3.3, 0.96% and 0.57% at 30°C and 20°C, respectively. The Panel noted that under conditions of pH 2.7 and a temperature of 30°C, there was a steady decline in the concentration of acesulfame K, reaching a value of 84.6% after 30 weeks. Taking into account that the sum of the degradation products acetylacetamide and acetoacetamide-N-sulfonic acid did not exceed 3.3%, the Panel noted that around a 12% of the food additive was degraded to unknown degradation products. This has been attributed to a further decomposition of acetylacetamide and acetoacetamide-N-sulfonic acid that occurs at acidic conditions (pH <3) (JECFA, 1991). Nevertheless, the report did not provide any details regarding the presence of these additional degradation products in E 950.

The formation of the degradation products acetylacetamide and acetoacetamide-N-sulfonic acid was also investigated under the sterilisation conditions that are normally applied during the manufacturing process of E 950 (Documentation provided to EFSA No 1). Samples of aqueous buffered solutions of acesulfame K (20,000 mg/kg) at pH-values ranging from 3 to 7 were heated in a laboratory autoclave at 100°C (pH 3–4) or 121°C (pH 4–7). At both temperatures, holding times of 10, 20, 30, 45 and 60 min were used. Samples were analysed by HPLC-DAD. After 60 min-heat treatment at 100°C, acetylacetamide content (calculated as % of the initial acesulfame K) ranged from 0.004% (pH 3.75) to 0.097% (pH 3). After 60 min-heat treatment at 121°C, acetylacetamide was only detected at pH 4 (0.015%). After 60 min-heat treatment at 100°C, acetoacetamide-N-sulfonic acid content ranged from 0.121% (pH 4) to 0.835% (pH 3). After 60 min-heat treatment at 121°C, only acetoacetamide-N-sulfonic acid was detected at pH 4 (0.128%) and pH 4.5 (0.044%). Acetylacetamide and acetoacetamide-N-sulfonic acid were not detected at pH-values above 4.5 and 5, respectively, at temperature of 121°C.

The Panel also noted that the loss of acesulfame K in acidic conditions designed to simulate cola beverages, is reported to be 15% after 1 year of storage at 25°C and 25% after 3 months at 40°C with the formation of degradation products such as acetoacetamide-N-sulfonic acid, acetylacetamide, acetoacetic acid and acetone (DuBois & Prakash, 2012). The Panel had not access to the original study to confirm these data.

Additional information on the stability of E 950 in food was also retrieved in the scientific literature reviewed performed by the Panel.

Acesulfame K was added into lime-lemon flavoured carbonated beverage samples and stored at 4, 27 and 37°C over a period of 60 days. The sweetener concentration was determined by HPLC-UV at 220 nm. A loss of up to 6.1% (at 37°C) was reported (Malik et al., 2000).

In another study (Buchgraber & Wasik, 2009), the stability of six sweeteners, including acesulfame K, was investigated in beverages and canned fruits stored at –20, 4 and 20°C for 3 days, 1, 2 and 4 weeks. A reference sample was maintained at –70°C as a benchmark temperature for stability. No changes in the levels of acesulfame K were reported under any of the time/temperature conditions.

A similar experiment was carried out by George et al. (2012). The stability of different sweetener blends (acesulfame K, aspartame, saccharin and sucralose) in *lassi* was assessed under refrigerated conditions (6–8°C) over a storage period of 15 days. The analysis was performed by HPLC-UV at 200 nm and 220 nm. Recovery rates of acesulfame K ranged from 98.3% (day 0) to 99.8% (day 15).

The stability of a sweetener blend composed of acesulfame K (E 950) and aspartame (E 951) (50:50) in whey lemon beverage was assessed under refrigerated conditions (6–8°C) over a storage period of 15 days (Arora et al., 2013). Analysis for acesulfame K was by HPLC-UV at 200 and 220 nm. Recovery rates of acesulfame K were 97%–98% at all sampling timepoints.

Bawane and Singhal (2019) studied the stability of acesulfame K in low-energy *kheer* (pH around 6), a traditional Indian dessert. The sweetener was incorporated into the rice used for formulating extruded noodles, which are used as a basis for creating ready-to-prepare *kheer*. Different extrusion temperatures (70, 90, 110, 130 and 150°C) and screw speeds (80 and 200 rpm) were used with a fixed moisture content of 200 g/kg. Acesulfame K was analysed by HPLC-DAD at 227 nm. The recovery rate for acesulfame K was higher than 90%.

The Panel considers that acesulfame K is degraded to a certain amount at pH lower than 3 with increasing temperatures. Information on the toxicity of acetylacetamide and acetoacetamide-N-sulfonic is provided in Sections 3.5.3 and 3.7, and in Appendix E.

3.2 | Authorised uses and use levels

Maximum levels of acesulfame K (E 950) have been defined in Annex II, Part E, to Regulation (EC) No 1333/2008[11] on food additives, as amended. In this document, these levels are called maximum permitted levels (MPLs).

Currently, acesulfame K (E 950) is an authorised food additive in the EU in 34 food categories (FCs) (corresponding to 47 authorised uses). It is authorised at MPLs ranging from 25 to 2500 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). Acesulfame K will dissociate in food into acesulfame and potassium.

The authorised food additive 'salt of aspartame-acesulfame' (E 962), for which the re-evaluation is ongoing and for which MPLs in food are expressed as 'acesulfame K equivalent' or 'aspartame equivalent' (Regulation (EC) No 1333/2008), is another source of exposure to acesulfame K. This salt dissociates and so the total amount of acesulfame in food will come from both food additives. For this reason, the exposure to acesulfame K from the use of both acesulfame K (E 950) and the salt of aspartame-acesulfame (E 962) is considered in this opinion.

Table 3 lists the food categories with their restrictions/exceptions that are permitted to contain acesulfame K (E 950) and the salt of aspartame-acesulfame (E 962) as defined in Annex II to Regulation (EC) No 1333/2008 and the MPLs. Note that the salt of aspartame-acesulfame (E 962) can only be added to food categories that are also authorised to contain acesulfame K (E 950).

TABLE 3 Food categories that are permitted to contain acesulfame K (E 950) and/or 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 (mg/L or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	E 950/E 962	Only energy-reduced products or with no added sugar	350[a]
03	Edible ices	E 950/E 962	Only energy-reduced or with no added sugar	800[a]
04.2.2	Fruit and vegetables in vinegar, oil or brine	E 950/E 962	Only sweet–sour preserves of fruit and vegetables	200[a]
04.2.3	Canned or bottled fruit and vegetables	E 950/E 962	Only fruit energy-reduced or with no added sugar	350[a]
04.2.4.1	Fruit and vegetable preparations excluding compote	E 950/E 962	Only energy-reduced	350[a]
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EC	E 950/E 962	Only energy-reduced jams, jellies and marmalades	1000[a]
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	E 950/E 962	Only energy-reduced jams, jellies and *marmalades*	1000[a]
04.2.5.3	Other similar fruit or vegetable spreads	E 950/E 962	Only energy-reduced fruit or vegetable spreads and dried fruit-based sandwich spreads, energy-reduced or with no added sugar	1000[a]
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	E 950/E 962	Only energy-reduced or with no added sugar	500[a]

[11]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.

TABLE 3 (Continued)

Food category number	Food category name	E-number	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
05.2	Other confectionery including breath refreshening microsweets	E 950/E 962	Only cocoa or dried fruit-based, energy reduced or with no added sugar	500
05.2	Other confectionery including breath refreshening microsweets	E 950	Only energy-reduced tablet form confectionery	500
05.2	Other confectionery including breath refreshening microsweets	E 950/E 962	Only confectionery with no added sugar	500[a]
05.2	Other confectionery including breath refreshening microsweets	E 950/E 962	Only cocoa, milk, dried fruit or fat-based sandwich spreads, energy-reduced or with no added sugar	1000[a]
05.2	Other confectionery including breath refreshening microsweets	E 950/E 962	Only starch-based confectionery energy reduced or with no added sugar	1000[a]
05.2	Other confectionery including breath refreshening microsweets	E 950/E 962	Only breath-freshening microsweets, with no added sugar	2500[a]
05.3	Chewing gum	E 950	Only with added sugar or polyols, as flavour enhancer	800
05.3	Chewing gum	E 950/E 962	Only with no added sugar	2000[a]
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	E 950/E 962	Only sauces	350[a]
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	E 950/E 962	Only confectionery with no added sugar	500[a]
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	E 950/E 962	Only cocoa or dried fruit-based, energy reduced or with no added sugar	500[a]
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	E 950/E 962	Only starch-based confectionery energy reduced or with no added sugar	1000[a]
06.3	Breakfast cereals	E 950/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	1200/1000[a]
07.2	Fine bakery wares	E 950	Only cornets and wafers, for ice-cream, with no added sugar	2000
07.2	Fine bakery wares	E 950/E 962	Only essoblaten – wafer paper	2000/1000[a]
09.2	Processed fish and fishery products including molluscs and crustaceans	E 950/E 962	Only sweet–sour preserves and semi-preserves of fish and marinades of fish, crustaceans and molluscs	200
11.4.1	Table-Top Sweeteners in liquid form	E 950/E 962		QS
11.4.2	Table-Top Sweeteners in powder form	E 950/E 962		QS
11.4.3	Table-Top Sweeteners in tablets	E 950/E 962		QS
12.4	Mustard	E 950/E 962		350[a]
12.5	Soups and broths	E 950/E 962	Only energy-reduced soups	110[a]
12.6	Sauces	E 950/E 962		350[a]
12.7	Salads and savoury based sandwich spreads	E 950/E 962	Only *Feinkostsalat*	350[a]
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	E 950/E 962		450[a]
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 950/E 962		450[a]
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	E 950/E 962	Only energy-reduced or with no added sugar	350[a]
14.1.4	Flavoured drinks	E 950/E 962	Only energy reduced or with no added sugar	350[a]
14.2.1	Beer and malt beverages	E 950/E 962	Only energy-reduced beer	25[a]

(Continues)

TABLE 3 (Continued)

Food category number	Food category name	E-number	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
14.2.1	Beer and malt beverages	E 950/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 for 'Obergäriges Einfachbier'; Beers with a minimum acidity of 30 milli-equivalents expressed as NaOH; Brown beers of the 'oud bruin' type	350[a]
14.2.3	Cider and perry	E 950/E 962	Excluding cydr jakościowy, perry jakościowe, cydr lodowy, perry lodowe	350[a]
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	E 950/E 962		350[a]
15.1	Potato-, cereal-, flour- or starch-based snacks	E 950/E 962		350/500[a]
15.2	Processed nuts	E 950/E 962		350/500[a]
16	Desserts excluding products covered in category 1, 3 and 4	E 950/E 962	Only energy-reduced or with no added sugar	350[a]
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children	E 950/E 962		500[a]
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children	E 950/E 962	Only food supplements in chewable form	2000[a]
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children	E 950/E 962		350[a]
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children	E 950/E 962	Only food supplements in syrup form	2000[a]

Abbreviations: MPL, maximum permitted level; QS, Quantum Satis.

[a]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 (E962), either alone or in combination with E 950 or E 951.

The Panel noted that for FCs 05.2 and 09.2, the MPLs for E 962/E 950 do not indicate the footnote (a). As it seems likely that this may be an omission, it was assumed that footnote (a) is also applicable to these food categories.

Acesulfame K (E 950) and the salt of aspartame-acesulfame (E 962) are not authorised to be used according to Annex III to Regulation (EC) No 1333/2008 i.e. these additives cannot be added into other food additives, food enzymes, food flavourings or nutrients. Therefore, all authorised uses of E 950/E 962 are reported in Table 3.

3.3 | Exposure data

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 one public call[12] for concentration data (use level and/or analytical data) in sweeteners, including acesulfame K (E 950) and salt of aspartame-acesulfame (E 962). In addition, analytical data on acesulfame K (E 950) and aspartame-acesulfame salt (E 962) can be submitted yearly to EFSA through the open calls for food additive occurrence data in food and beverages intended for human consumption.[13]

In response to these calls, use levels were made available by industry stakeholders and analytical data were made available by Member States, on both acesulfame K (E 950) and aspartame-acesulfame salt (E 962). Both analytical data and use

[12]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.

[13]05 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.

level data on acesulfame K (E 950) and aspartame-acesulfame salt (E 962) in food and beverages as available in EFSA's Data Warehouse on April 11th, 2024 and September 12th, 2024 respectively, were considered for the present assessment.

Reported use levels of acesulfame K (E 950) and salt of aspartame-acesulfame (E 962)

Industry provided EFSA with 519 use levels of acesulfame K (E 950) in foods and beverages for 26 out of the 47 authorised uses, corresponding to 21 out of the 34 authorised food categories[14] according to Annex II to Regulation (EC) No 1333/2008 (Table 3).

The use levels of acesulfame K (E 950) were provided by Association of the European Self-Medication Industry (AESGP), Cloetta Suomi Oy, European Dairy Association (EDA), European Fruit Juice Association (AIJN), Food Drink Europe (FDE), Food Supplement Europe (FSE), International Chewing Gum Association (ICGA Europe), International Sweetener Association (ISA), Produlce, Specialised Nutrition Europe (SNE), Total Diet & Meal Replacements Europe (TDMR) and Unione Italiana Food (AIDEPI) (Documentation provided to EFSA No 3 to 12).

The Panel noted that for FC 05.1 'Cocoa and Chocolate products as covered by Directive 2000/36/EC' and FC 14.1.3 'Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products', the use levels provided referred only to niche products. Since analytical data were also available for these food categories, the Panel did not use these reported use levels in the exposure assessment, but used the analytical data instead (EFSA, 2020b).

Additionally, 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 (EC) No 1333/2008 (Table 3). 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 13 to 15).

Annex A, Table A1 summarises the reported use levels of acesulfame K (E 950) and salt of aspartame-acesulfame (E 962) in foods and beverages.

Analytical results of acesulfame K (E 950) and salt of aspartame-acesulfame (E 962) provided by Member States

Four analytical results of the salt of aspartame-acesulfame (E 962) were reported to EFSA between 2013 and 2024.[15] These results were reported by Slovakia and were all left-censored (< LOD).

Regarding acesulfame K (E 950), 44,294 analytical results were reported to EFSA between 2013 and 2024.[16] The Panel considered the analytical results for foods and beverages sampled during the last 10 years in the assessment. Those sampled before 2013 were considered outdated.

The reporting countries were Germany ($n=33{,}057$), Slovakia (2430), Austria ($n=1911$), Hungary ($n=1877$), Italy ($n=1218$), Ireland ($n=1013$), Belgium ($n=585$), Spain ($n=507$), Lithuania($n=415$), United Kingdom ($n=296$; data received before 1st January 2021 (Brexit)), Luxembourg ($n=270$), Croatia ($n=215$), Portugal ($n=215$), Cyprus ($n=187$), Czechia ($n=37$), Greece ($n=30$), Denmark ($n=27$) and France ($n=4$). In total, 84.5% of these analytical results were left-censored (47.8% < LOD and 36.7% < LOQ).

Sweeteners exposure assessment is based on the assumption that foods containing the sweetener are identified from the food consumption database and that the levels of the sweeteners in these foods are derived from the quantified analytical results only (EFSA, 2020a, 2020b). Therefore, these left-censored data ($n=6810$) and values not fit for the purpose for the exposure assessment (e.g. binary data, values from a non-EU country, $n=19$) were excluded. This resulted in 6891 analytical values for acesulfame K (E 950) to be considered.

Of these 6891 analytical values, 24 were from a non-accredited laboratory or were obtained without an internally validated method, and 148 were obtained via suspect sampling. These values were also discarded, together with four additional values, which were found to be duplicates.

The Panel noted that 393 quantified analytical values were reported in food categories in which acesulfame K (E 950) is not authorised for direct addition according to Annex II of Regulation (EC) No 1333/2008), including FC 01.7.1 'Unripened cheese', FC 07.2 'Fine bakery wares' (other than 'cornets and wafers, for ice-cream' and 'essoblaten – wafer paper', see Table 1), FC 12.9 'Protein products, excluding products covered in category 1.8', FC 14.1.1 'Water', FC 14.1.2 'Fruit juices as defined by Directive 2001/112/EC and vegetable juices' and FC 14.1.5.2 'Other'. In addition, 32 quantified analytical values sampled before 2018 refer to 'Fine bakery products for special nutritional uses' (FC 07.2) in which E 950 and E 962 where authorised until then.[17] All these results ($n=601$) were not considered in the exposure assessment.

In addition, 402 analytical values for authorised food categories were above the MPL and were not considered. These results were mainly for FC 14.1.4 'Flavoured drinks', FCs 17, 17.1 and 17.2 'Food supplements supplied in solid and liquid form', FC 05.2 'Other confectionery including breath-freshening microsweets', FC 12.7 'Salads and savoury based sandwich spreads', and FC 09.2 'Processed fish and fishery products including molluscs and crustaceans'.

[14]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.

[15]Extraction of the analytical data from the EFSA database on 12/09/2024.

[16]Extraction of the analytical data from the EFSA database on 11/04/2024.

[17]https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0097.

Overall, 5888 analytical results were available for foods in which acesulfame K (E 950) is authorised to be added according to Annex II to Reg No1333/2008. This corresponds to 26 food categories out of the 34 in which acesulfame K (E 950) is authorised and 33 of its 47 authorised uses.

The majority of these 5888 analytical values were for FC 14.1.4 'Flavoured drinks' ($n = 4091$), followed by FC 05.3 'Chewing gum' ($n = 330$), FC 05.2 'Other confectionery including breath-freshening microsweets' ($n = 258$), FC 01.4 'Flavoured fermented milk products' ($n = 268$) and FC 17 'Food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children' ($n = 163$). For other food categories the number of analytical values varied from 1 to 107.

The Panel noted that, although the analytical data were reported for acesulfame K (E 950) and salt of aspartame-acesulfame (E 962), all analytical data could be derived from the use of either of the two additives.

Details on the analytical results of acesulfame K (from acesulfame K (E 950) and salt of aspartame-acesulfame (E 962)) available for the exposure assessment are provided in Annex A, Table A2.

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

For the purpose of this Scientific Opinion, Mintel's Global New Products Database (GNPD) was used for checking the labelling of food and beverage products and food supplements for acesulfame K (E 950) and the salt of aspartame-acesulfame (E 962) within the EU's food market as the database contains the required ingredient information on the label. Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide, currently having 24 out of its 27 EU member countries and Norway included.[18]

In Mintel's GNPD[19], acesulfame K (E 950) was labelled on 6168 products between January 2020 and January 2025. These products belong mainly to 'Carbonated Soft Drinks' ($n = 1332$), 'Nutritional & Meal Replacement Drinks' ($n = 758$), 'Gum' ($n = 464$), 'Energy Drinks' ($n = 426$) and 'Vitamins & Dietary Supplements' ($n = 392$).

Mintel's GNPD did not contain any food products labelled to contain the salt of aspartame-acesulfame (E 962).

Annex A Table A3 lists the percentages of the food products labelled to contain acesulfame K (E 950) out of the total number of food products per food sub-category according to Mintel's GNPD food classification. The percentages ranged from 0.01% in 'Hard Cheese & Semi-Hard Cheese', 'Vegetables', 'Poultry Products' and 'Chocolate Tablets' to 62% in the food sub-category 'Gum'. The average percentage of foods labelled to contain acesulfame K (E 950) was 2.5%.

Table A3 also contains the list of corresponding food categories according to Annex II to Regulation (EC) No 1333/2008. Mintel's GNPD indicated uses of acesulfame K (E 950) in two authorised food categories for which no use levels/analytical data were reported to EFSA (i.e. foods from FC 15.1 'Potato-, cereal-, flour- or starch-based snacks' ($n = 21$, from six Mintel sub-categories) and FC 06.3 'Breakfast cereals' ($n = 15$, from two Mintel sub-categories)).

The Panel noted also that for a few food sub-categories in which the use of acesulfame K (E 950) is not authorised, foods were found labelled to contain this additive (i.e. 'Baking Ingredients & Mixes' ($n = 31$), Sweet Biscuits/Cookies' ($n = 19$, excluding two waffles products) and 'Plant Based Spoonable Yogurts (Dairy Alternatives)', 'Meat Snacks', 'Poultry Products', 'Soft Cheese & Semi-Soft Cheese', 'Fresh Cheese & Cream Cheese', 'Hard Cheese & Semi-Hard Cheese', 'Vegetable Snacks and Creamers' ($n = 1$ for each Mintel sub-category)). However, as the linkage between Mintel's GNPD food sub-categories and the food categories according to Annex II to Regulation No 1333/2008 cannot be considered as completely accurate, these results should only be considered indicative of possible unauthorised use of acesulfame K (E 950) in foods. Furthermore, the Panel noted that the percentage of products containing acesulfame K (E 950) from (potentially) non-authorised sub-categories out of the total number of products labelled as containing E 950 on the market was 1%. The Panel also noted that products from 'Baking Ingredients & Mixes' and 'Sweet Biscuits/Cookies' (belonging to FC 07.2) were reported to contain acesulfame K (from either of the two food additives) by Member States (see Annex A, Table A2).

3.3.3 | Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011)). The version of the Comprehensive database taken into account in this assessment was published in December 2022.[20] Data from EU Member States were considered for the estimations.

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for

[18] Missing Cyprus, Luxembourg and Malta.

[19] https://www.gnpd.com/sinatra/home/ accessed on 3/1/2025.

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

exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database includes the currently best available food consumption data across Europe.

Food consumption data from infants, toddlers, children, adolescents, adults and the elderly were used in the exposure assessment. For the present assessment, food consumption data were available from 43 different dietary surveys carried out in 22 Member States (Table A4). Not all Member States provided consumption information for all population groups, and in some cases food consumption data from more than one consumption survey of one country was available. In most cases, when different dietary surveys were available for one country and age class, the data from the most recent survey were used. However, when two national surveys from the same country gave a better coverage of the age range than using only the most recent one, both surveys were kept. For details on each survey, see Annex A, Table A4.

TABLE 4 Population groups considered for the exposure estimates of acesulfame K.[21]

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

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

Since 2018, all consumption records in the Comprehensive Database have been codified according to the FoodEx2 classification system (EFSA, 2015). Nomenclature from the FoodEx2 classification system has been linked to the food categorisation system of Annex II to Regulation (EC) No 1333/2008, part D, to perform exposure assessments of food additives. In practice, the FoodEx2 food codes were matched to the food categories. For a detailed description of the methodology used to link these codes to the food categories, see section 5.2.1 of EFSA 2020 (EFSA, 2020b). In FoodEx2, facets are used to provide further information about different properties and aspects of foods recorded in the Comprehensive Database. Facets have been used in the exposure assessment of acesulfame K (E 950) to further identify foods to be included in the assessment (e.g. sweetener-related facets for foods in relevant food categories, see details in Annex A, Table A5).

Food categories considered for the exposure assessment of acesulfame K[20]

Food categories for which concentration data of acesulfame K were provided, were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx2 classification system), at the most detailed level possible (up to FoodEx2 Level 7) (EFSA, 2015).

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 acesulfame K (E 950) (see details in Table 3). 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, 2024).

As FC 17 'Food supplements' does not consider food supplements for infants and toddlers as defined in the legislation, therefore, the exposure estimates of acesulfame K for these two population groups did 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. meal replacement drink reclassified as flavoured drink).

Some restrictions/exceptions of certain food categories are not referenced in the EFSA Comprehensive Database, and therefore the whole food category was considered in the exposure assessment (Annex A, Table A5). This may have resulted

[21]See Section 4.

in overestimation/underestimation of the exposure via six food categories, namely FCs 05.2 'Other confectionery including breath refreshening microsweets', 05.4 'Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4', 14.2.1 'Beer and malt beverages', 14.2.3 'Cider and Perry', 17.1 'Food supplements supplied in a solid form, excluding food supplements for infants and young children' and 17.2 'Food supplements supplied in a liquid form, excluding food supplements for infants and young children'. An example of a possible underestimation is the exposure to acesulfame K for consumers of 'Brown beers of the 'oud bruin' type', falling within FC 14.2.1. 'Brown beers of the 'oud bruin' type' is a specific type of beer that is not referenced in the EFSA Comprehensive database. Due to this, the MPL for other beer and malt beverages within FC 14.2.1, which is lower than the one set for 'Brown beers of the 'oud bruin' type', was considered in the exposure assessment.

Use of acesulfame K (E 950) and the salt of aspartame-acesulfame (E 962) in FC 06.3 'Breakfast cereals' is restricted to 'only breakfast cereals with a fibre content of more than 15%, and containing at least 20% bran, energy reduced or with no added sugar (see Table 3). Considering the whole food category would result in a large overestimation of the exposure to acesulfame K, and therefore this food category was not considered in any of the exposure assessment scenarios.

Food supplements in solid and liquid form have a different MPL which is applicable to supplements in a chewable or syrup form, respectively (see Table 1). According to Mintel GNPD, less than 10% of the solid food supplements are in a chewable form and only two food supplements in liquid form are syrup. Therefore, the MPLs considered for FCs 17.1 and 17.2 were respectively 500 and 350 mg/kg. This may lead to a minor underestimation.

For a few food categories, no concentration data were available and could therefore not be considered in the refined exposure assessment (see Section 3.4): 12.4 'Mustard' and 15.1 'Potato-, cereal-, flour- or starch-based snacks'. Furthermore, the Panel noted that for some food categories, the number of analytical results was limited (< 5).

Overall, out of the 34 food categories in which acesulfame K (E 950) and the salt of aspartame-acesulfame (E 962) are authorised, 31 food categories were included in the *regulatory maximum level exposure scenario* (28 food categories with an MPL and the maximum use levels for FCs 11.4.1, 11.4.2 and 11.4.3 ('Table-top sweeteners')). For the refined scenarios (see Section 3.4), 29 food categories were included. Compared to the *regulatory maximum level exposure scenario*, FCs 06.3 'Breakfast cereals' and 15.2 'Processed nuts' were not included due to lack of concentration data.

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

3.4 | Exposure estimates

Since the analytical data can derive from the use of either acesulfame K (E 950) or the salt of aspartame-acesulfame (E 962), and the MPLs are expressed on the maximum of acesulfame K coming from both additives, the Panel considers that the exposure assessment calculated for acesulfame K includes both the use of acesulfame K (E 950) and aspartame-acesulfame salt (E 962).

The Panel considered appropriate, in the remit of the re-evaluation of sweeteners, to estimate the chronic exposure to acesulfame K (EFSA FAF Panel, 2024). 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 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 acesulfame K, 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 protocol (EFSA FAF Panel, 2024).

Chronic dietary exposure was calculated by combining MPLs/concentration levels of acesulfame K (from E 950 and E 962) in each food with the average daily consumption of each food at individual level in each dietary survey and population group. Exposure estimates per individual were divided by the individual's body weight resulting in a distribution of daily individual average exposures per kilogram body weight. Based on these distributions, the mean and 95th percentile (P95) exposures were calculated per survey and per population group. Mean and P95 estimates based on dietary surveys/population groups with less than six or 60 consumers, respectively, are not presented (EFSA, 2011).

In this evaluation, as stated in section 5.2.3 in the protocol (EFSA FAF Panel, 2024), the dietary exposure was assessed for consumers only of at least one food category that could contain acesulfame K.[22] Exposure estimates for these population groups are assumed to be the best approximate reflecting the exposure levels in diabetics, which is considered to be the population with the highest exposure to sweeteners (EFSA, 2020b). 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 a higher number of non-consumers within certain food categories.

[22]Part of the survey population may have no exposure to the additive because they did not report eating a food from one of the food categories that could contain the additive (these are 'non-consumers'). The sub-group who reports eating these foods are called consumers.

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, also the exposure to acesulfame K for consumers only of each single food category (but still considering their whole diet) was 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. More explanation is given below.

Regulatory maximum level exposure assessment scenario

The *regulatory maximum level exposure assessment scenario* is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008 and in case of *QS*, on maximum reported use levels/the highest reliable percentiles of the analytical levels when available. For acesulfame K, the MPLs, as listed in Table A5 of Annex A, were used and for the three food categories of FC 11.4 'Table-top sweetener', in which E 950 and E 962 are authorised according to *QS*, the highest reliable percentile of the reported analytical results was used.

Refined brand-loyal exposure assessment scenario

The *refined brand-loyal exposure assessment scenario* for acesulfame K was based on use levels reported by food industry or analytical results reported by Member States. This exposure scenario considers only those food categories for which these data were provided to EFSA. In this refined scenario, it was assumed that a consumer is exposed long-term to acesulfame K (from E 950 and/or E 962) present at the maximum reported use level/the highest reliable percentile of the analytical data for one food category and at the mean of typical use levels/mean of analytical data for the other authorised food categories. For more details, see the protocol (EFSA FAF Panel, 2024).

Annex A, Table A5 summarises the concentration levels of acesulfame K used in the *refined brand-loyal exposure assessment scenario*.

Refined regulatory maximum level exposure assessment scenario

Results of the regulatory maximum level exposure assessment scenario are not comparable to the exposure estimates of the refined brand-loyal exposure assessment scenario. Since the number of food categories considered per scenario is different ($n = 31$ and 29, respectively), the underlying populations of consumers only are not the same. For this reason, the Panel also performed a refined regulatory maximum level exposure assessment scenario based on the same population group as included in the refined brand-loyal exposure assessment scenario (Annex A, Table A5).

3.4.1 | Dietary exposure to acesulfame K from the use of E 950 and E 962

Table 5 summarises the estimated dietary exposure to acesulfame K from the use of E 950 and E 962 as food additives in six population groups (Table 4) according to three exposure scenarios among consumers only of at least one food category containing E 950 and/or E 962.

TABLE 5 Summary of chronic dietary exposure to acesulfame K from the use of acesulfame K (E 950) and the salt of aspartame- acesulfame (E 962) 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 acesulfame K (minimum–maximum across the dietary surveys in mg/kg bw per day and number of surveys in brackets).

	Infants (12 weeks-11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario[a]						
• Mean[b]	0.1–1.9 (10)	0.3–3.8 (15)	0.3–3.0 (19)	0.1–1.7 (21)	0.2–1.7 (22)	0.1–1.6 (23)
• 95th percentile[c]	0.4 (1)	0.6–14.1 (14)	0.9–9.8 (19)	0.4–5.8 (20)	0.7–6.8 (22)	0.3–8.9 (22)
Refined regulatory maximum level exposure assessment scenario						
• Mean[b]	0.1–4.7 (5)	0.3–5.6 (13)	0.1–3.2 (19)	0.04–2.0 (21)	0.1–2.9 (22)	0.1–3.2 (21)
• 95th percentile[c]	0.5 (1)	0.9–16.7 (4)	0.2–10.4 (15)	0.7–5.9 (14)	0.2–12.7 (19)	0.9–12.7 (12)
Refined brand-loyal exposure assessment scenario						
• Mean[b]	0.1–4.7 (5)	0.3–5.2 (13)	0.1–3.0 (19)	0.04–1.9 (21)	0.1–2.8 (22)	0.1–3.2 (21)
• 95th percentile[c]	0.5 (1)	0.8–15.7 (4)	0.2–9.4 (15)	0.7–5.9 (14)	0.2–12.2 (19)	0.9–12.7 (12)

[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 not considered in the regulatory maximum level exposure assessment scenario.

[b]Mean estimates based on dietary surveys/population groups up to and including five 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.

The Panel noted that the exposure to acesulfame K calculated in this opinion was very likely mainly due to the use of acesulfame K (E 950) since very limited uses (three food categories) were indicated for the salt of aspartame-acesulfame (E 962) and the reported use levels were lower than those reported for acesulfame K (E 950) in the same food categories. This is supported by the information from Mintel's GNPD, in which no product labelled with the salt of aspartame-acesulfame (E 962) is reported (Section 3.3.2).

In the *regulatory maximum level exposure assessment scenario*, mean exposure to acesulfame K ranged from 0.1 mg/kg bw per day in infants, adolescents and the elderly to 3.8 mg/kg bw per day in toddlers. The 95th percentile of exposure ranged from 0.3 mg/kg bw per day in the elderly to 14.1 mg/kg bw per day in toddlers.

In the *refined regulatory maximum level exposure assessment scenario*, mean exposure to acesulfame K ranged from 0.04 mg/kg bw per day in adolescents to 5.6 mg/kg bw per day in toddlers. The 95th percentile of exposure ranged from 0.2 mg/kg bw per day in adults to 16.7 mg/kg bw per day in toddlers.

In the *refined brand-loyal scenario exposure assessment scenario*, mean exposure to acesulfame K ranged from 0.04 mg/kg bw per day in adolescents to 5.2 mg/kg bw per day in toddlers. The 95th percentile of exposure ranged from 0.2 mg/kg bw per day in children and in adults to 15.7 mg/kg bw per day in toddlers.

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

Main food categories contributing to the exposure to acesulfame K (from E 950 and E 962)

For the *regulatory maximum level exposure assessment scenario*, the main food categories contributing to the exposure to acesulfame K was FCs 14.1.4 'Flavoured drinks' for all population groups except infants and 12.6 'Sauces' for all population groups. In addition, for adults and the elderly, FC 11.4.3 'Table-top sweeteners in tablets' was the third main contributing food category. For infants, the main contributing food categories were FCs 12.6 'Sauces', 04.2.5.2 'Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC' and 04.2.5.3 'Other similar fruit or vegetable spreads'.

The main contributor in the *refined regulatory maximum level exposure assessment scenario* was FC 14.1.4 'Flavoured drinks' in all population groups. In adults and the elderly, also FC 11.4.3 'Table- top sweeteners' and FC 11.4.3 'Table- top sweeteners in tablet form' were important contributors to the exposure. In infants, toddlers and children, FC 05.2 'Other confectionery including breath-freshening microsweets' were also important contributors to the exposure, as well as FC 01.4 'Flavoured fermented milk products including heat-treated products' for toddlers and FCs 04.2.5.2 'Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC' and 04.2.5.3 'Other similar fruit or vegetable spreads' for infants. In children, FC 05.3 'Chewing gum' was also an important contributor.

For the *refined brand-loyal scenario*, the main food category contributing to the exposure to acesulfame K was FC 14.1.4. 'Flavoured drinks' for all population groups. The second main contributing food categories were FC 11.4.3 'Table-Top Sweeteners in tablets' for the elderly, adults and adolescents; FCs 04.2.5.2 'Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC' and 04.2.5.3 'Other similar fruit or vegetable spreads' for infants; FCs 01.4 'Flavoured fermented milk products including heat-treated products' for toddlers, and FC 05.2 'Other confectionery including breath-freshening microsweets' for children and toddlers.

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

Dietary exposure for consumers of a single food category containing acesulfame K (from E 950 and E 962)

Exposure 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. Table A11 of Annex A 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. The 'consumers only' scenario, as defined in the exposure protocol (EFSA FAF Panel, 2024), is based on the population of consumers of any food category containing the sweetener. The consumers of specifically one food category (e.g. table-top sweeteners) are a sub-population of the population of interest (i.e. consumers of any sweetened foods). These additional estimates are calculated to support the interpretation of the dietary exposure results (see Table 5) and they will not be considered for the risk assessment which is based on the population of consumers of any food category containing the sweetener.

For most of the exposure estimates for consumers only of one food category, the mean exposure was comparable to those for consumers only of at least one food category, considering the uncertainties related to the exposure estimates (see Section 3.4.2). However, mean exposure of consumers only of FC 11.4.2 'Table-top sweeteners in tablets' could exceed the mean dietary exposure for consumers of at least one food category by a factor of 2.9 (exposure estimates up to 8.1 mg/kg bw day) (Table A11 of Annex A). At the P95, exposure for consumers of the following single food categories 'Table-top sweeteners in tablets' and 'food supplements in solid form' was a factor 1.5 to 2 higher than the exposure of consumers only of at least one food category for adults and the elderly.

3.4.2 | Uncertainty analysis

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

TABLE 6 Qualitative evaluation of influence of uncertainties on the chronic dietary exposure estimates.

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]
Use of the additive in table-top sweeteners regardless of the type of the sweetener consumed	+
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	+/–
Use of the MPLs for food supplements in solid and liquid form (not considering the higher MPLs for chewable and syrup, based on Mintel information)	–
Food categories with less than 5 analytical values ($n = 4$ food categories) considered in the exposure assessment	+/–
29 out of the 34 food categories authorised to contain acesulfame K (E 950) and the salt of aspartame-acesulfame (E 962) were considered in the refined scenarios	–
Regulatory maximum level and brand-loyal exposure assessment scenario: number of Mintel food sub-categories in which acesulfame K (E 950) was labelled that were included in the current exposure assessment: 64 out of 82 food sub-categories, representing 98% of the products labelled with acesulfame K (E 950)	–
Use levels/MPLs considered applicable to all foods in 5 food categories and to all foods meeting the facets criteria in the other food categories, while the percentage of foods labelled with acesulfame K (E 950) in a corresponding food sub-category labelled with acesulfame K (E 950) in Mintel was maximally 62% (Gums)	+
Methodology	
Regulatory maximum level exposure assessment scenario: – exposure calculations based on the MPLs according to Annex II to Regulation (EC) No 1333/2008 and maximum use levels for the FC 11.4	+
Refined brand-loyal exposure assessment scenario: – exposure calculations based on the maximum/highest reliable percentile or mean levels	+/–
Use of data from food consumption surveys 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 Panel considered the refined brand-loyal exposure assessment scenario the most appropriate exposure scenario for the risk assessment of acesulfame K (from E 950 and E 962) as it considered concentration levels, and this exposure scenario covers the majority of food categories in which the sweeteners are authorised (29 out of the 34 authorised food).

The Panel acknowledged that the assumption that 100% foods within five food categories and in all foods meeting the facets criteria in the other food categories and for which concentrations were available, are assumed to contain E 950 and/or E 962 has resulted in an overestimation of the exposure to acesulfame K. According to Mintel, the percentage of foods containing acesulfame K (E 950) compared to foods available on the market is maximally 62% (for the sub-category of gums), while the average percentage is 2.5% among all Mintel sub-categories.

Overall, the Panel considered that the dietary exposure to acesulfame K from the use of E 950 and E 962, using the *refined brand-loyal scenario* gives the most appropriate estimates that should be used for risk assessment. These estimates are regarded to overestimate the current dietary exposure to acesulfame K.

3.4.3 | Concentrations and dietary exposure to acesulfame K in the EU literature

A literature search (Appendix A) was carried out to gather information to collect data on levels of acesulfame K in food and beverages, and on dietary exposure estimates to this sweetener in Europe published between 1999 and 2024.

Occurrence data

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

In two Portuguese studies, acesulfame K was reported in non-alcoholic beverages (Basílio et al., 2020; Silva et al., 2021). In Basílio et al. (2020), a total of 56 samples 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. For 48 out of 56 samples (86%) acesulfame K was detected in concentrations ranging from < LOQ – 641.5 mg/L. The maximum reported concentration referred to a concentrated beverage, with an acesulfame K concentration of 91.6 mg/L in the drinkable product after dilution according to label instructions. Silva et al. (2021) analysed soft drinks ($n = 68$) 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 ($n = 11$). Acesulfame K was found in all the samples, at concentrations ranging from 23 to 142 mg/L.

The concentration of acesulfame K in food and food supplements on the Italian market was reported by Janvier et al. (2015). Foods included were flavoured drinks ($n = 57$), fruit nectars ($n = 18$), syrups ($n = 3$), jams ($n = 14$), ketchups ($n = 1$), confectionary ($n = 84$), yogurt ($n = 42$), ice creams ($n = 3$), table-top sweeteners ($n = 14$) and food supplements ($n = 54$), with no quantification of acesulfame K in the jams and ketchup analysed. In 47 flavoured drinks (82%), acesulfame K was found at a mean concentration of 126 mg/L. Out of 14 samples of table-top sweetener formulations, acesulfame K was found in six (43%) at a mean concentration of 67 mg/kg. Acesulfame K was found in 23 solid food supplements (43%) at a mean concentration of 4004 mg/kg which exceeded the MPL of 500 or 2000 mg/kg.

Székelyhidi et al. (2023) analysed 69 sugar-free (diet, light and zero) beverages purchased at supermarkets and one of the largest chains of fast-food restaurants in Hungary. Acesulfame K was quantified in 62 (78%) of the beverages with concentrations between 14 and 238 mg/L.

Analysis of artificial sweeteners in 66 beverage products from local markets in Santiago de Compostela in Spain included energy drinks, soft drinks, juices, teas, soy beverages, dairy-based drinks, beers and spirit alcoholic drink (Lorenzo et al., 2015). In 26 (39%) of the beverages, acesulfame K was present with concentrations ranging from 24 to 283 mg/L. The lowest concentrations were for beer and alcoholic beverages and the highest values (above 200 mg/L) were found in five of the soft drinks and in one grapefruit juice.

Buffini et al. (2018) analysed a total of 377 samples from the Irish market, which included 17 food categories. The authors describe that some of the solid food supplements contained acesulfame K at levels above the MPLs (mean of 6100 mg/kg). This was also the case for syrup-type or chewable food supplements (mean of 3000 mg/kg), and for one product from the wafer category, one soup sample and two sauce samples. The reported mean concentrations include also results reported below the LOD and so they cannot be compared to the values reported in this opinion.

Krmela et al. (2020) reported results for 76 samples collected at a typical Czech supermarket: 14 soft drinks, 19 energy drinks and 43 alcoholic beverages, with 11 of them containing acesulfame K. The concentrations ranged from 22 to 206 mg/L with the highest values in energy drinks.

Kubica et al. (2015) analysed different food products with a multi-method able to determine steviol glycosides as well as other sweeteners, such as acesulfame K. They analysed 21 samples of different soft and alcoholic drinks as well as three drink powders from the market in Poland. The samples were mainly selected to be those labelled with steviol glycosides and this is likely the reason why only two samples (both carbonated alcoholic drinks) were found to contain acesulfame K, at 9 and 23 mg/L.

Seven out of 8 analysed samples of sugar-free beverages (energy drinks, iced teas, carbonated drinks), purchased from a supermarket located in Brno, Czech Republic, contained acesulfame K (Diviš et al., 2020). Compared to four other sweeteners tested, acesulfame K was the most common sweetener in the selected beverages with concentrations ranging from 186 to 278 mg/L.

In summary, the quantified levels of acesulfame K 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 MPL in the reported literature as well as in the analytical data submitted to EFSA (see Section 3.3.1).

Dietary Exposure

The publications summarised below report dietary exposure assessments performed using methodologies different from the one described in the re-evaluation protocol.

Carvalho et al. (2022) estimated the exposure to acesulfame K based on the Portuguese dietary survey IAN-AF 2015–2016. Using the MPLs as defined in Regulation No 1333/2008, mean dietary exposure estimates of acesulfame K ranged from 0.58 to 3.85 mg/kg bw per day and from 1.58 to 7.77 mg/kg bw per day at the 95th percentile in the different age groups. Considering MPLs only for food categories where acesulfame K (E 950) is labelled, exposure estimates varied within the age groups between 0.08 and 0.37 mg/kg bw per day at the mean, and 0.36 and 1.23 mg/kg bw per day at the 95th percentile. Using analytical data only for food categories where acesulfame K (E 950) is labelled, exposure estimates varied within the age groups between 0.04 and 0.17 mg/kg bw per day at the mean and from 0.18 to 0.69 mg/kg bw per day at the 95th percentile.

Based on the analytical data and food consumption data from the National Adults Nutrition Survey described by Buffini et al. (2018; see above), the exposure to acesulfame K in the adult Irish population was estimated, resulting in a mean exposure of consumers at 0.11 mg/kg bw per day and at 1.18 mg/kg bw per day at the P99.

Chazelas et al. (2021) reported an acesulfame K exposure of 0.06 mg/kg bw per day at the mean and of 0.33 mg/kg bw per day at the P95 for French adults that participated in the NutriNet-Santé cohort and are consumers of foods containing food additives. The approach used by the authors deviates from the EFSA approach. Contribution of acesulfame K from

acesulfame-aspartame salt to the exposure was not considered in the NutriNet-Santé; furthermore, it is not clear whether the adults are consumers of acesufame K only or of all sweeteners and the concentration data used in the NutriNet-Santé cohort was a mix of analytical data, use levels and MPLs.

Estimates of acesulfame K exposure in Irish children (1–4 years) were reported by Martyn et al. (2016) using food survey data and analytical data in foods and beverages of the Irish market. Mean acesulfame K exposure of consumers only was 0.58 mg/kg bw per day and 2.1 mg/kg bw per day at the 95th percentile.

Acesulfame K exposure of Belgian children with type 1 diabetes was assessed based on analytical data and a specific food survey within three age groups: 4–6 years, 7–12 years and 13–18 years (DeWinter et al., 2015). The mean exposure to acesulfame K for the three age groups ranged from 1.26 to 2.3 mg/kg bw per day with children aged 4–6 years having the highest exposure levels. The 95th percentile estimates for consumers only was between 5.22 to 10.43 mg/kg bw per day, also with the highest for children aged 4–6 years.

Estimates of acesulfame K exposure in a 3 to > 65 years old Italian population ranged from 0.10 (mean of total population) to 0.45 mg/kg bw per day (95th percentile of total population) (Le Donne et al., 2017). The estimates were based on analytical data and a food label survey.

An exposure assessment combining national individual food consumption data of the Belgian adult population with MPLs resulted in mean acesulfame K exposure of 2 mg/kg bw per day and a P95 of 6.6 mg/kg bw per day for the total adult population (Van Loco et al., 2015).

In summary, it is not possible to directly compare dietary exposure estimates from the EU literature to those estimated in this opinion, 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 the population groups considered are different. Nonetheless, the Panel noted that estimates from the current opinion tend to be in the same order of magnitude as those from the literature.

3.5 | Biological and toxicological data

The biological and toxicological studies that were assessed as relevant according to the inclusion criteria established in the revised protocol on hazard identification and characterisation of sweeteners (EFSA, 2020a; EFSA FAF Panel, 2023), are listed in Annex B. The identified studies were provided to EFSA following the public call for biological and toxicological data[6] and in response to related clarification requests and/or also identified by EFSA from the literature.

An evaluation of the RoB was performed (Annex D) and a WoE approach for the reliable studies 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 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 derivation of a reference point when considered as supporting information (see Appendix A), are reported in Appendix B and Appendix C.

In the case of genotoxicity, studies were evaluated according to the approach outlined in the revised protocol (EFSA FAF Panel, 2023).

3.5.1 | Absorption, distribution, metabolism and excretion

No new studies on ADME were submitted by the IBOs. The Panel noted that several ADME data in human and animals were considered and evaluated by the SCF or JECFA or were among the publications retrieved in the systematic literature search (see Appendix A).

According to SCF (SCF, 2000), acesulfame K was administered to animals of both sexes, during 2 weeks, at two dose levels; rats: 840 mg/kg bw/day and 1325 mg/kg bw per day via the diet (Troschau & Acesulfame, 2000) and dogs: 900 and 1500 mg/kg bw per day as bolus dose (Stammberger, 1998). After 2 weeks of treatment (no further details provided), the plasma concentration-time profiles were determined for 24 h. There were no major sex differences in the plasma concentration-time profiles in either species. Only steady state, and not peak plasma concentration (C_{max}) values, could be obtained for rats because administration was by continuous feeding. The increases in AUC (0–24 h) in both species and in Cmax in the dogs were approximately proportional to the doses.

According to JECFA (JECFA, 1981), following intravenous administration of 14C acesulfame K to rats (10 mg/kg bw) (Kellner & Christ, 1975a), the plasma radioactive half-life was determined to be 0.23 h and the radioactivity was fully accounted for in the urine. Radioactivity was detected in urine and faeces of rats and dogs orally administered 14C acesulfame (10mg/kg bw) (Kellner & Christ, 1975a). Radioactivity was detected in urine and bile in pigs orally administered 14C acesulfame (3.6–4.5 mg/kg bw) (Kellner & Christ, 1975b). Only intact acesulfame was detected in the urine of all species and the bile of pig using thin-layer chromatography, mass spectrometry and isotope dilution technique. Therefore, the radioactivity measurements represent also the concentration of the compound. A single oral dose of 30 mg C14-acesulfame K, given to three human volunteers, was almost completely absorbed, with 99% of the dose being excreted in urine and less than 1% in faeces (Christ & Rupp, 1976). Maximum blood radioactivity concentration was reached after 1–1.5 h and elimination occurred with a plasma radioactivity half-life of the 2–2.5 h. No metabolites could be identified in serum and urine

(Volz, 1976). Therefore, maximum concentration in blood of radioactivity measurements represent also the concentration of the compound.

According to JECFA (JECFA, 1983), pretreatment in rats with unlabelled acesulfame K (300 mg/kg diet for 60 days) (Volz & Eckert, 1981) did not modify rate and amount of excretion into the urine of 25 mg C14 acesulfame K, compared to rats without pretreatment. A total of, 96%–99% of the radioactive dose was excreted in the urine and the urine excretion half-life was 4–4.5 h. No metabolites could be detected in the urine.

According to JECFA (JECFA, 1991), in a further study in rats with pretreatment (7 days, 1% acesulfame K in the diet; equivalent to a dose of 1200 mg/kg bw per day) followed by 500 mg C14 acesulfame K (Volz et al., 1983), the results of the study reported in 1983 (JECFA, 1983) were confirmed. Low concentrations were found in the rat foetus by autoradiography following the administration of a single oral dose of C14 acesulfame K of about 10 mg/kg bw per day (Kellner & Eckert, 1983a); the ratio foetus: mother being 1:14 and 1:3 at 0.5 and 2 h, respectively. C14 acesulfame K was found in the milk of nursing rats given a single oral dose of about 10.6 mg/kg bodyweight (Kellner & Eckert, 1983b); the concentrations being about 6 times higher than in the blood of mothers. Half-lives in blood and milk were similar, 5.6 h in the milk and 4h in the blood. About 1% of the dose was excreted in the milk within 24 h.

New relevant studies identified in the literature are summarised below.

Wilson et al. (1999) measured the urinary excretion of acesulfame K in urine, collected after 24 h, following oral intake of acesulfame K (doses 2.2–102 mg) by healthy volunteers (2–4 subjects per dose). Measurement was performed after extensive cleaning up procedure by HPLC- UV. The data show that the urinary excretion within 24 h was dose-linear and that acesulfame K is absorbed to 100% and excreted in the urine as the parent compound to about 100%.

Stampe et al. (2022) investigated the concentration-time profile of acesulfame K together with other artificial sweeteners in plasma and in breast milk of breast-feeding women in an experimental clinical study. An additional aim of the study was to determine whether BMI and/or a diagnosis of type 1 diabetes mellitus (T1DM) had any influence on artificial sweetener toxicokinetics.

The study was performed in 49 women, 20 with a BMI < 25 kg/m^2, 21 with BMI > 27 kg/m^2 and 8 with T1DM. With the exception of women with T1DM (to prevent potential hypoglycemic episodes), participants were fasted overnight prior to ingestion of 200 mL of a mixture consisting of 85 mg acesulfame K, 75 mg sucralose, 60 mg cyclamate and 20 mg saccharin mixed with 60 mL of unsweetened cranberry juice (for taste). In total, eight blood samples and eight breast milk samples were collected at times $t = 0$, 30, 60, 120, 180, 240, 300 and 360 min after ingestion. It was noted by the authors that breasts were not always completely emptied at all samplings (nursing women were permitted to breastfeed their offspring throughout the study period). Artificial sweeteners were quantified by high performance liquid chromatography with tandem mass spectrometry (LC–MS/MS).

Only sample mean values were reported (i.e. participant sample variability via SD or SEM was not reported). It is described how the kinetic parameters, including the AUC, were obtained. For acesulfame K, the plasma peak concentration (C_{max}) was 1548 ng/mL and the time to reach the peak concentration (tmax) was 120 min. The milk Cmax was 936 ng/mL and the milk tmax was 240 min. The ratio of AUCs (AUC milk/AUC plasma) was 0.89.

The Panel noted both mean acesulfame K plasma and milk concentrations were appreciable at the final sample time point (both 60 ng/mL at 360 min) and therefore the AUC determinations are underestimates because they do not cover the full plasma concentration-time curve.

The Panel noted that the tmax in plasma and milk were markedly different (1 h in plasma and 4 h in milk) and considered this might be explained by the fact that breasts were not always completely emptied at each sampling. Concentration-time profiles for acesulfame K in plasma and milk were independent of participant BMI or T1DM in mothers.

Based on their data, the authors (Stampe et al., 2022) calculated the number of acesulfame K-containing drinks needed for mothers to be exposed at a level of 9 mg/kg bw per day (the ADI set by SCF, 2000). They also calculated the number of acesulfame K-containing drinks needed for nursing infants to be exposed to a similar level, although the Panel considered the authors' calculation was not clearly explained.

Leth-Møller et al. (2023) conducted an open-labelled clinical investigation in women planned for caesarean section (C-section). Eligible participants were pregnant women, aged 18 or older with allocation to a control group or intervention group. Participants in both the intervention and control groups were asked to refrain from intake of diet drinks for 48 h before the C-section. Participants allocated to the intervention group were given 250 mL of unsweetened blackcurrant-flavoured juice containing 85 mg acesulfame K, 100 mg aspartame, 60 mg cyclamate, 20 mg saccharin and 75 mg sucralose. Participants were instructed to drink it 2 h before the C-section and maternal blood samples were obtained immediately before C-section. During the C-section, amniotic fluid was obtained. Fifteen minutes into the C-section procedure, the umbilical cord was clamped and cord blood obtained 30 min after the start of C-section. There were 35 participants in total (19 in the intervention group and 9 in the control group).

Maternal mean plasma acesulfame K concentration was 848.5 ng/mL (95% CI: 695.7–1001.3 ng/mL), and the mean foetal cord acesulfame K plasma concentration was 643.9 ng/mL (95% CI: 546.3–741.4 ng/mL) which gave a cord/maternal plasma concentration ratio of 0.80 (95% CI: 0.70–0.90). Foetal cord and maternal acesulfame K plasma concentrations were correlated (correlation coefficient of 0.53, range 0.30–0.76).

The amniotic fluid concentration was 494.7 ng/mL (95% CI: 230.5–759.0 ng/mL). The mean cord plasma/amniotic concentration ratio was 0.83 but varied widely (95% CI: 0.33–1.32) and lacked any discernible correlation (0.26 (95% CI: –1.34–1.87)).

The Panel considered that this study showed that acesulfame K crosses the placenta and is present in the foetal circulation. When given to pregnant women 2 h before obtaining amniotic fluid, acesulfame K is detectable in the amniotic fluid, indicative of foetal systemic exposure and urinary excretion.

Halasa et al. (2023) obtained amniotic fluid samples ($n = 13$) from 12 participants during either caesarean births (full term elective) or during clinically indicated third trimester amnioreduction. All except four women were in a fasted state prior to the procedures. Cord blood samples were obtained from an independent cohort of new-borns ($n = 15$) whose mothers were enrolled in a separate clinical study. Acesulfame K was detected in seven amniotic samples (range 0.7–78.9 ng/mL). All 15 cord blood samples contained low concentrations of acesulfame K (range 0.7–6.5 ng/mL).

Sylvetsky et al. (2015) examined the breast milk from 20 lactating women for the presence of acesulfame by LC–MS without prior knowledge of non-nutrient sweetener consumption. Acesulfame was present in breast milk from 15 of the women (range from < LOQ – 2.22 ug/mL), including 4 who reported no non-nutrient sweetener-containing food and drink consumption over the 24 h preceding the milk donation (0.02–0.09 ug/mL).

Rother et al. (2018) performed a study with 34 exclusively breast-feeding women (14 normal weight, 20 obese) who consumed a drink sweetened with 68 mg sucralose and 41 mg acesulfame K, prior to consumption of a standardised breakfast meal. Breast milk was collected from the same breast prior to beverage ingestion and hourly for the following 6 h and acesulfame concentrations determined by LC–MS. Peak breast milk acesulfame concentrations ranged from 299.0 to 4764.2 ng/mL (median peak concentration 945.3 ng/mL). One mother's milk had notably higher (four to fivefold those of the 33 other participants) concentrations of acesulfame. Concentrations of breast milk acesulfame were higher in those of normal weight compared to those who were obese (peak median: 1244 vs. 877 ng/mL respectively).

Sylvetsky, et al. (2024) performed a study with 40 breast-feeding mothers, aged 32.4 ± 4.9 years, BMI 28.6 ± 9.4 kg/m^2. The mothers were given a juice drink, containing 95 mg sucralose and 17 mg acesulfame K. Blood was taken at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h and milk samples at 1, 2, 3, 4, 6, 8 and 12 h after ingestion of the beverage. In the infants, one blood sample per infant (2.9 ± 1.6 months) was taken within 1.5 and 14 h following ingestion of acesulfame K by the mothers. The concentration of acesulfame K was determined by LC–MS, and the authors reported a minimum measurable concentration of 0.1 ng/mL. The peak plasma concentration in mothers (C_{max}) occurred at 4 h and its geometric mean was 379 µg/L, the mean half-life was 3.6 h, both with wide coefficient of variation (CV) (28% C_{max}, 64% half-life). The geometric mean AUC in plasma was 2030 µg/L × h and 3066.7 µg/L × h in milk with high CVs (29% for AUC in plasma; 87% for AUC in milk). The measurements of the plasma concentrations in infants were reported as mean, 9.2 µg/L (CV 14.8%). From the data, the mean milk-plasma-ratio (AUCmilk/AUCplasma) was calculated to be 1.75 ± 1.17 (mean ± SD). The mean relative infant dose (infant dose/maternal dose) was 1.59 ± 1.7% (mean ± SD). The infant dose was calculated by multiplying the concentration in the milk at every nursing time by the volume at every nursing time which was consumed by the infant. Since the mean dose in nursed infants is below 5% of the mother's dose, the Panel notes that nursing by mothers who consume acesulfame K raises no concern for their infants.

Summary and conclusion on ADME

Based on the available data, the Panel considered that acesulfame K is almost fully absorbed (at least at doses up to 430 mg/kg bw in human; 2000 mg/kg bw in rats). The Panel considered that acesulfame K is not metabolised, has a half-life of 2–4 h and is excreted into the urine. The newly available data from humans focussed on the exposure of the foetus and the breast-fed infant to acesulfame K. Acesulfame K is capable of passing the placenta, as indicated by detection in amniotic fluid and cord blood samples and entering the foetal circulation. Acesulfame K also passes into breast milk and the breast-fed infant is exposed, however to a low extent which accounts for 1.6% of the mother's dose.

3.5.2 | Animal toxicity

No data on acute toxicity were received by IBOs; no new data were identified in the literature. Repeated dose toxicity studies were assessed systematically and are discussed in Section 3.5.4.1.

3.5.3 | Genotoxicity

The genotoxicity of acesulfame K was previously evaluated by the SCF (2000) and JECFA (1991). In these two assessments various genotoxicity studies (reported between 1974 and 1986) were reviewed. The SCF concluded that acesulfame k was not mutagenic based on the data available at the time. The studies included a mouse micronucleus assay, a dominant lethal assay and a chromosome aberration test in vivo. In vitro studies included a mammalian gene mutation assay, assessment of cell transformation, unscheduled DNA synthesis and three Ames tests. These tests are either not recommended by OECD or were performed prior to the respective current OECD guidelines and are therefore of limited relevance. However, one study cited in JECFA 1991 (Jung & Hollander, 1986) which reported negative findings in the gene mutation assay in Salmonella Typhimurium strains TA97, TA100, TA 1535, TA1537 and TA 1538 and in *E. Coli* WP2uvrA (all in the presence and absence of S9) is considered by the Panel to be reliable without restrictions and the results obtained of high relevance according to current criteria.

The genotoxicity studies published since the year 2000 were retrieved through a systematic literature search (Appendix A). A total of 11 studies related to in vitro genotoxicity and six related to genotoxicity in vivo were identified. The studies are described in detail in Annex C. The main findings from studies with limited or high relevance are summarised below (Tables 7 and 8). Studies evaluated to have results of low relevance due to important flaws or limitations were not considered in the WoE and are not included in Tables 7 and 8.

TABLE 7 Summary table of the in vitro genotoxicity studies on Acesulfame K (E 950). A comprehensive summary of the extracted data and detailed account of the evaluations performed is provided in Annex C.

Test system	Exposure conditions	Information on the characteristics of the test substance	Result	Reliability/ comments[23]	Relevance of the test system/ relevance of the result[24]	Reference
Micronucleus test in peripheral human lymphocytes	1st assay: 500, 1000 and 2000 µg/mL Exposure: 3 h (±S9); and 24 h after the addition of cytochalasin B Positive controls: mitomycin C and colchicine (-S9); cyclophosphamide (+S9) 2nd assay: 500, 1000 and,000 µg/mL Exposure: 24 h after the addition of cytochalasin B (-S9) Positive controls: mitomycin C and colchicine	Acesulfame K Purity: 100.1%	Negative	Reliable without restrictions	High/High	Documentation provided to EFSA No 16
Gene mutation assay (Ames test) Salmonella typhimurium strains TA97a and TA100	Concentrations: 0, 100, 250, 500, 1000, 10,000 µg/plate (±S9)	Acesulfame K Hoechst AG (Frankfurt, Germany) Purity: not stated	Negative	Reliable with restrictions	High/Limited	Bandyopadhyay et al. (2008)
DNA Repair assay: measurement of DNA damage in primary rat hepatocytes (from male F344 and Sprague–Dawley rats)	Concentrations: 0.005, 0.01, 0.02, 0.04 M Exposure: 20 h	Acesulfame K (Purity: not stated)	Negative	Reliable with restrictions	Limited/ Limited	Jeffrey and Williams (2000)

[23]See Annex C for comments.

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

TABLE 8 Summary table of the in vivo genotoxicity studies on Acesulfame K (E950). A comprehensive summary of the extracted data and detailed account of the evaluations performed is provided in Annex C.

Test system	Exposure conditions	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of the test system/ relevance of the result	Reference
Comet assay: mouse organs incl. glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow. – Male ddY mice (4 mice)	Acesulfame K was administered at the limit dose of 2000 mg/kg b.w. Oral dosing to a group of four mice. Exposure: 3 and 24 h	Acesulfame K Wako Pure Chemical Industry Ltd. (Osaka, Japan) Purity >98.0%	Negative	Reliable with restrictions	High/ Limited	Sasaki et al. (2002)
P53 expression in rat liver. Rats	Acesulfame K was administered at 0, 30 and 90 mg/kg b.w. Gastric intubation to two groups of rats (mature and immature). Exposure: daily, 5 days/week for 12 weeks	Acesulfame K (purity ≥ 99%), obtained from Sigma-Aldrich Company	Increase in P53 gene expression in liver. Lymphocyte proliferation (low dose only), macrophage activation, microscopic histopathological alterations in the liver.	Reliable with restrictions	Limited/ Limited	Mohammed et al. (2024)

In the studies available since the SCF (2000) opinion, overall negative results were obtained in Ames tests. Negative results were obtained in an assay of UDS in primary rat hepatocytes (Jeffrey & Williams, 2000), however, such assay can only be considered as supportive information since corresponding TG has been suppressed in OECD.

Negative findings were also reported in a micronucleus assay in human lymphocytes (Documentation provided to EFSA No 16) which was submitted following EFSA's call for data[8] to complete the in vitro basic test battery (tier 1) for hazard identification as recommended by EFSA Scientific Committee (2011). This study is considered reliable without restrictions and the result of high relevance. In vivo, there were studies with results of limited relevance showing negative responses in the Comet assay in a range of tissues (Sasaki et al., 2002) and a report of an increase in p53 expression in rat liver (Mohammed et al., 2024).

Based on the overall negative results obtained in a battery of tests, including results of Ames (Jung and Hollander, cited in JECFA, 1991) and in vitro micronucleus assay (Documentation provided to EFSA No 16) that are considered of high relevance, the Panel concluded that acesulfame K does not raise a concern for genotoxicity. This concurs with the conclusion of the previous SCF Opinion (SCF, 2000).

3.5.3.1 | *Genotoxicity assessment of acesulfame K degradation products and impurities*

The mutagenicity of acesulfame K degradation products was previously evaluated by JECFA (1991), that reviewed toxicological studies, including genotoxicity, on the breakdown products acetylacetamide and acetoacetamide- N-sulfonic acid. For acetylacetamide, gene mutation in bacteria and mammalian cells, in vitro chromosomal aberrations and UDS were assessed; for acetoacetamide-N-sulfonic acid, the same battery of tests and, in addition, the mouse MN assay were considered. Based on the results reported, JECFA concluded that these compounds are not mutagenic.

For acetylacetymide and acetoacetamide-N-sulfonic acid no experimental data on genotoxicity were retrieved in a systematic literature search. In the present assessment, QSAR analyses were performed to further evaluate the potential genotoxicity of the two main degradation products (acetylacetamide and acetoacetamide-N-sulfonic acid). A suite of VEGA models predicting mutagenicity in the Ames test, chromosomal aberrations and in vitro and in vivo micronucleus activity, and the OECD QSAR Toolbox profilers for DNA binding, DNA alerts for in vitro mutagenicity and protein binding, were applied (Appendix D).

In VEGA models, no in vitro mutagenicity in the Ames test was predicted for both substances by models with moderate or high reliability (the latter for the ISS model applied to acetylacetamide). Negative predictions with high or moderate reliability for in vitro and in vivo micronucleus activity were also formulated for both substances by the IRFMN models. A single positive prediction for potential clastogenicity was obtained for acetylacetamide by the CORAL model, for which however some critical aspects were highlighted (Table D.1). The application of the OECD QSAR ToolBox did not identify

structural alerts for DNA binding potential and in vitro mutagenicity (Ames, chromosomal aberrations and micronuclei), in either chemical structure analysed (Appendix D).

Overall, QSAR analyses did not provide indications for genotoxicity for both degradation products. This supports the conclusion of the previous JECFA opinion (JECFA, 1991) that were briefly summarised above.

In this assessment the Panel also considered 5-chloro-acesulfame as a relevant impurity (see Section 3.1.1). No experimental data on genotoxicity were retrieved in a systematic literature search. An assessment was performed using the in silico tools (OECD QSAR Toolbox and VEGA models). A structural alert for genotoxicity was identified by the QSAR Toolbox in 5-chloro-acesulfame, which was predicted as mutagenic in the Ames test and in vivo micronucleus test in VEGA models (Appendix D). The Panel noted that such predictions only had moderate reliability, but concluded that in the absence of experimental data 5-chloro-acesulfame is considered to raise concern for genotoxicity. Therefore, to ensure that the exposure to this impurity from the use of E 950 does not pose a concern (remaining below the TTC value of 0.0025 μg/kg body weight per day), the presence of 5-chloro-acesulfame in the food additive should be less than 0.1 mg/kg); alternatively, appropriate genotoxicity data for 5-chloro-acesulfame should be generated. For details please refer to Appendix E.

3.5.4 | Synthesis of systematically appraised evidence

For acesulfame K (E 950), a total of 1412 references were screened based on title and abstract. These references included studies retrieved from the literature (timeframe: 1.1.1999–2.12.2024) as well as the key studies on which the derivation of the ADIs set by SCF (2000) and JECFA (1991) were based. No new studies were submitted by the IBOs; the studies provided by one IBO (Documentation provided to EFSA No 17) were already considered and evaluated by the SCF or were among the publications retrieved in the systematic literature search (see Appendix A). After title and abstract screening, 679 studies were screened at the full-text level; 148 studies were further subjected to categorisation and confirmation of relevance screening, resulting in 35 studies (20 animal studies, 14 human studies; 1 study reporting both human and animal data) for inclusion in RoB assessment (Appendix A, Figure A.1).

According to the protocol (EFSA, 2020a; EFSA FAF Panel, 2023), the key study previously considered by the SCF (SCF, 2000) for deriving the current ADI, a 2-year study in dogs (Documentation provided to EFSA No 17) was evaluated for RoB, along with relevant literature published since the previous evaluation by the SCF, allowing 1 year of overlap (cut-off date: 1999). The toxicity study in dogs considered key in the SCF (2000) evaluation was allocated to tier 3 (high RoB). The Panel noted that a 2-year chronic toxicity and carcinogenicity study in rats was also evaluated by the SCF in its assessment (SCF, 2000). This study was seen as key for setting the ADI for acesulfame K by JECFA (JECFA, 1991). The study in rats (Documentation provided to EFSA No 17) therefore was assessed by the Panel for RoB. It was allocated to tier 2 (moderate RoB) for the majority of endpoints investigated (see Annex D); three endpoints were allocated to tier 3 (high RoB). Following the approach described in the protocol (EFSA, 2020a; EFSA FAF Panel, 2023), the systematic approach for the appraisal of the evidence was applied to: (i) the new evidence available since the cut-off date and (ii) the previously identified key study in rats (Documentation provided to EFSA No 17).

3.5.4.1 | *Animal studies*

The studies included in the assessment encompassed 11 animal studies *(Shi et al., 2019; Glendinning et al., 2020; Mendoza-Pérez et al., 2021a; Chiang et al., 2022; Hayes et al., 2022; Cai et al., 2024; Zhai et al., 2024; Documentation provided to EFSA no 17; Rathaus et al., 2024; Shou et al., 2024).* Among these studies, four were allocated to tier 1 *(Glendinning et al., 2020; Hayes et al., 2022; Mendoza-Pérez et al., 2021a; Shi et al., 2019)* and six to tier 2 *(Mendoza-Pérez et al., 2021a; Chiang et al., 2022; Cai et al., 2024; Zhai et al., 2024; Documentation provided to EFSA NO 17; Shou et al., 2024*) following a RoB evaluation. One study was allocated tier 1 or tier 2 depending on the measured endpoint (Rathaus et al., 2024).

TABLE 9 Animal studies included in the assessment.

Authors	Study type[a]	Exposure duration	Species/strain	No of animals	Dose level	Dose level in mg/kg bw per day	RoB tier
Glendinning et al. (2020)	Sub-acute	28 days	Mice, C57BL/6 (B6)	5 animals/sex/control group; 10 males and 11 females/acesulfame K group	100 mM in deionised water	Equivalent to: 0, 3600 mg/kg bw per day acesulfame K	1
Hayes et al. (2022)	Sub-acute	43 days	Rats, Sprague–Dawley	9 animals/sex/group	0.1% Acesulfame K solution in drinking water	Equivalent to: 0, 120 mg/kg bw per day approximately	1
Shi et al. (2019)	Sub-acute	84 days	Mice, ICR	10 males/group	1 g/L in drinking water	Equivalent to: 0, 174 mg/kg bw per day	1
Mendoza-Pérez et al. (2021a)	Sub-acute (i) Sub-chronic (ii) Chronic (iii)	21, 35, 63 (i), 210 (ii) and 480 (iii) days	Rats, Wistar Hsd:Han	10 animals/sex/group for childhood, adolescence and young adult groups; 5 animals/sex/group for adulthood and reproductive senescence groups	0.05% in drinking water	Equivalent to: (i) 0, 60 mg/kg bw per day acesulfame K for sub-acute, (ii) 45 mg/kg bw per day acesulfame K for sub-chronic exposures and (iii) 25 mg/kg bw per day acesulfame K for chronic exposures	1
Rathaus et al. (2024)	Sub-chronic	140 days	Mice, C57BL/6	10 males/group	535.25 mg/L in drinking water	Equivalent to: 0, 77 mg/kg bw per day	1 and 2
Zhai et al. (2024)	Sub-acute	Experiment 1: 28 days Experiment 2: 14 days	Mice, C57BL/6 J	Experiment 1: 5 males/group Experiment 2: 3 animals/sex/group	Experiment 1: 7 and 21 mg/L AceK in drinking water Experiment 2: 1 g/L AceK in drinking water	Equal to: Experiment 1: 0, 1.36 and 3.69 mg/kg bw per day Experiment 2: 0, 180 mg/kg bw/day	2
Cai et al. (2024)	Sub-acute	28 days	Mice, C57BL/6J	Experiment 1: 10 males/group Experiment 2: 5 males/group	In both experiments: 0, 0.2 and 10 mM AceK dissolved in deionised water, in drinking water	Equivalent to: 0, 7 and 350 mg/kg bw per day	2
Chiang et al. (2022)	Sub-acute	56 days	Rats, Sprague–Dawley	5–6 females/group	Gavage in water	Equal to: 0, 10 and 100 mg/kg bw per day acesulfame K	2
Shou et al. (2024)	Sub-acute	77 days	Mice, C57BL/6	8 males/group	Gavage with water	Equal to: 0, 40 mg/kg bw (low-dose group) and 120 mg/kw bw (high-dose group) in drinking water	2
Mendoza-Pérez et al. (2021b)	Sub-chronic	104, 197 and 288 days	Rats, Wistar Hsd:Han	15 males/group up to day 104, 10 males/group up to day 197 and 5 males/group up to day 288	0.015% in drinking water	Equivalent to: 0 and 13.5 mg/kg bw per day acesulfame K	2
Documentation provided to EFSA NO 17	Chronic/carcinogenicity study	More than 2 years (120 weeks approximatively)	Rats, CPB-WU Wistar	4 groups of 60 males +60 females each	0, 0.3, 1.0 and 3.0% in diet	Equal to 0, 150, 500, 1500 mg/kg bw per day	2

[a]Studies were classified according to duration into (i) sub-acute (less than 90 days), (ii) sub-chronic (from 90 days to less than 1 year) and (iii) chronic (1 year or longer).

The WoE assessment of the 11 animal studies Table 9 are described in detail in Annex (E1). This annex shows the WoE rating according to predefined downgrading and upgrading elements for each HOC. Each HOC consists of groups of endpoints (see Table 10), each endpoint being addressed in one or more of the included animal studies.

TABLE 10 Health outcome categories and related endpoints of the appraised animal studies subjected to WoE evaluation[a].

Health outcome categories (HOCs)	Endpoints
General toxicity	Clinical signs and survival, body weight, liquid/water intake, energy/feed intake
Additional clinical chemistry[b]	Serum triglyceride levels, cholesterol level (total, LDL, HDL)
Haematotoxicity	Haematology parameters (haemoglobin, haematocrit, RBC count, packed cell volume, neutrophils)
Liver toxicity	Serum triglyceride levels, cholesterol level (total, HDL and LDL), bilirubin (total and direct), total bile acid, liver weight (relative and absolute), liver triglyceride content, macroscopic changes, histopathology[c], clinical chemistry (ALP, ALT (SGOT), AST (SGPT), total serum protein, serum albumin)
Nephrotoxicity	Clinical chemistry (creatinine and BUN), ion concentration in the urine (Na+, K+, Cl-, Ca++), relative urine output (RUO), urinalysis (urinary ALT (UGOT), specific gravity, osmolarity, composition), kidney weight (relative), macroscopic changes, histopathology.[c]
Other organ toxicity	Colon length, colon microscopic non-neoplastic changes, disease activity index (DAI) of the colon (stool consistency, rectal bleeding, colon length), histopathology of remaining 41 organs/tissues (other than liver and kidney)
Glucose/insulin homeostasis	OGTT and IGTT, ITT, glucose and insulin

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferases; AST, aspartate aminotransferases; IGTT, impaired glucose tolerance test; ITT, insulin-tolerance test; OGTT, oral glucose tolerance test.

[a]Annex E1.

[b]'Additional clinical chemistry' denotes the clinical chemistry not covered under other HOCs.

[c]Histopathology includes non-neoplastic, pre-neoplastic and neoplastic changes.

In addition to the apical and related endpoints reported in Table 10, other endpoints were measured in the included studies which 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 health outcome categories; see Table 11. The 'final confidence rating' was based on the 'initial confidence rating' followed by downgrading and upgrading considering elements that decrease or increase the confidence in the body of evidence across studies of the same HOC (Appendix A and Annex E1, modified from NTP OHAT, 2019). In particular, rating the confidence in the evidence of each identified relevant outcome begins with consideration of the study design and then addresses four elements to possibly downgrade the confidence in the body of evidence (RoB, unexplained inconsistencies across the studies, relevance of the studies and imprecision) and three elements to possibly upgrade the confidence in the body of evidence (magnitude of effects, dose–response and consistency across study population/study design). The confidence in the evidence for the presence or absence of adverse effects was evaluated for (i) each study, by (ii) groups of endpoints and (iii) across all endpoints within a HOC. The rating of confidence may be different for the individual endpoints. The final confidence in the body of evidence was then translated into a level of evidence for the presence or absence of adverse effects, as outlined in the revised protocol (EFSA, 2020a; EFSA FAF Panel, 2023) and in Section 2.2.

TABLE 11 Summary table of rating confidence in the body of evidence for each health outcome category: Animal studies. For a detailed assessment, see Annex E1) .

		Elements for downgrading[b]					Elements for upgrading[b]					
Health outcome categories (HOCs) investigated[a]	**Initial rating (No. of studies)[a]**	**Concern for risk of bias**	**Concern for unexplained inconsistency**	**Concern related to relevance of studies**	**Concern for imprecision**	**Downgrading**	**Magnitude of effect**	**Dose–response**	**Consistency across study population/ study design**	**Upgrading**	**Final rating of confidence**	**Effect/no effect**
General toxicity	High (11)	Not serious	Not serious	Not serious	Not serious	No	Not large	No	Yes	No	High	No effect
Additional clinical chemistry	High (4)	Not serious	Not serious	Not serious	Serious	Yes	Not large	No	No	No	Moderate	No effect
Haematotoxicity	High (1)	Not serious	N.A.	Not serious	Not serious	No	Not large	No	N.A.	No	High	No effect
Liver toxicity	High (5)	Not serious	Not serious	Not serious	Not serious	No	Not large	No	Yes	No	High	No effect
Nephrotoxicty	High (4)	Not serious	Not serious	Not serious	Not serious	No	Not large	No	Yes	No	High	No effect
Other organ toxicity (colon and 41 organs/ tissues in Documentation provided to EFSA No 17)	High (2)	Not serious	Not serious	Not serious	Not serious	No	Not large	No	Yes	No	High	No effect
Glucose/insulin homeostasis	High (9)	Not serious	Not serious	Not serious	Serious	Yes	Not large	No	Yes	No	Moderate	No effect

Abbreviation: N.A.: not applicable.

[a]The total number of studies assessed was 11. The number in parentheses refers to studies considered under the specific HOC.

[b]Please refer to Appendix A, Annex E1 and to the protocol (EFSA, online) for further explanations on what is assessed under each element and on the wording used for the grading of the elements.

General toxicity

Adverse effects related to general toxicity (clinical signs, mortality, body weight, as well as water, feed and energy intake) were evaluated in mice and rats in 11 studies of different duration. Doses varied by three orders of magnitude (see Table 9).

Clinical signs and mortality

Clinical signs and mortality of rats exposed to acesulfame K up to 1500 mg/kg bw per day were reported in a combined chronic toxicity and carcinogenicity (2-year) study (Documentation provided to EFSA No 17). Clinical overt signs were equally distributed among control and treated rats. Mortality was low in all groups during the first 1.5 years. In the 1500 mg/kg bw per day dose group, mortality was relatively low in both sexes, with significantly lower rates in females than in males.

Body weight and water, feed and energy intake

No adverse changes in body weight were noted in mice or rats in studies of different duration and doses of acesulfame K (Shi et al., 2019; Glendinning et al., 2020; Mendoza-Pérez et al., 2021a, 2021b; Chiang et al., 2022; Mendoza-Pérez et al., 2021; Hayes et al., 2022; Cai et al., 2024; Zhai et al., 2024, Documentation provided to EFSA No 17; Rathaus et al., 2024; Shou et al., 2024). Except for one combined chronic toxicity and carcinogenicity study (Documentation provided to EFSA No 17) and one sub-acute (28 days) study (Glendinning et al., 2020), doses were generally low. Only two studies lasted longer than 480 days (Mendoza-Pérez et al., 2021a, Documentation provided to EFSA No 17). Changes in body weight were within 10%, including a transient weight loss reported in weeks 10 and 12 of the long-term 2-year study (Documentation provided to EFSA No 17). Intake of water added acesulfame K increased about 60% (not statistically significantly) relative to water control in male mice (Shi et al., 2019) and was statistically significantly increased relative to water intake in the controls of mice (both sexes evaluated together) (Glendinning et al., 2020). However, daily intake of a beverage added acesulfame K was not consistently significantly increased across life stage and sex, relative to control in other studies (e.g. 936 Mendoza-Pérez et al., 2021a). Water intake (measured in week 63) was statistically significantly increased in rats of both sexes exposed to acesulfame K in the diet at 1500 mg/kg bw per day in the 2-year study (Documentation provided to EFSA No 17).

Overall, no treatment-related general toxicity effects were noted in studies of durations up to 140 days and 2 years in mice and rats, respectively, and with doses of acesulfame K from about 1 to 3600 mg/kg bw per day. Considering the final rating of confidence in the body of evidence for the HOC 'general toxicity' as 'high' (Annex E1) and the absence of adverse effects (see Table 11), the Panel considered that there is high confidence in the body of evidence that exposure to acesulfame K is not associated with general toxicity effects (Table 12), i.e. mortality and changes in clinical signs, body weight and water, feed and energy intake.

Additional clinical chemistry

The clinical chemistry parameters related to triglycerides, total cholesterol, HDL- and LDL-cholesterol reported in four studies (Mendoza-Pérez et al., 2021a, 2021b; Rathaus et al., 2024; Shou et al., 2024) are described in the section 'Liver toxicity' (for the evaluation of WoE for these endpoints in a separate HOC, see Annex E1).

No treatment-related adverse changes in the additional clinical chemistry parameters triglycerides, total cholesterol, HDL- or LDL-cholesterol levels were noted in studies in rats up to 480 days dosed up to 60 mg/kg bw per day or in mice up to 20 weeks dosed up to 120 mg/kg bw per day. Considering the final rating of confidence in the body of evidence for the HOC 'additional clinical chemistry' as 'moderate' (downgraded for imprecision; Annex E1) and the absence of adverse effects (see Table 11), the Panel considered that there is moderate confidence in the body of evidence (Table 12) that exposure to acesulfame K is not associated with adverse changes in triglycerides, total cholesterol, HDL- or LDL-cholesterol levels.

Haematotoxicity

In a 2-year combined chronic toxicity and carcinogenicity study in male and female rats (Documentation provided to EFSA No 17) with a dietary exposure to acesulfame K at doses amounting up to 1500 mg/kg bw per day, no adverse effect was observed on haematological parameters (haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count) in weeks 33, 53, 78, 102 and 119.

The Panel noted some statistically significant changes in haematological parameters: decreases in Hb in high-dose males (−7%) and high-dose females (−8%) in week 102 and in high-dose females (−6%) in week 119, in PCV in low-dose and high-dose males (−6% and − 8%, respectively) in week 102 and in high-dose females (−9%) in week 119, in RBC count (−8%) and neutrophils (−32%) and increases in lymphocytes (+34%) in high-dose males in week 102. Neutrophils were increased in mid-dose females (+43%) in week 102 and in low-dose males (+31%) in week 119. The Panel considered the changes as not toxicologically relevant, as these were small (Hb, PCV, RBC) (WHO, 2015), without dose–response relationship (PVC in males, neutrophils in females in week 102 and in males in week 119); some effects were observed in one sex only (lymphocytes in males in week 102, neutrophils in females in week 102 and in males in week 119), but the changes were transient

(lymphocytes, RBC, PCV in males in week 102), and there were no changes in other relevant parameters (for lymphocytes and neutrophils in the total WBC count).

Considering the final rating of confidence in the body of evidence for the HOC 'haematotoxicity' as 'high' (Annex E1) and the absence of adverse effects after 2-year exposure up to 1500 mg acesulfame K/kg bw per day (Table 11), the Panel considers that there is high confidence in the body of evidence (Table 12) that the exposure to acesulfame K (E 950) is not associated with haematotoxicity.

Liver toxicity

Three studies in rats covering durations of exposure of 104, 197 and 288 days (Mendoza-Pérez et al., 2021b), 480 days (Mendoza-Pérez et al., 2021a) and 2 years (Documentation provided to EFSA No 17), and two studies in mice with a duration of 11 weeks (Shou et al., 2024) or 20 weeks (Rathaus et al., 2024) were assessed for adverse effects in liver.

Serum triglycerides were unchanged in male rats (no female rats were included in the study) after exposure to 13.5 mg acesulfame K/kg bw per day in drinking water for 104 or 197 days, but they were statistically significantly increased on day 288 (Mendoza-Pérez et al., 2021b). Triglyceride levels remained unchanged in the 480-day study in male or female rats receiving acesulfame K in drinking water at doses of 60, 40 or 25 mg/kg bw per day during sub-acute (21, 35 and 63 days), sub-chronic (210 days) and chronic (480 days) exposures, respectively (Mendoza-Pérez et al., 2021a). No effect on serum triglycerides was reported in male mice (no female mice were included in the study) exposed to 77 mg acesulfame K/kg bw per day in drinking water for 20 weeks (Rathaus et al., 2024). In contrast, serum triglycerides and triglyceride content in liver tissue were statistically significantly increased in male mice (no female mice were included in the study) administered 40 or 120 mg acesulfame K/kg bw by gavage with water every 2 days for 11 weeks (Shou et al., 2024).

Serum cholesterol of male rats was not affected by sub-chronic exposures to 13.5 mg acesulfame K/kg bw per day (days 104 and 288) although a transient increase relative to water control (but not to a sucrose control) was recorded (day 197) in this study (Mendoza-Pérez et al., 2021b).

Clinical chemistry parameters, studied in male but not in female mice, such as alanine and aspartate aminotransferases (ALT, AST), total cholesterol (chol), high density lipoprotein (HDL) or HDL- chol, low density lipoprotein (LDL) or LDL-chol were not affected by exposure to 77 mg acesulfame K/kg bw per day in drinking water for 20 weeks (Rathaus et al., 2024) or when 40 or 120 mg acesulfame K/kg bw per day was administered by gavage with water control every 2 days for 11 weeks (Shou et al., 2024). In the latter study, total bile acid, total and direct bilirubin were not affected, but statistically significant, although not toxicologically relevant, increases in serum ALP (8% and 15%, respectively, as compared to the control group) were reported (Shou et al., 2024). Furthermore, serum lipopolysaccharide (LPS) at both acesulfame K doses was reported to be small but statistically significantly increased in a dose-dependent manner relative to the control and without concomitant clinical signs. The Panel noted, that LPS in serum originates only from the membrane of gram-negative bacteria, likely from the gastrointestinal (GI) tract. Thus, the increase might indicate some leakiness of the GI barrier, and/or insufficient clearing by the liver. This could be due to inflammation as serum levels of TNF-α and IL-6 (but not of IL-1β) were also statistically significantly increased at the high dose in these male mice, and microscopic examination revealed inflammatory cell infiltrations in the liver tissue from the high-dose group. Other findings in this study were: a statistically significantly increase in absolute liver weight (about 14% relative to the control group; a relative liver weight was not reported), a nuclear condensation of hepatocytes at low and high dose, and an increased fat deposition in hepatocytes, both observed by microscopy. The increased liver fat content was in accordance with a statistically significantly increase in triglyceride content in the liver (see above the text on serum and liver tissue triglycerides in Shou et al. (2024)). The Panel noted that reporting of all microscopic changes was only supported by micrographs (Figures 1A and 2A) and no information on incidence and severity of the microscopic changes was provided. The Panel considered absence of these data and of information on blinding during the microscopic examination as a high RoB (tier 3). Furthermore, the Panel noted that the clinical chemistry data did not support a (severe) liver damage, as the only change was a slight increase in ALP and there were no changes in AST, ALT, total bile acid or bilirubin.

In a 2-year study in rats with dietary exposure to acesulfame K at doses up to 1500 mg/kg bw per day (Documentation provided to EFSA No 17), there were no adverse effects on liver enzymes (ALP, ALT, AST) or serum protein and albumin or on the relative liver weight (absolute weight was not presented). No gross or microscopic non-neoplastic or neoplastic changes attributable to acesulfame K were seen in the liver (Documentation provided to EFSA No 17).

Considering the final rating of confidence in the body of evidence for the HOC 'liver toxicity' as 'high' (Annex E1) and the absence of adverse effects (see Table 11), the Panel considered that there is high confidence in the body of evidence (Table 12) that the exposure to acesulfame K is not associated with adverse effects on liver.

Nephrotoxicity

Nephrotoxicity was addressed in four studies of different duration and design in mice and rats (Cai et al., 2024; Chiang et al., 2022; Documentation provided to EFSA No 17; Shou et al., 2024).

The 28-day exposure to 7 or 350 mg acesulfame K/kg bw per day in drinking water of male mice was associated with a statistically significant and dose dependent increase in the relative urine output (RUO). The urinary concentration of potassium was statistically significantly reduced while no changes relative to the control group were reported for urinary concentrations of Na^+, Cl^-, Ca^{2+} at either dose (Cai et al., 2024).

No effect on creatinine or BUN was observed in male mice exposed to acesulfame K at 40 or 120 mg/kg bw administered by gavage every 2 days for 11 weeks (Shou et al., 2024).

Serum creatinine and blood urea nitrogen (BUN) levels were statistically significantly increased in female rats dosed by gavage with 100 mg acesulfame K/kg bw per day for 56 days (Chiang et al., 2022). The Panel noted that the levels of creatinine and BUN in the control and the treated groups were within the physiological ranges for female rats (Delwatta et al., 2018; Han et al., 2010; Keppler et al., 2007; Thammitiyagodage et al., 2020), while no other parameters related to kidney function were investigated in this study (Chiang et al., 2022).

In a 2-year study in male and female rats (Documentation provided to EFSA No 17) with a dietary exposure to acesulfame K at doses up to 1500 mg/kg bw per day, no effects were observed on BUN, urinalysis parameters (glutamic-oxalacetic transaminase, osmolality, composition, hereunder protein content) examined in weeks 35, 52, 78, 93 and 104, or on relative kidney weight. There were no treatment-related gross or microscopic non-neoplastic or neoplastic changes in the kidneys.

Based on the duration of exposure and the doses of the test compound in the combined chronic and carcinogenicity study in rats (Documentation provided to EFSA No 17) with no effects on creatinine or BUN in male mice in a 77-day sub-acute study (Shou et al., 2024), the Panel considered that the results from both studies superseded the findings of the low increases in serum creatinine and BUN in female rats exposed to acesulfame K in a sub-acute study (56 days) (Chiang et al., 2022), in which other endpoints related to nephrotoxicity were not studied.

Considering the final rating of confidence in the body of evidence for the HOC 'nephrotoxicity' as high (Annex E1) and the absence of adverse effects after 2-year exposure up to 1500 mg acesulfame K/kg bw per day (see Table 11), the Panel considers that there is high confidence in the body of evidence (Table 12) that the exposure to acesulfame K (E 950) is not associated with nephrotoxicity.

Other organ toxicity

One sub-acute study (14 and 28 days) in mice (Zhai et al., 2024) and one chronic (2 years) study in rats (Documentation provided to EFSA No 17), covering a dose range from about 1 to 1500 mg acesulfame K/kg bw per day, investigated adverse effects in gross or microscopic changes in organs other than liver and kidney (assessed separately above).

Colon

Colon length was not affected by a dose of 180 mg acesulfame K/kg bw per day given for 14 days to mice (three animals per sex per group) (Zhai et al., 2024). In another experiment in the same publication, the authors reported increased infiltration of inflammatory cells in the intestinal epithelium and decreased secretion of protective mucin produced by goblet cells using histological examinations (five male mice per group) at a dose of 3.69 mg acesulfame K/kg bw per day. Such findings were not observed in histopathological examinations in rats dosed from 150 to 1500 mg/kg bw per day for up to 2 years (60 animals per sex and group) (Documentation provided to EFSA No 17). In the WoE the Panel put more weight on the long-term study with a higher number of animals (see WoE Summary Annex E1).

Other organs and tissues

In the combined chronic toxicity and carcinogenicity study with acesulfame K in rats pretreated in utero (Documentation provided to EFSA No 17) no gross lesions attributable to exposure to the test item were reported. The microscopic examination of samples from 41 organs and tissues (colon included, kidney and liver assessed separately) did not reveal any histopathological changes which could be ascribed to the test item exposure.

Considering the final rating of confidence in the body of evidence for the HOC 'other organ toxicity' as 'high' and the absence of adverse effects (Table 11, Annex E1), the Panel considered that there is high confidence in the body of evidence (Table 12) that exposure to acesulfame K (E 950) is not associated with adverse effects in the colon or in any of the organs and tissues assessed in rodent models.

Glucose/insulin homeostasis

Nine studies of different duration and design measuring endpoints relevant for the assessment of effects on glucose and insulin homeostasis were included in the assessment. The species tested were rat (Mendoza-Pérez et al., 2021a, 2021b; Chiang et al., 2022; Hayes et al., 2022; Documentation provided to EFSA No 17) and mouse (Glendinning et al., 2020; Rathaus et al., 2024; Shi et al., 2019; Shou et al., 2024). Fasted blood glucose was reported in 4 studies (Mendoza-Pérez et al., 2021a, 2021b; Rathaus et al., 2024), whereas in 3 studies (Chiang et al., 2022; Documentation provided to EFSA No 17; Shou et al., 2024), animals were not fasted or fasting was not reported.

Blood glucose levels in fasted or not fasted animals

No effect on fasting blood glucose was reported in male rats exposed to 13.5 mg acesulfame K/kg bw per day in drinking water for 104 days and for 288 days; a statistically significant increase was recorded at 197 days, but values were equal to control at the end of the study (288 days) (Mendoza-Pérez et al., 2021a). Fasting blood glucose levels were not affected in

male or female rats by exposure to acesulfame K in drinking water for 480 days (25 mg/kg bw per day), except for transient changes; in detail, the blood glucose of males was statistically significantly lower than the control at 14 months, while in females it was increased after 2 months of exposure (Mendoza-Pérez et al., 2021a). There was no effect on blood glucose in female rats treated orally by gavage with 100 mg acesulfame K/kg bw per day for 56 days in water (Chiang et al., 2022). The 43-day exposure of male and female rats to approximately 15 mg acesulfame K/kg bw per day in drinking water had no effect on glucose tolerance investigated 4 days after cessation of acesulfame K intake (Hayes et al., 2022). Two-year dietary exposure of male and female rats to acesulfame K at doses up to 1500 mg/kg bw per day had no effect on blood glucose (Documentation provided to EFSA No 17). There were no effects on fasting blood glucose during an oral glucose tolerance test (OGTT) in male and female mice exposed to 3600 mg acesulfame K/kg bw per day for 28 days in drinking water (Glendinning et al., 2020). Neither was there any effect on blood glucose in male mice receiving 40 or 120 mg acesulfame K/kg bw by gavage every 2 days for 11 weeks (Shou et al., 2024). Furthermore, there was no effect on blood glucose after 6 h of food withdrawal measured weekly or in intraperitoneal glucose tolerance test in male mice exposed to acesulfame K in drinking water for 20 weeks, equivalent to 77 mg acesulfame K/kg bw per day (Rathaus et al., 2024). In contrast, one study in male mice reported an increase of about 30% in fasting blood glucose during an oral glucose tolerance test following exposure to acesulfame K in drinking water at a dose equivalent to 174 mg acesulfame K/kg bw per day for 12 weeks (Shi et al., 2019).

Blood insulin and insulin sensitivity

No effect on blood insulin was reported in male rats exposed to 13.5 mg acesulfame K/kg bw per day in drinking water for 3, 6 or 9 months (Mendoza-Pérez et al., 2021a). There were no effects in an intraperitoneal insulin-tolerance test in male mice at week 15 of exposure to 77 mg acesulfame K/kg bw per day in drinking water (Rathaus et al., 2024).

Due to uncertainty about fasting status of blood glucose in two studies and downgrading for imprecision (overall low number of animals in the studies and only one sex in two studies), the final rating of confidence in the body of evidence for the HOC 'glucose/insulin homeostasis' was rated 'moderate' (Annex E1, Table 11). There were no adverse effects in eight of nine studies, of which five in rats (Mendoza-Pérez et al., 2021a, 2021b; Chiang et al., 2022; Hayes et al., 2022; Documentation provided to EFSA No 17) and three in mice (Glendinning et al., 2020; Rathaus et al., 2024; Shou et al., 2024). The Panel considered that there is moderate confidence in the body of evidence (Table 12) that the exposure to acesulfame K (E 950) is not associated with an impairment of glucose/insulin homeostasis.

TABLE 12 Translation of confidence ratings into level of evidence for conclusions of adverse effects or no adverse effects for the included animal studies for each of the health outcome category (HOC) considered in the assessment.

HOC	Final rating of confidence	Level of evidence
General toxicity	High	High There is high confidence in the body of evidence that the exposure to acesulfame K (E 950) is not associated with general toxicity effects.
Additional clinical chemistry	Moderate	Moderate There is moderate confidence in the body of evidence that exposure to acesulfame K (E 950) is not associated with adverse changes in triglycerides, total cholesterol, HDL- or LDL-cholesterol levels.
Haematotoxicity	High	High There is high confidence in the body of evidence that the exposure to acesulfame K (E 950) is not associated with haematotoxicity.
Liver toxicity	High	High There is high confidence in the body of evidence that the exposure to acesulfame K (E 950) is not associated with adverse effects on liver.
Nephrotoxicity	High	High There is high confidence in the body of evidence that the exposure to acesulfame K (E 950) is not associated with nephrotoxicity.
Other organ toxicity	High	High There is high confidence in the body of evidence that exposure to acesulfame K (E 950) is not associated with adverse effects in colon, or any other of the 43 organs/tissues examined for neoplastic and non-neoplastic changes described (Documentation provided to EFSA No 17).
Glucose/insulin homeostasis	Moderate	Moderate There is moderate confidence in the body of evidence that the exposure to acesulfame K (E 950) is not associated with an impairment of glucose/insulin homeostasis.

Abbreviation: HOC, health outcome category.

3.5.4.2 | *Human studies*

The studies included in the assessment comprised 13 human studies (Bryant et al., 2014; Chiang et al., 2022; Chien et al., 2023; Debras et al., 2023; Debras et al., 2022a, 2022b; Duran Agüero et al., 2014; El Helou et al., 2019; Hess et al., 2018; Meyer-Gerspach et al., 2018; Olabi et al., 2018; Steinert et al., 2011; Wu et al., 2024). Among these studies, seven were allocated to tier 1 (Chien et al., 2023; Debras et al., 2023; Debras et al., 2022a, 2022b; El Helou et al., 2019; Meyer-Gerspach et al., 2018; Steinert et al., 2011) and 6 to tier 2 (Bryant et al., 2014; Chiang et al., 2022; Duran Agüero et al., 2014; Hess et al., 2018; Olabi et al., 2018; Wu et al., 2024) following a RoB evaluation. Annex E2 reports all the human studies evaluated, clustered by endpoint within the different HOCs, for which a WoE analysis was performed.

TABLE 13 Human studies included in the assessment.

Authors	Type of study design	Dose (g/person or g/kg bw)[a]	Intervention/exposure	Number of subjects	Population (mean age in years)	RoB tier
El Helou et al. (2019)	HCT (Randomised controlled trial) (single exposure per test preparation)	3.5 g of acesulfame K, equivalent to 49.4 mg/kg bw for lean participants and 30.9 mg/kg bw for obese participants based on reported mean body weights	Acesulfame K in vanilla custard, 1 day per test preparation	30 M (15 lean and 15 obese)	20.1 ± 0.4 for lean participants and 21.7 ± 0.9 for obese participants	1
Meyer-Gerspach et al. (2018)	HCT – randomised (counterbalanced), placebo-controlled, cross-over trial (single exposure per test beverage)	3.14 mg/kg bw (220 mg acesulfame K in 250 mL tap water) (based on a 70 kg adult)	Single exposure per test beverage	12 (6 M)	Adults Mean: 23 (19–25)	1
Steinert et al. (2011)	HCT-randomised, placebo-controlled, cross-over trial (single exposure per test beverage)	220 mg acesulfame K in 250 mL tap water (equivalent to 3.14 mg/kg bw, based on a 70 kg adult)	Single exposure per test beverage	12 (6 M)	Adults mean: 23.3 (19–29)	1
Chien et al. (2023)	Cohort study (Co)	0.0007 mg for girls and 0.0023 mg Acesulfame K intake for boys (respectively 0.004 and 0.0094% ADI).	Exposure was estimated through a food frequency questionnaire performed every 3 months between the recruitment and the end of puberty.	1893 (65% F)	Children/adolescents 9.69 ± 1.82 (F) and 11.78 ± 1.93 (M); 10.41 ± 2.11 (6–15 years)	1
Debras et al. (2022b)	Cohort study (Co)	Mean: 4.64 ± SD: 15.14 mg per day (lower consumers: 2.74 ± 2.86 mg per day; higher consumers: 22.39 ± 29.01 mg per day). Equivalent to mean 0.07 ± 0.22 mg/kg bw per day (lower consumers: 0.04 ± 0.04 mg/kg bw per day; higher consumers: 0.32 ± 0.41 mg/kg bw per day	Study from 2009 to 2021 (median follow-up: 7.8 years); acesulfame K was assessed by multiple 24 h recall.	102,865 (80.7% F)	Adults, 42.22 ± 14.5 years	1
Debras et al. (2022a)	Cohort study (Co)	Mean: 4.60 ± SD: 14.90 mg per day (lower consumers: 2.71 ± 2.79 mg per day; higher consumers: 22.14 ± 28.43 mg per day). Equivalent to mean 0.07 ± 0.21 mg/kg bw per day (lower consumers: 0.04 ± 0.04 mg/kg bw per day; higher consumers: 0.32 ± 0.41 mg/kg bw per day)	Study from 2009 to 2021; median follow-up: 9.0 years. acesulfame K was assessed by multiple 24 h recall.	103,388 (20,903 M and 82,485 F)	Adults: 42.22 ± 14.41 years	1

TABLE 13 (Continued)

Authors	Type of study design	Dose (g/person or g/kg bw)[a]	Intervention/exposure	Number of subjects	Population (mean age in years)	RoB tier
Debras et al. (2023)	Cohort study (Co)	Mean: 4.58 ± SD: 14.9 mg per day (2.68 ± 2.76 mg/day for lower consumers and 22.0 ± 28.4 mg/day for higher consumers)	Study from 2009 to 2022; mean follow-up of 8.97 ± 2.33 years. acesulfame K was assessed by multiple 24 h recall	105,588 (83,625 F and 21.963 M)	Adults: 42.5 ± 14.6 years	1
Bryant et al. (2014)	HCT (Randomised controlled trial) single exposure per test beverage	85 mg Acesulfame K with 45 g glucose in 250 mL water. Equivalent to 1.21 mg/kg bw (based on 70 kg adult)	Acesulfame K with glucose in water, 1 day per test (at least 3 days between visits)	10 (60% M)	Adults, 21 ± 2.4	2
Olabi et al. (2018)	HCT (Randomised controlled trial) single exposure per test preparation	46.2 mg/kg bw based on 3.5 g intake and study mean body weight of 75.7 kg	Single exposure per test preparation	11 M	Adults, 20.8 ± 0.9 (20–23 years)	2
Chiang et al. (2022)	Cohort study (Co)	T0 (no consumption), T1 (0–0.05% ADI), T2 (0.05–0.27% ADI) and T3 (> 0.27% ADI), considering JECFA ADI for acesulfame K (15 mg/kg bw) and participants' body weight.	Study from 2018 to 2021. acesulfame K was assessed by a food frequency questionnaire in pregnancy	613 (100% F)	Pregnant women	2
Wu et al. (2024)	Cross-sectional study (CrSe)	0.6543 mg acesulfame K median intake (0.14 %ADI)	Not specified (NNS food frequency questionnaire)	884 F	Children, adolescents (6–12 years) (9.75 ± 1.8)	2
Duran Agüero et al. (2014)	Cross-sectional study (CrSe)	NA	NA, Food frequency questionnaire	571 (281 M and 290 F)	Adolescents (13.2 ± 6.3 years)	2
Hess et al. (2018)	Cross-sectional study (CrSe)	Consumer intake ≥ 3 mg	2-week data collection period (3 × 24 h recall)	125 (54 M and 71 F)	Adults, 36.7 ± 16.5	2

Abbreviations: F, females; HCT, human controlled trial; M, males; NA, Not applicable; RoB, risk of bias.

[a]As reported by the authors.

The outcomes addressed in the studies summarised in Table 13 were aggregated into five different HOCs, (Table 14). The human HOCs were constructed to align, to the extent possible, with the HOCs defined for the animal studies (Table 10). 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 15).

TABLE 14 Health outcome categories and related endpoints of the appraised human studies subjected to WoE evaluation.

Health outcome categories (HOCs)	Endpoints
Cancer	All cancer, which were then divided into breast cancer, obesity-related cancer, prostate cancer
Cardiovascular risk factors and disease	Metabolic syndrome (MetS) trygliceride values, waist circumference in unadjusted and adjusted models, cardiovascular disease, coronary heart disease, cerebrovascular disease, body composition (fat mass, fat-free mass, BMI and Waist-to-Height Ratio), type II diabetes
Glucose/insulin homeostasis	GLP-1, blood glucose measurements (OGTTs, glucose value (spot samples), insulin, plasma glucagon, type 2 diabetes
Birth outcomes	Preterm births
Development	Precocious puberty

Abbreviations: GLP-1, glucagon-ike peptide-1; HOC, health outcome categories; OGTT, oral glucose tolerance test.

TABLE 15 Rating of the confidence in the body of evidence for each health outcome category investigated. For a detailed assessment see Annex E2.

Health outcome categories (HOCs) investigated	Initial rating (No. of studies)	Elements for downgrading concern for:						Elements for upgrading					
		Risk of bias	Unexplained inconsistency	Relevance of studies	Imprecision	Downgrading[a]	Residual confounding	Magnitude of effect	Dose–response	Consistency acrossstudy population/ study design	Upgrading	Final rating of confidence	Association observed (yes/no)
Cancer	Moderate ($n=1$)	Not serious	N.A.	Not serious	Not serious	Yes	No	Not large	Yes	N.A.	No	Low	Yes
Cardiovascular risk factors and disease	Moderate ($n=4$)	Not Serious	Serious	Not serious	Not serious	Yes	Yes	Large	Yes	N.A.	No	Low	Yes
Glucose/insulin homeostasis	High ($n=8$)	Not serious	Not serious	Not serious	Serious	Yes	No	Not large	Yes	No	No	Moderate	No
Birth outcome (preterm)	Moderate ($n=1$)	Serious	N.A.	Not Serious	Serious	yes	No	Large	Yes	N.A.	No	Very low	Yes
Development	Low ($n=1$)	Not serious	N.A.	Not serious	Not serious	No	No	Large	Yes	N.A.	No	Low	Yes

[a]For reasons for downgrading see also main text.

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 will be presented in Sections 3.5.4.3 and 3.7.

Cancer

Cohort studies

Debras et al. (2022b) conducted a prospective cohort study ($N = 102.865$; median follow-up time 7.8 years) in France on adult volunteers (78.5% females, mean age 42.2 years) with internet access to investigate the association between artificial sweeteners consumption from all dietary sources and cancer incidence. Dietary intake including the use of table-top sweeteners was assessed by multiple 24-h recalls. After adjusting, in the Cox-proportional hazards models for age, sex, education, physical activity, smoking, body mass index, height, percentage weight gain during follow-up, diabetes, family history of cancer, intake of aspartame and sucralose, number of 24-h dietary records and baseline intake of energy, alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods and dairy products, an association with an increased risk of overall cancer (HR:1.13, 95% CI: 1.01–1.26, p-trend = 0.007) was observed for high acesulfame K consumption (≥ 5.06 mg/day in men and ≥ 5.50 mg/day in women, mean intake 22.39 mg/day) compared to non-consumers. When analysed separately by types of cancer, an increased risk, although not statistically significant, was found for prostate (HR: 1.18 (0.82–1.71)) and breast cancer (HR: 1.17, 95% CI (0.96–1.43). When the authors grouped in obesity[25] related-cancers similar results were found (HR: 1.13, 95% CI: 0.97–1.30). The main limitation of the study is the selection bias (volunteers) followed by multiple imputation for missing values without saying what variables were included in the imputation procedure and the number of missing information for each variable.

For the outcome cancer, only one study that was rated as tier 1, was available (Debras., 2022b). In this study a dose–response association was found between acesulfame intake and overall cancer (HR:1.13, 95%CI: 1.01–1.26, p-trend = 0.007). For separate cancer types (prostate, breast and obesity-related cancer), no statistically significant findings were found. Conclusions cannot be drawn regarding the association between acesulfame K and cancer due to the availability of only one study with intrinsic limitations and the lack of association between different cancer types.

Cardiovascular risk factors and disease

For the outcome cardiovascular risk factors, one cohort study investigated the association between acesulfame K and cardiovascular disease (Debras et al., 2022a) while one cohort (Chien et al., 2023) and two cross-sectional studies (Duran Agüero et al., 2014; Hess et al., 2018) investigated the association between acesulfame K and cardiovascular risk factors. Debras et al. (2022a) found no association between acesulfame K, modelled as continuous variable and cardiovascular disease risk (HR: 1.18, 95% CI: 0.98–1.4). However, when the associations were assessed for different cardiovascular disease subtypes, an increased risk was reported for coronary heart disease (HR: 1.40; 95% CI: 1.06–1.84) but not for cerebrovascular diseases (HR: 1.01, 95% CI: 0.79–1.29). When acesulfame K was treated as categorical variable an increased risk of cardiovascular diseases was found for a 'high'[26] intake of acesulfame K (≥ 5.42 mg/day in men and ≥ 5.43 mg/day in women versus no consumption) HR: 1.30, 95% CI: 1.09–1.55, p-trend = 0.001). Chien et al. (2023) conducted a study among young subjects (6–15 years old) and observed a decreased BMI (T1 β: −0.17 95% CI: 0.30–0.04) for 'moderate', but not for 'high', intake of acesulfame K. Duran Agüero et al. (2014) also conducted a study on young subjects and found no association between acesulfame K and 'overweight-obesity' (OR:1.12; 95% CI:0.73–1.63). Hess et al. (2018) found no association between acesulfame K intake and metabolic syndrome risk factors ($p = 0.28$) in adults but intake of acesulfame K was associated with high waist circumference ($p = 0.024$).

Cohort studies

Debras et al. (2022a) studied the association between artificial sweeteners from all dietary sources and table-top sweeteners, and the risk of cardiovascular diseases in a French prospective cohort study ($N = 103.388$ adult volunteers with internet access, 79.8% females, mean age 42.22 years, median follow-up time 9 years). Dietary intake including the use of table-top sweeteners was assessed by multiple 24-h recalls. After adjusting for age, sex, physical activity, smoking, education, family history of cardiovascular disease, energy intake, intake of aspartame and sucralose, alcohol, sodium, saturated fatty acids, polyunsaturated fatty acids, fibre, sugar, fruit and vegetables, red and processed meat, in a Cox-proportional hazard model, a not statistically significant trend of increased risk for cardiovascular diseases was reported for acesulfame intake (HR: 1.18, 95% CI: 0.98–1.41). When the models were run separately for coronary heart and cerebrovascular diseases, an increased risk was observed for coronary heart disease (HR: 1.40, 95% CI: 1.06–1.84) but not for cerebrovascular diseases (HR: 1.01, 95% CI: 0.79–1.29). When acesulfame K intake was categorised into three groups, an association with an increased risk for cardiovascular diseases was observed for low (below the sex-specific median: < 5.42 mg/day for men, < 5.43 mg/day for women, HR: 1.20, 95% CI: 1.05–1.37) and high consumers (the sex-specific median or mor: ≥ 5.42 mg/day for men, ≥ 5.43

[25]Defined as: colorectal, stomach, liver, mouth, pharynx, larynx, oesophageal, breast, ovarian, endometrial and prostate cancers.
[26]'High' and 'moderate' intakes as defined by the authors.

mg/day for women; HR:1.30, 95% CI: 1.09–1.55, *p*-trend = 0.001). When the models were run separately for coronary heart diseases and cerebrovascular diseases, an association with an increased risk was observed for cerebrovascular diseases for low (HR: 1.29, 95% CI: 1.07–1.57) and high acesulfame K intake (HR: 1.31, 95% CI: 1.05–1.63 *p*-trend = 0.003). An increased risk that did not reach statistical significance was seen for coronary heart diseases (HR: high vs. non-consumers, HR: 1.27, 95% CI: 0.99–1.64).

Chien et al. (2023) conducted a cohort study from 2018 to 2021 in Taiwan (*N* = 1893) to study the association between sweeteners intake (including acesulfame K) and BMI, waist-to-height ratio and body composition changes during pubertal growth in children and adolescents (6–15 years). A food frequency questionnaire was used to assess sweeteners intake every 3 months. Proportions of daily intake with respect to ADI was categorised into tertiles. After controlling for parental education time, family income, sleep quality, amount of exercise and total energy intake, no clear association was found between acesulfame K intake and BMI, waist-to-height ratio, fat-free mass and fat mass. A decrease in BMI (β: −0.17 95% CI: −0.30 to 0.04) was observed for moderate intake but not for high intake. One limitation of the study was the lack of control for other dietary items in the model; another was the exposure assessment.

Cross-sectional studies

Hess et al. (2018), conducted a cross-sectional study (*N* = 125) in adults (mean age 36.7, SD = 16.5) in USA to examine the association between 'metabolic syndrome risk factors' and intake of non-nutritive sweeteners (NNS) including acesulfame K. Diet was assessed by three 24-h food recalls. NNS consumption was estimated by foods, beverages and table-top sweeteners. After controlling for age, sex, total daily caloric intake, total Healthy Eating Index scores, physical activity and total NNS, acesulfame K was not associated with risk factors for metabolic syndrome ($p = 0.28$). However, acesulfame K was associated with high waist circumference ($p = 0.024$). The limitations of the study were the lack of sampling of the study population and the small sample size.

Duran Agüero et al. (2014) conducted a cross-sectional study among 571 students (mean age of 13.2, SD = 6.3 years; 281 males and 290 females) from the cities of Viña del Mar and Santiago de Chile. The aim of the study was to examine the association between nutritional status (Body Mass Index for age) and non-nutritive sweeteners, including acesulfame K. Students were classified as normal weight, underweight, overweight and obese according to United States Centers for Disease Control (CDC) growth charts. Sweeteners consumption was assessed by a food frequency questionnaire that included only foods containing sweeteners. After controlling for sex, age and education, no association (OR: 1.12; 95%: 0.734–1.63) was found between acesulfame K intake and overweight-obesity. Among female students, the normal weight group showed a higher consumption of acesulfame K per kilogram of body weight in comparison to the overweight group ($p < 0.01$). Main limitations of the study were the lack of control for other dietary components and physical activity.

All studies that were included in the RoB for the outcome cardiovascular risk factors were rated as moderate RoB (tier 2) except for the study of Debras et al. (2022a) that was rated as low RoB (tier 1). The use of a cross-sectional design in most studies and the use of non-apical outcomes (e.g. metabolic syndrome risk factors, BMI and body composition) and selection bias were the main issues of the studies included.

Glucose homeostasis

Four short-term cross-over trials (*N* = 11–15 subjects) (El Helou et al., 2019; Meyer-Gerspach et al., 2018; Olabi et al., 2018; Steinert et al., 2011) and a randomised trial (*N* = 10) were considered for the assessment of the effect of acesulfame K on glucose homeostasis. Out of the five studies, four showed no effect (El Helou et al., 2019; Meyer-Gerspach et al., 2018; Steinert et al., 2011), while Bryant et al. (2014) through an intervention study on 4 separate days among healthy fasted subjects (*N* = 10), showed a larger glycemic response (17.45%) in comparison to glucose alone (147.1 ± 15.9 vs. 125.3 ± 16.2 mmol/L) after acesulfame K intake (85 mg).

Most of the studies considered for the outcome glucose homeostasis were tier 1 (three out of five). Limitations of the studies were the small sample sizes and the short-term nature of the studies.

Randomised trials

Bryant et al. (2014) conducted in USA an intervention study on 4 separate days among healthy fasted subjects (*N* = 10) of mean age 21 years (SD = 2.4 years) to examine the effect of aspartame (150 mg aspartame), saccharin (20 mg) and acesulfame K (85 mg) on glycemic responses and appetite on regular intervals (0, 5, 15, 30, 45, 60 min). Each sweetener was given in combination with glucose (45 g). There was no effect of any sweetener on the blood glucose response at any time point, except for acesulfame K that showed a larger glycemic response (17.45%) in comparison to glucose alone (147.1 ± 15.9 vs. 125.3 ± 16.2 mmol/L). None of the sweeteners had an effect on perceptions of hunger or fullness. The main limitation of the study was the small sample size.

Olabi et al. (2018) conducted a randomised crossover design in Lebanon (*N* = 11, BMI: 19–25 kg/m^2, age 18–50 years) on men to assess the effect of food acceptability on serum glucose, postprandial ghrelin and insulin levels and on appetite score. Subjects were randomly assigned to 1 of 2 meals: vanilla custard with acesulfame K (LA) or without it (HA). Blood samples were collected before meal (time 0) and after 15, 30, 60, 120, 180 and 240 min. Ghrelin levels were significantly higher ($p < 0.05$) for LA meal at 180 and 240 min in comparison to the HA meal while insulin levels were significantly higher

($p < 0.05$) for HA meal at 15 and 30 min. Serum glucose did not differ between meals. Appetite scores did not vary between meals at different time points. The main limitation of the study is the sample size and attrition (out of 20 subjects included in the study only 11 completed it).

Steinert et al. (2011) conducted in Switzerland a six-way cross-over trial ($N = 12$, 19–23 years) to assess human gut response to artificial sweeteners and carbohydrate sugars. On separate days (3–5 days), each subject received an intragastric infusion of 50,000 mg glucose, 25,000 mg fructose, 169 mg aspartame, 220 mg acesulfame K or 62 mg sucralose dissolved in 250 mL of water or water only (control). Blood samples were taken at regular time intervals (5, 10, 15, 20, 30, 45, 60, 75, 90 and 120 min) after the infusion was completed. A shorten feeling of fullness was observed for acesulfame and sucralose but it did not reach statistically significant results. In contrast with glucose, sucralose, aspartame or acesulfame K did not affect plasma concentrations of glucagon-like peptide, peptide tyrosine and ghrelin when compared to water. A decrease in glucose and insulin levels were not affected by aspartame, acesulfame K or sucralose. The main limitations of the study were the sample size and the very short wash out period between treatments.

Meyer-Gerspach et al. (2018) conducted a randomised, double-blind, cross-over trial ($N = 12$, 19–25 years) to study the effect of the administration (4 separate occasions) of intragastric sweeteners (50 g glucose plus 250 mL tap water; 25 g fructose plus 250 mL tap water; 220 mg acesulfame K plus 250 mL tap water; 250 mL tap water (placebo) on GI motility, plasma motilin, ghrelin, GLP-1, CCK, gastrin, glucose and hunger in humans. Gastroduodenal manometry was used to record contractions and usual analog scales were used to rate hunger and satiety feelings. No effect after acesulfame K administration was seen for antral motility, motilin and cholecystokinin secretion. However, an initial decrease in hunger feelings and stronger increase in satiety was observed ($p < 0.05$) after acesulfame K administration compared with placebo, followed by a fast return of hunger and decrease of satiety ($p < 0.05$). The main limitations of the study are the sample size and the very short wash out period between treatments.

El Helou et al. (2019) conducted a randomised cross-over trial (15 normal weight subjects and 15 obese, 18–50 years) to study the effect of a low acceptability food (custard with 3.5 g acesulfame K) on glucose, insulin, ghrelin and glucagon-like peptide 1 (GLP-1). Appetite scores and subsequent energy intake were also recorded. Subjects were randomly assigned to either custard or custard with acesulfame K. Custard with acesulfame K did not change postprandial status of glucose, insulin, ghrelin and GLP-1 or sensation of hunger, satiety or fullness in normal and obese individuals. Energy intake was higher in participants with obesity but did not reflect postprandial hormones and appetite scores and energy intake was not statistically significantly different between meals. The main limitations of the study were the sample size and the very short wash out period between treatments.

Cohort studies

Debras et al. (2023), within the French cohort study NutriNet-Santé, ($N = 172{,}456$ subjects with internet access) studied the association between artificial sweeteners, including acesulfame-K and the risk of type 2 diabetes (T2D) ($N = 107.561$ mean age 42.5 years, 79.2% women, median follow-up time 8.97 years). Dietary Intake which included table-top sweeteners, was assessed from repeated 24-h recall. In a cox-proportional model after controlling for age, sex, BMI, weight change, physical activity, smoking, education, family history of diabetes, presence of cardiovascular disease, hypertension, dyslipidaemia, number of 24-h dietary records, energy intake, alcohol saturated fatty acids, fibre, sugar, fruit and vegetables, red and processed meat, and dairy products, high intake of acesulfame-K (> 16.4 mg/day in men, > 18.5 mg/day in women), was associated with an increased risk of type 2 diabetes (HR: 1.70; 95% CI 1.42–2.04, p-trend < 0.001). The main limitation of the study was the generalisability of the study since only subjects with internet access were enrolled in the study and only 62.37% (only subjects with at least two 24 h recall) of the initial sample were included in the analysis.

Birth outcomes

Cohort studies

Chiang et al. (2022) conducted a cohort study in Taiwan on 613 pregnant women (mean age 33.9 years old) to investigate the relation between acesulfame K consumption and preterm delivery (< 37 gestational weeks). Acesulfame K intake was assessed by a food frequency questionnaire (305 items) during the past 3 months and a 3-day dietary recall. Table-top sweeteners use was not mentioned. The amount of daily consumption of Acesulfame K was then divided by body weight to calculate the percentage of ADI (15 mg/BW). After controlling for age, education and family income, height, pre-pregnancy BMI and total calorie intake, an increased risk was found for moderate (T2 vs. T1% ADI ≤ 0.0027; OR: 3.21, 95% CI: 1.02, 10.12) and high levels of acesulfame K intake (T3 vs. T1: %ADI > 0.0027; OR: 3.33 95% CI: 1.17, 9.47). Limitations: Out of 1170 initially enrolled only 613 women had births records (52.4%); loss of follow-up of 47.6%, missing values mentioned but no number provided, lack of control for important confounders such as pregnancy disorders (e.g. placental dysfunction, pre-eclampsia) and maternal chronic diseases (e.g. diabetes, hypertension), smoking and caffeine consumption.

For the Birth outcome, only one study, rated as tier 2, (moderate RoB) was available. In this study an increased risk was found for acesulfame K consumption and preterm delivery.

Development

Cross-sectional

In Taiwan a cross-sectional study (Wu et al., 2024) was conducted on 884 girls (mean age 9.75 years) from paediatric endocrinologist clinics to study the association between sweeteners, including acesulfame K consumption and central precocious puberty (e.g. breast development, pubic hair, armpit hair, menstruation and acne in girls < 8 years; advanced bone age of ≥ 2, basal luteinizing hormone ≥ 0.3 IU/L, LH levels ≥ 5 IU/L or peak stimulated LH/follicle-stimulating hormone (FSH) IU/L ≥ 0.66 after exogenous GnRH stimulation). A food frequency questionnaire of 34 food items was used to assess intake of acesulfame K. After controlling for age, BMI *z*-score, sleep quality, total energy intake and parental education level, high acesulfame K intake (> 0.6543 mg daily) was associated with higher precocious puberty risk in girls (OR: 2.01, 95% odds ratio: 1.25–3.23, *p*-trend = 0.003). The main limitation of the study was the use of a non-random sample and the lack of control for other potential risk factors (e.g. nutritional deficiencies early in life, psychological distress).

For the Development outcome, only one study, rated as tier 2 (moderate RoB), was available. In this study an increased risk was found for acesulfame K consumption and higher precocious puberty risk (Table 16).

TABLE 16 Translation of confidence ratings into level of evidence for conclusions of adverse effects or no adverse effect on health for the included human studies for each of the health outcome category (HOC) considered in the assessment.

HOC	Final rating of confidence	Level of evidence
Cancer	Low	Low A positive association with cancer was reported in one prospective cohort study. As consumption of acesulfame K is likely linked to certain lifestyle and dietary habits which, despite adjustments for diet may still leave room for confounding, limited conclusions can be drawn from a single observational study. These findings would therefore need to be replicated in an independent setting. The Panel therefore considers that the level of evidence for an association with cancer is low.
Cardiovascular risk factors and disease	Low	Low Overall findings from different studies within this health outcome category were inconclusive. One large prospective study showed no association between acesulfame K and cardiovascular disease. However, in a stratified analysis of the same data an increased risk was observed for acesulfame K and coronary heart disease. With no clear support from other studies the Panel concluded that the overall confidence in the evidence for an effect on cardiovascular risk factors and disease is low.
Glucose/insulin homeostasis	Moderate	Moderate Several small intervention studies reported on the effect on postprandial changes in glucose or insulin or other measures of glucose homeostasis. One large prospective cohort study reported association with type 2 diabetes. With no evidence from existing studies on the effect of acesulfame K on short-term glucose homeostasis, the biological explanation for an association with type 2 diabetes is not well defined and unclear. Limited evidence can be drawn from a single observational study and the findings of no effect from several intervention studies on short-term glucose homeostasis are given more weight. The Panel concluded that there is moderate confidence in the evidence for no effect on glucose/insulin homeostasis.
Birth outcomes	Very low	Inadequate The Panel considered that the single study reporting association with preterm delivery provides inadequate confidence in the evidence for an association between acesulfame K exposure and preterm delivery. The main reasons for this conclusion are limited confounder control (lack of reporting and/or adjustment for underlying maternal pregnancy complication and underlying diseases), and the fact that limited conclusions can be drawn from a single observational study.
Development	Low	Low One cross-sectional study reported a positive association between consumption of acesulfame K and precocious puberty in girls. As dietary habits of children and teenagers change rapidly with age, the current dietary habits as reflected in intake of acesulfame-K may not be reflective of past intakes, which would be more likely to influence onset of puberty. Given the cross-sectional study design and lack of other human studies, the panel concluded that the confidence in the evidence for precocious puberty in girls is low.

Abreviation: HOC, health outcome category.

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

Human and animal evidence streams were integrated for similar HOCs, in accordance with Appendix A and with definition given in the Protocol (EFSA, 2020a; 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 were expressed in terms of likelihood of an association between the intake of acesulfame K (E 950) and an adverse effect on human health. Consideration was given to the conclusions of previous evaluations (JECFA, 1991; SCF, 2000) to assess whether the new data support them. In the case of the HOCs with data available only from animal studies (namely 'haematotoxicity', 'liver toxicity', 'nephrotoxicity', 'general toxicity (all but body

weight)' and 'other organ toxicity (all but carcinogenicity)', the integration of evidence in Table 18 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 re-evaluation in accordance with the revised protocol (EFSA, 2020a; EFSA FAF Panel, 2023) (Table 17).

TABLE 17 Overview of the health outcome category (HOC) for which human and animal evidence streams were integrated.

HOC human studies	HOC animal studies
Cancer	Other organ toxicity
Glucose/insulin homeostasis	Glucose/insulin homeostasis
Cardiovascular risk factors and disease	General toxicity Additional clinical chemistry

The Panel considered it appropriate to integrate the (i) endpoint 'neoplastic changes' from the animal studies HOC 'other organ toxicity' with the human HOC 'cancer' and (ii) the endpoint bodyweight from 'general toxicity' and triglycerides and total cholesterol 'additional clinical chemistry' from animal studies with the HOC 'cardiovascular risk factors and diseases' addressed in human studies (Tables 10 and 14).

Cancer/other organ toxicity

The HOC 'cancer' from human studies was integrated with the endpoint histopathological changes of the HOC 'other organ toxicity' in animal studies. The Panel considered the level of evidence in human data (one study only) to be low to assess whether the exposure to acesulfame K is associated with cancer. The level of evidence in the included animal studies for the lack of carcinogenic effects was 'high', and the Panel considered that there is a high confidence in the body of evidence that exposure to acesulfame K is not associated with histopathological changes including carcinogenicity. Integrating the evidence from human and animal studies, the Panel concluded that it is unlikely that exposure to acesulfame K (E 950) is associated with cancer in humans.

Glucose and insulin homeostasis

The HOC 'glucose and insulin' was evaluated in human and animal studies. The level of evidence was rated as 'moderate' for the lack of adverse effects both in human and animal studies. Therefore, the Panel concluded that it is unlikely that exposure to acesulfame K (E 950) is associated with disturbances of glucose/insulin homeostasis in humans.

Cardiovascular risk factors and disease

The human studies which included one large prospective cohort study showed no association between acesulfame K (E 950) and overall cardiovascular disease. In a stratified analysis of the same data an increased risk was observed for acesulfame K (E 950) and coronary heart disease. The proposed association with coronary heart disease, stemming from the single available study in humans, would need to be confirmed. With no clear support from other studies, the Panel concluded the level of evidence for adverse effects was low. None of the 11 animal studies in which a wide dose range was tested, reported an adverse effect on body weight.[27] Triglyceride and total cholesterol levels in four of the same studies were not adversely increased. The level of evidence for no effect for the endpoints body weight under HOC 'general toxicity' and triglyceride and total cholesterol under HOC 'additional clinical chemistry' combined was evaluated as high. The Panel concluded that it is unlikely that acesulfame K (E 950) is associated with cardiovascular risk factors and disease.

Birth outcomes (preterm)

A single observational study constituted the body of evidence of human studies in which intake of acesulfame K was associated with preterm delivery. The level of evidence for this adverse effect was evaluated as inadequate. There was no supporting evidence from animal studies. The Panel considered that it was not possible to conclude that acesulfame K (E 950) is associated with adverse events in birth outcomes, due to the lack of support from other studies and the fact that there is no evidence from animal studies which could support the findings from the only eligible human study for this endpoint.

[27]Generally, changes in body weight in laboratory rodents of this magnitude are not considered adverse (van Berlo et al., 2022; WHO, 2015).

Development (precocious puberty)

The evidence consisted of a single cross-sectional study that reported on precocious puberty in girls. The level of evidence was rated as low. There was no supporting evidence from animal studies. The Panel considered that it was not possible to conclude that acesulfame K (E 950) is associated with onset of puberty in girls, due to the lack of support from other studies, limitations of the cross-sectional study design and the fact that there is no evidence from animal studies which could support the findings from the only eligible human study for this endpoint.

General toxicity (all but body weight)

The level of evidence of the HOC 'general toxicity' in animal studies, consisting of several endpoints (Table 11), was evaluated as high. The Panel concluded that it is unlikely that acesulfame K (E 950) is associated with general toxicity in humans.

Liver toxicity

In the assessed animal studies, no adverse test substance-elated effects were observed for several endpoints comprising the HOC liver toxicity (Table 11). The level of evidence was evaluated as high. Effects on liver were not reported in the human studies. The Panel concluded that it is unlikely that acesulfame K (E 950) is associated with liver toxicity in humans.

Nephrotoxicity

No adverse test substance related effects were observed for several endpoints of the HOC nephrotoxicity (Table 11) in the assessed animal studies. Single incidents could be explained by possible adaptive responses. The level of evidence was evaluated as high. Effects on kidneys were not reported in the human studies. The Panel concluded that it is unlikely that acesulfame K (E 950) is associated with renal toxicity in humans.

Haematotoxicity

No adverse test substance related effects on a number of haematological parameters (Table 11) were observed in the combined chronic toxicity and carcinogenicity study (Documentation provided to EFSA No 17). The level of evidence was evaluated as high. Haematological parameters were not reported in human studies. The Panel concluded that it is unlikely that acesulfame K (E 950) is associated with haematological effects in humans.

Other organ toxicity (all but carcinogenicity)

No treatment-related changes including relative organ weight in any of the organs or tissues were observed in the animal studies of which one was the combined chronic toxicity and carcinogenicity study (Documentation provided to EFSA No 17). The level of evidence was evaluated as high. Other toxicity effects on organs or tissues were not reported in the human studies. The Panel concluded that it is unlikely that acesulfame K (E 950) is associated with toxicity in any organ or tissue in humans.

3.5.5 | Hazard characterisation and identification of a reference point

For the selection of the reference point (RP), considering the outcome of the assessment of HOCs, 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 18).

TABLE 18 Overview of the conclusions on the level of likelihood of an association or absence of association between the intake of acesulfame K (E 950) and an adverse effect on human health for each health outcome category (HOC).

Evidence stream	HOC (endpoint)	Effect or association observed (yes/no)	Level of evidence[a]	Integration of the evidence (likelihood)
Human	Cancer	Yes	Low	It is **unlikely** that intake of acesulfame K (E 950) is associated with cancer.
Animal	Other organ toxicity (carcinogenicity)	No	High	
Human	Glucose/insulin homeostasis	No	Moderate	It is **unlikely** that intake of acesulfame K (E 950) is associated with disturbances of glucose or insulin homeostasis.
Animal		No	Moderate	

TABLE 18 (Continued)

Evidence stream	HOC (endpoint)	Effect or association observed (yes/no)	Level of evidence[a]	Integration of the evidence (likelihood)
Human Animal	Cardiovascular risk factors and disease General toxicity (body weight) Additional clinical chemistry (triglycerides, total cholesterol)	Yes No	Low High	It is **unlikely** that that intake of acesulfame K (E 950) is associated with effects on cardiovascular risk factors and disease.
Human Animal	Birth outcomes (preterm)	Yes	Inadequate	It is **not possible to conclude** that intake of acesulfame K (E 950) is associated with adverse birth outcomes.
Human Animal	Development (precocious puberty)	Yes	Low Missing data	It is **not possible to conclude** that intake of acesulfame K (E 950) is associated with onset of puberty in girls.
Human Animal	General toxicity (all but body weight)	No	Missing data High	It is **unlikely** that acesulfame K (E 950) is associated with general toxicity in humans.
Human Animal	Nephrotoxicity	No	Missing data High	It is **unlikely** that acesulfame K (E 950) is associated with nephrotoxicity in humans.
Human Animal	Liver toxicity	No	Missing data High	It **is unlikely** that acesulfame K (E 950) is associated with liver toxicity in humans.
Human Animal	Other organ toxicity (all but carcinogenicity)	No	Missing data High	It is **unlikely** that acesulfame K (E 950) is associated with toxicity in any organ or tissue in humans.
Human Animal	Haematotoxicity	No	Missing data High	It is **unlikely** that acesulfame K (E 950) is associated with haematological effects in humans.

Abbreviation: HOC, health outcome category.

[a]'Missing data' pertains to data missing in the body of evidence in the current re-evaluation in accordance with the revised protocol (EFSA, 2020a; EFSA FAF Panel, 2023).

The Panel therefore addressed attention to the key studies in the previous evaluations by JECFA and SCF which were used for the derivation of the ADI for acesulfame K: a 2-year study in dogs (Documentation provided to EFSA No 17; cited in SCF, 2000) and a 2-year combined chronic toxicity and carcinogenicity study in rats (Documentation provided to EFSA No 17; cited in JECFA, 1991). In these two studies the NOAEL was 3% acesulfame K in the diet, the highest dietary concentration tested, corresponding to doses of 900 mg/kg bw per day in the dog and to 1500 mg/kg bw per day in the rat. Originally, the NOAEL from the 2-year study in dogs was considered as RP for derivation of the ADI of 0–9 mg/kg bw per day by both committees (JECFA, 1983; SCF, 1985) according to the view of that time that the dog was the most sensitive species. In the subsequent re-evaluations, JECFA based the ADI on the NOAEL from the rat study recognising that acesulfame K is not metabolised in any tested species and that the 2-year study in rats represented a greater portion of the life span than did the 2-year study in dogs (JECFA, 1991). The SCF maintained the ADI based on the RP from the dog study, as the Committee concluded that the peak plasma concentrations in the dog was higher than in the rat, and the acesulfame K bolus administration in feed for dogs might better reflect intake pattern of acesulfame K in humans (SCF, 2000). In the current assessment, the RoB in the 2-year dog study (Documentation provided to EFSA No 17) was assessed as tier 3 (high RoB) due to selection, performance and detection bias (Annex D), and the study was therefore not further considered. The 2-year study in rats (Documentation provided to EFSA No 17) was evaluated as RoB tier 2 (moderate) and was included in the WoE assessment. As no new evidence challenged the findings in this study, the Panel continues to regard this as the study on which the derivation of the ADI is to be based. Consequently, the 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 in the 2-year study in the rat of 3% acesulfame K in the diet, corresponding to 1500 mg/kg bw per day.

3.6 | Environmental considerations

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 a food additive under Regulation (EU) No 257/2010, EFSA has not received any information from IBOs or any other interested party in relation to any environment risks of acesulfame K (E 950) however it became aware of a large amount of data and information available in the public domain.

A systematic review collating published data on acesulfame K (E 950) was performed to identify evidence of potential adverse effects on the environment (Agriculture and Environment Research Unit, University of Hertfordshire (AERU), 2021)

resulting from the use of acesulfame K (E 950) as a food additive. This review was complemented by additional papers retrieved in the updated literature search in the present assessment (see Appendix A).

As reported in Section 3.5.1, the Panel considered that acesulfame K is not metabolised in human, has a half-life of 2–4 h and is primarily excreted via the urine. Therefore, acesulfame K, when ingested as a food additive, has the potential to reach the environment via wastewater. It is expected that the main receiving compartment will be the aquatic environment. Acesulfame K used as a food additive may also reach the terrestrial environment (e.g. via agricultural fertilisation with sewage sludge or flood events), however, these routes are expected to be less relevant than the direct emission from wastewater facilities into surface water. Considering the physicochemical properties of acesulfame K (i.e. high solubility in water, low log K_{ow}, low adsorption coefficient and low vapour pressure; see AERU, 2021), it is expected that acesulfame K mainly partitions to water.

The amount of acesulfame[28] that may reach the environment depends on (i) how efficiently wastewater treatment plants can remove it from their influent and (ii) on the subsequent (bio)degradation in the environmental compartments.

Regarding the fate of acesulfame following wastewater treatment, according to the above-mentioned review, the removal efficiency for acesulfame is generally quite poor (AERU, 2021). Luo et al. (2019), cited in the AERU review, provided a comprehensive review of the various methods for the removal of acesulfame from waters and reported removal efficiencies varying between 17 and 100%. Some of the reviewed papers dealt also with the potential formation of transformation products following the water treatment due to the reaction with disinfection products and with disinfection by-products (Nawaz and Sengupta, 2019, Li et al., 2017). In the updated literature search, many additional studies, including laboratory simulation studies, exploring the removal of acesulfame from the aquatic medium (including wastewater) were identified (Wang et al., 2019; Toth et al., 2012; Scheurer et al., 2012; Punturat & Huang, 2017; Phattarapattamawong et al., 2018; Nam et al., 2018; López-Muňoz et al., 2018; Liu et al., 2019; Ghosh et al., 2019; Ghosh et al., 2018; Dulova et al., 2019; Calza et al., 2017; Jahani et al., 2020; Pstrowska et al., 2021; Li et al., 2023; Mendez-Arriaga and Vecitis, 2021; Shao et al., 2019; Ben Mordechay et al., 2022; Kattel et al., 2017; Xue et al., 2020; Zhang et al., 2022; Mejia et al., 2021; Castronovo et al., 2023; Law & Leung, 2019; Chow & Leung, 2019; Bonatelli et al., 2023; Gajdos et al., 2023; Branco et al. 2023; Plantard et al., 2024; Vaidya et al., 2020; Bracamontes-Ruelas et al., 2023; Vidal et al., 2023; Farinelli et al., 2024; van Brenk et al., 2024; Liu et al., 2024; Kleinsteuber et al., 2019; Huang et al., 2021; Huang et al., 2022; Qiao et al., 2024). These papers showed that the removal efficiency varied and was dependent on the applied method and conditions. In the REACH registration dossier, a mean removal rate from wastewater of 8% was reported.[29] Some studies dealt with the potential formation of transformation products, potentially with higher toxicity than acesulfame (assessed with the Microtox assay (Chow et al., 2021; Sang et al., 2014; Yin et al., 2017), following the water treatment (Chow et al., 2021; Li et al., 2016; López-Muňoz et al., 2018; Perkola et al., 2016; Sang et al., 2014; Scheurer et al., 2012; Scheurer et al., 2014; Yin et al., 2017). The type of transformation products of acesulfame depends on the applied method (e.g. ozonation, UV treatment, chlorination). Kahl et al. (2018) reported that in biological wastewater treatment, the main transformation product of acesulfame is sulfamic acid. A similar conclusion was reached by Castronovo et al. (2017). Further degradation of the transformation products was mentioned in other papers and was reported to depend on the treatment applied (e.g. Chow & Leung, 2019; Law & Leung, 2019). Li et al. (2016) (cited also in AERU, 2021) performed a FET test (Fish Embryo Toxicity test, OECD, 2013) on acesulfame and on some of its photodegradation products obtained in a laboratory simulation test (TiO2-assisted photodegradation). The lowest no observed effect concentration (NOEC) obtained for the phototransformation products (5 g/L) was lower than the one obtained for acesulfame (10 g/L). Nevertheless, it is noted that in both cases the NOEC was in the order of g/L.

Following its release into the environment via, e.g. waste water treatment plants effluents, acesulfame can undergo some transformation or (bio)degradation, but this seems to be a rather slow process (AERU, 2021). Some papers addressing the (bio)degradability and transformation of acesulfame were retrieved in the review (AERU, 2021). Bergheim et al. (2015) performed a biodegradability test in line with OECD TG 301 D, F and 302B (OECD, 1992a; OECD, 1992b) and found that acesulfame could not be classified as 'readily biodegradable'. In the same study, Bergheim et al. (2015) compared the transformation of acesulfame after irradiation with Xe lamp (300–800 nm; no information on flux, simulating sunlight conditions) and Hg lamp (200–600 nm; 47 W)[30] and concluded that phototransformation products of acesulfame are likely to be formed mostly during high-energy UV-water treatment processes and, to a lesser extent, also by sunlight. Similarly, in Perkola et al. (2016) the photolysis of acesulfame under different conditions simulating sunlight and water treatment processes was assessed. The photolytic half-life (DT50) under simulated sunlight was determined to be slower ranging between 250 and 420 days depending on the conditions (deionised, river, lake water and in presence of ferric iron). Gan et al. (2013) reported that under natural sunlight, both direct and indirect photolysis of acesulfame were found to be negligible in sterilised systems at neutral or alkaline pH, whereas direct photolysis occurred at pH 4 in deionised water. In unsterilised systems, photolysis was substantially enhanced, implying, according to the authors, a joint effect of photolysis and biodegradation or that the sterilisation process had the secondary effect of inactivating some photosensitisers. The near-surface summer half-life (DT50) of acesulfame in the water from the Haihe River, China was found to be 9 days. Regarding the persistence of acesulfame in soil, Biel-Maeso et al. (2019) evaluated the degradation of acesulfame in two

[28]It is expected that acesulfame K rapidly dissociates in the environment (e.g. water) to acesulfame and potassium, and therefore the in the literature, authors analysed acesulfame and this term is used in this section.

[29]Mean removal rate of acesulfame K in a wastewater treatment plant determined over a period of 6 month. Samples were taken from the influent and the secondary effluent. https://chem.echa.europa.eu/100.054.269/overview?searchText=55589-62-3; original studies not available to EFSA.

[30]Dosimetry of the irradiation sources was not fully described.

types of soils. The degradation half-life (DegT50) under aerobic conditions was found to be between 11 and 19 days, in the same order of magnitude as in the studies by Buerge et al. (2011; DegT50 3–49 days) and Ma et al. (2017; DegT50 8.4–12.3 days). Some papers (e.g. Storck et al. (2016)) suggest that acesulfame K does not readily degrade under typical environmental conditions, but under certain conditions which favour modifications of the microbial community composition. This was also suggested in some papers retrieved in the updated literature search (e.g. Coll et al., 2020). Coll et al. (2020) reported that the dissipation half-lives (DT50s) obtained in sediment–water incubations according to OECD TG 308 (OECD, 2002) ranged between 11 and 152 days depending on the sediment source (sediments from two different rivers collected both up and downstream of the waste treatment plant).

Additional papers dealing with the environmental fate of acesulfame following its release from waste water treatment plants were retrieved in the updated literature search. Jahani et al. (2020) reported that acesulfame is not readily biodegradable. Bracamontes-Ruelas et al. (2023) (1698) reported that the inoculum's community composition and the redox state plays a key role on micropollutant biodegradation performance in laboratory experiments. In Minella et al. (2017), the photodegradation of acesulfame in surface waters was assessed, combining kinetic measurements and photochemical modelling and comparing the model predictions with field data. According to the authors, with the possible exception of shallow water bodies containing low dissolved organic carbon, acesulfame is a refractory (i.e. unreactive, resistant) compound in environmental waters. Similarly, in Zou et al. (2015) acesulfame was found to have a long persistence in Norra Bergundasjön (Swedish lake). According to the REACH registration dossier,[31] the calculated photodegradation of acesulfame K according to Atkinson (ref) resulted in a degradation half-life of about 8.5 h under the conditions of the northern hemisphere and assuming a 12-h day. In another study, no hydrolytic degradation was observed under environmentally relevant conditions, and acesulfame was not biodegradable (OECD TG 301 and 302B).

Several studies retrieved in AERU (2021) and in the updated literature search reported analytical results of the measurement of acesulfame in environmental matrices (see Annex F). In the studies reporting on acesulfame measurements both in the wastewater influent and effluent, the concentration of acesulfame in the effluent was was generally comparable or slightly lower to the concentration in the influent, indicating that the removal of acesulfame from wastewater does not occur or poorly. The highest concentration of acesulfame measured in surface water from the available literature studies was 122 µg/L (Montes et al., 2019)[32] noting that in some instances, acesulfame was reported as not detected in surface water. In Belton et al. (2020) review, a sample-weighted average of acesulfame concentration in surface water of 2.9 µg/L (range from < LOQ to 53.7 µg/L) was reported. Acesulfame was also measured in marine/costal water (range 0.0097–9.9 ug/L). The concentration of acesulfame in surface water sediment was reported in two of the available literature studies (Fu et al., 2020; Valdes et al., 2023). Acesulfame was detected only in one of the two studies at maximum 1.81 µg/kg dry weight (Fu et al., 2020). Acesulfame was measured and reported in groundwater in some studies concentrations above 0.1 ug/L were measured in some cases (range 0.0009–12 ug/L, see Annex F), such levels would trigger further assessment in other regulatory areas (see EFSA FEEDAP Panel, 2019). None of the included studies measured the concentration of acesulfame in soil. In the literature there is also some indication of uptake of acesulfame by plants (Ma et al., 2023).

The international platform of chemical monitoring (IPCHEM)[33] includes data on the concentration of acesulfame in different environmental compartments from several EU and non-EU countries (from EMPODAT[34]). The maximum reported concentration for surface water was 2.4 µg/L. According to the same database, acesulfame was not detected in sediment and groundwater.

The review from AERU (2021) identified several ecotoxicological studies on acesulfame. The review reported that the acute toxicity values for most aquatic organisms is greater than 0.1 mg/L and reported an LC50 for both Golden Orfe (*Leuciscus idus*) and zebrafish (*Danio rerio*) greater than 1000 mg/L (Markert & Weigand, 1979a, 1979b; Lambert et al., 2010: cited in AERU (2021). Zelinski et al., 2018 reported a EC50 for *Artemia salina* higher than 1000 mg/L. Kobeticova et al. (2018) performed a growth inhibition test with the macrophyte duckweed (*Lemna minor* L.) in which the aquatic plants were exposed to acesulfame at concentrations ranging from 6.25 mg/L to 100 mg/L for 7 days. The NOEC for frond numbers and frond area was 100 mg/L. Stolte et al. (2013) assessed the toxicity of acesulfame towards green algae (*Scenedesmus vacuolatus*), water fleas (*Daphnia magna*) and duckweed (*Lemna minor*) and in an activated sewage sludge microbial community. The lowest observed effect concentrations (LOEC) for all tested organisms/communities were reported as > 1000 mg/L. The review also reports some studies measuring oxidative stress biomarkers in fish indicating that acesulfame K or its transformation products could enhance oxidative stress in fish (e.g. Cruz-Rojas et al., 2019; Ren et al., 2016; Yin et al., 2017). The relevance of such changes for the environment is unclear. In the updated literature search, additional papers were retrieved addressing effects in zebrafish and *Daphnia magna* for which the relevance for the environment is unclear (Colin-Garcia et al., 2023; Dong et al., 2020; Kim et al., 2015; Saputra et al., 2021; Wiklund et al., 2023). In Lin et al. (2024), the survival rate, body weight changes and cocoon production/weight in earthworms (*Eisenia fetida*) exposed for 28 days to 0.1, 1 and 10 mg/kg of acesulfame were assessed. The survival rates of earthworms in acesulfame treated soils were not significantly different from the control group. Average body fresh weight of earthworms significantly increased (20.5%) at concentration of 1 mg/kg. The cocoon production was significantly increased at 10 mg/kg while the average weight of cocoons

[31] https://chem.echa.europa.eu/100.054.269/overview?searchText=55589-62-3; original studies not available to EFSA.

[32] The authors reported that this value was exceptionally higher than the other values measured for acesulfame K which were 'in general, in agreement with those found in surface waters from other origins'. The sampling site was a surface water collected near an urban settlement.

[33] https://ipchem.jrc.ec.europa.eu/#databaseConsole/EMPODAT/acesulfame/33665-90-6/none/Water accessed on 19.2.2025.

[34] EMPODAT compiles data from monitoring, survey, research/technical studies provided by NORMAN member organisations (https://www.normandata.eu/?q=Home). The reason of data collection therefore may vary by dataset and/or data provider.

significantly decreased. The same paper addressed additional effects such as changes in the gut microbiota of earthworms. Ecotoxicological tests on aquatic plants (*Lemna minor*, 7 days) and crustaceans (*Thamnocephalus platyurus*, 24 h and *Daphnia magna*, 24–48 h) were reported (Kerberová et al., 2022). In all cases the obtained inhibition/effect concentrations (IC50 and EC50) were above 1000 mg/L.

The REACH registration dossier[35] for acesulfame K reports the summaries of several ecotoxicological studies. Regarding the aquatic organisms, the following acute toxicity endpoints[36] are reported: LC50 (zebrafish, 96 h) = 1800–2500 mg/L and EC50 (*Daphnia magna*, 24 h) > 1000 mg/L. Chronic toxicity studies were also available for the three main taxonomic groups providing the following endpoints: NOEC for fish (fish early-life stage toxicity test on zebrafish) = 22 mg/L; NOEC for aquatic invertebrates (*Daphnia magna* reproductive toxicity study) = 100 mg/L; EC50 (growth rate and biomass) for algae > 100 mg/L. Studies on sediment dwellers or terrestrial organisms were not reported.

The concentrations measured in surface water (highest concentration 122 µg/L; Montes et al., 2019) are lower than the available 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. A environmental risk assessment for acesulfame K (E 950) was not be performed by the Panel.

3.7 | Discussion

The present opinion deals with the re-evaluation of acesulfame K (E 950), authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No. 1333/2008 on food additives.

Acesulfame K (E 950) is the potassium salt of 6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide, produced through chemical synthesis in different reaction steps using dichloromethane as a solvent. The product undergoes several purification processes, including crystallisation, filtration, recrystallisation and centrifugation. The results of the analysis of dichloromethane in samples of acesulfame K (E950) was reported as below the LOD of 1 ppm. Considering the different purification steps in the manufacturing process of acesulfame K, the Panel considered the presence of residual solvents in acesulfame K as unlikely.

In the EU specifications, a limit of 20 mg/kg is set for UV-active organic impurities; however, no specific limits exist for individual organic impurities. The European Pharmacopoeia monograph on acesulfame K (E 950) identifies two organic impurities: acetylacetamide, also a degradation product of the food additive, and 5-chloro-acesulfame. The formation of 5-chloro-acesulfame is associated with the generation of chlorinating agents (e.g. chloromethyl chlorosulfate and methyl bis chlorosulfate) as a result of a side reaction between sulphur trioxide and dichloromethane, when used as a solvent (Boehshar & Burgard, 2003).

Analysis of these impurities was documented in certificates of analysis for six samples of E 950. Acetylacetamide was reported as 'below the LOD', though the LOD for the HPLC method was not specified. A limit of 0.5–1 mg/kg was stated in the certificates of analysis. 5-chloro-acesulfame was reported at levels below 2 mg/kg, the reported limit indicated in the certificates of analysis. The potential exposure to these impurities (at their maximum reported limits of 1 mg/kg for acetylacetamide and 2 mg/kg for 5-chloro-acesulfame) resulting from the use of E 950 was assessed as a worst-case scenario.

No experimental toxicity data including genotoxicity were retrieved in a systematic search of published literature for acetylacetamide (Appendix A). Acetylacetamide was assessed by JECFA (1991) and considered to be of a low toxicity and not mutagenic. In silico QSAR analyses performed by the Panel did not provide indications for genotoxicity (Appendix D). To assess the risk that would result if it is present in E 950, the Panel used a NOAEL of 20 mg/kg bw per day, estimated from a 28-day study (performed according to the OECD Guideline 422 as reported in an ECHA dossier publication[37]) with a factor of 5 applied to extrapolate from sub-acute to chronic exposure, as a reference point (Appendix E). Considering the presence of acetylacetamide at 1 mg/kg in E 950, the MOE was calculated as 10^6, indicating no safety concern. Additional exposure to acetylacetamide is expected as a degradation product of acesulfame K, but due to the high MOE, no safety concerns arise based on available data.

No experimental toxicity data including genotoxicity were retrieved in a systematic search of published literature for 5-chloro-acesulfame. However, the QSAR analysis triggered some genotoxicity alerts and therefore a TTC of 0.0025 µg/kg bw per day is applicable when assessing the safety of this potential impurity in the food additive (EFSA Scientific Committee, 2019). The Panel noted that the potential exposure to this impurity, assuming its presence at the reported limit of 2 mg/kg, is above the TTC value. In order to ensure that potential exposure to this impurity resulting from the use of E 950 would not raise a concern, a maximum limit of 0.1 mg/kg for 5-chloro-acesulfame should be included in the specifications for the food additive. Alternatively, appropriate genotoxicity data for 5-chloro-acesulfame should be generated.

[35] https://chem.echa.europa.eu/100.054.269/overview?searchText=55589-62-3; original studies not available to EFSA.

[36] Intended here as the ecotoxicological values resulting from the available ecotoxicological tests (e.g. LC50, EC50, NOEC), see also section 'Glossary and/or abbreviations and/or acronyms for detailed definitions'.

[37] https://chem.echa.europa.eu/100.025.250/dossier-view/8724681f-86c0-4dcd-bf88-758cb88707bd/ec65bb2e-413f-4e99-8d0f-374294ab7787_9e1be6ad-081b-4921-bfe8-e4ed1d2af284?searchText=5977-14-0 [Accessed on 22 January 2025].

Analytical results for arsenic, lead, cadmium, mercury and fluoride in commercial samples of E 950 were submitted. The Panel noted that among these potential inorganic impurities, only lead, mercury and fluoride have defined limits in the EU specifications. Based on the additional data provided for arsenic and cadmium, as well as the production process involving organic synthesis followed by crystallisation and recrystallisation steps (which minimise systematic contamination), the Panel considered there is no need for additional specification limits for arsenic or cadmium.

For fluoride, exposure was calculated based on its presence at the existing EU limit of 3 mg/kg acesulfame K. The resulting exposure (0.0002 mg/day and 0.0006 mg/day for the mean and 95th percentile in toddlers, respectively) was found to be four orders of magnitude below the tolerable upper intake level of 1.6 mg/day for children (1–3 years of age) proposed by the EFSA Scientific Committee (EFSA Scientific Committee, under public consultation).

The Panel assessed the risk that would result if lead and mercury were present in E 950 at: (i) the existing limit in EU specifications; (ii) the reported limit values (0.1 mg/kg for lead and 0.01 for mercury). Taking into account the calculations performed by the Panel, the fact that the food additive is not the only potential dietary source of toxic elements, and that the maximum limits should be established based on actual levels in the commercial food additive, the Panel recommended lowering the specification limits for lead and mercury. If the European Commission decides to revise the current limits in the EU specifications, the estimates of exposure to toxic elements intake could be considered.

Based on the submitted microbiological data and the manufacturing process, the Panel considered microbiological contamination unlikely and considered there is no need to include microbiological criteria in the EU specifications for E 950.

The Panel noted that the ultrafiltration step recommended in EFSA Guidance (EFSA Scientific Committee, 2021) to remove small particles was not included in the solubility test provided. However, the reported solubility of E 950 in water (237 g/L), measured according to European Commission Regulation (EC) No. 440/2008 and OECD TG 105 methods, is substantially higher than the 33.3 g/L threshold requiring additional assessment for the fraction of small particles including nanoparticles. Thus, the Panel considered that E 950 is fully dissolved when consumed as a food additive, and the potential presence of small particles, including nanoparticles, does not pose a concern. Acesulfame K can therefore be assessed following the conventional risk assessment, i.e. EFSA Guidance for submission for food additives evaluations (EFSA ANS Panel, 2012).

The Panel noted that acesulfame K is degraded to a certain amount under acidic conditions (pH < 3) at elevated temperatures, producing acetylacetamide and acetoacetamide-N-sulfonic acid as identified degradation products. As indicated above, no safety concern arised for acetylacetamide. Acetoacetamide-N-sulfonic acid also was reviewed by JECFA (JECFA, 1991); and found to be of a low toxicity and not mutagenic. No new evidence from animal toxicological studies including genotoxicity or human data were retrieved in a systematic search of published literature. In silico QSAR analyses performed by the Panel did not provide indications for genotoxicity for this degradation product (Appendix D).

The biological and toxicological dataset available to the Panel for the re-evaluation of acesulfame K (E 950) comprised evidence from animal toxicological studies and human data, both published and unpublished, made available to EFSA in response to calls for data and related clarification requests and/or also identified from the published literature. The selection, appraisal and integration of the evidence was performed according to the principles outlined in the revised protocol on hazard identification and characterisation of sweeteners (EFSA, 2020a; EFSA FAF Panel, 2023) and reported in Appendix A.

Regarding the ADME of acesulfame K, the 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.

Based on the overall negative results obtained for acesulfame K in a battery of genotoxicity tests, including robust Ames and in vitro micronucleus assays, the Panel concluded that acesulfame K (E 950) does not raise a concern for genotoxicity. This concurs with the conclusion of the previous SCF Opinion (SCF, 2000).

An evaluation of the RoB in each reliable study was performed (Annex D), and a WoE approach across studies considered of low (tier 1) or moderate (tier 2) RoB was applied for each of the identified HOC for both human and animal studies (Appendix A, Annexes E1 and E2). Based on the outcome of the WoE, the Panel noted that across multiple studies in animals involving different doses and durations, no treatment-related adverse effects were observed for acesulfame K (E 950).

A high level of evidence was established for the absence of adverse effects grouped under (i) general toxicity, (ii) haematotoxicity, (iii) nephrotoxicity, (iv) liver toxicity and (v) other organ toxicity. The level of evidence was moderate for endpoints grouped under (i) glucose/insulin homeostasis and (ii) additional clinical chemistry. The moderate level was reached due to shortcomings in the RoB assessment (internal validity), investigations with low number of animals and only one sex, short study durations and exposure with single doses. The characterisation of the exposure to the test substance often represented shortcomings in the animal studies. Some of the studies did not provide sufficient information about doses (in mg/kg bw per day), and exposure characteristics, such as homogeneity of substance in feed, purity and analysis of test substance were not reported. Despite these shortcomings, the evidence was sufficient for the Panel to conclude that acesulfame K (E950) is not associated with adverse effects in animals.

Concerning the evidence of adverse health effects related to the exposure to acesulfame K based on the included human studies, the Panel noted that the level of evidence related to the HOC cancer was considered to be low due to the conclusions drawn from an observational study reporting on data from a single cohort (Debras et al., 2022b). These

findings would require replication in an independent setting. The Panel also noted that no adverse effects in relation to cancer were reported in a 2-year combined chronic toxicity and carcinogenicity study in rats (Documentation provided to EFSA No 17). Furthermore, the Panel noted that acesulfame K was not found to raise a concern for genotoxicity in the present assessment (see Section 3.5.3).

Regarding cardiovascular risk factors and disease, the level of evidence for an effect was low, as findings from different studies are inconclusive. One study indicated an association with coronary heart disease, but no firm conclusions (low level of evidence) can be drawn in the absence of other studies. For glucose/insulin homeostasis, the level of evidence is moderate, with several small intervention studies showing no effect on short-term glucose homeostasis and a single large cohort study suggesting an association with type 2 diabetes; however, the biological explanation remains unclear. The level of evidence is low and inadequate for development and birth outcomes, respectively, as findings rely on single studies.

Following an integration of the evidence as described in the revised protocol on hazard identification and characterisation of sweeteners (EFSA FAF Panel, 2023), summarised in Table 18, the Panel considered that it is unlikely that intake of acesulfame K is associated with (i) cancer, (ii) disturbances of the glucose or insulin homeostasis, (iii) cardiovascular risk factors and disease, (iv) general toxicity, (v) heamatological effects, (vi) nephrotoxicity, (vii) liver toxicity or (viii) toxicity in any organ or tissue. It is not possible to conclude that intake of acesulfame K (E 950) is associated with (i) preterm delivery and (ii) precocious puberty in girls, due to study limitations (single cross-sectional studies) and the lack of supporting evidence from animal studies.

Based on the integration of the body of evidence from human and animal studies, the Panel concluded that no new studies were relevant for the identification of a RP based on adverse effects (no integrated evidence conclusions were 'likely' or 'very likely'). The Panel therefore addressed attention to the two key studies in the previous evaluations by JECFA and SCF; a 2-year study in dogs (Documentation provided to EFSA No 17), and a 2-year combined chronic toxicity and carcinogenicity study in rats (Documentation provided to EFSA No 17). In these two studies, the NOAEL was 3% acesulfame K in the diet, the highest dietary concentration tested, corresponding to doses of 900 mg/kg bw per day in the dog and to 1500 mg/kg bw per day in the rat. Because the two-year dog study could not be considered due to a high RoB (tier 3), the Panel included only the 2-year rat study (tier 2) in the WoE. Consequently, the Panel establishes an ADI for acesulfame K (E 950) of 15 mg/kg bw per day based on application of a default 100-fold uncertainty factor to the NOAEL identified in rats fed a diet with 3% acesulfame K for 2 years, corresponding to 1500 mg/kg bw per day.

The authorised food additive 'salt of aspartame-acesulfame' (E 962) is another source of exposure to acesulfame K. This salt dissociates and so the total amount of acesulfame in food will come from both food additives. For this reason, the exposure to acesulfame K from the use of both acesulfame K (E 950) and the salt of aspartame-acesulfame (E 962) is considered in this opinion. The dietary exposure to acesulfame K from the use of E 950 and E 962 was estimated according to different exposure scenarios based on consumers only as described in Section 3.4. Currently, the use of acesulfame K (E 950) and the salt of aspartame-acesulfame (E 962) is authorised in the EU in 34 food categories, and concentration data were available for 29 categories as described in Section 3.3.3. In all scenarios, the exposure estimates were regarded to overestimate the current exposure to acesulfame K from the use of E 950 and E 962 in the EU. The Panel considered *the refined brand-loyal exposure assessment scenario*, the most appropriate exposure scenario for the risk assessment. In this *scenario*, mean exposure to acesulfame K ranged from 0.04 mg/kg bw per day in adolescents to 5.2 mg/kg bw per day in toddlers. The 95th percentile of exposure ranged from 0.2 mg/kg bw per day in children and adults to 15.7 mg/kg bw per day in toddlers. The main food category contributing to the exposure to acesulfame K was FC 14.1.4. 'Flavoured drinks' for all population groups. The Panel noted that the refined exposure estimates are based on information provided on the reported use levels of acesulfame K from the use of E 950 and E 962. If actual practice changes, these refined estimates may no longer be representative and should be updated.

4 | UNCERTAINTY

The uncertainties, and the direction of the uncertainty, related to the exposure assessments are summarised in Table 6 of Section 3.4.2. Overall, the Panel considered that the dietary exposure estimates of acesulfame K from the use of E 950 and E 962, based on the *refined brand-loyal exposure assessment scenario*, are the most appropriate to be used for the risk assessment of the food additive. These estimates are considered to overestimate the current dietary exposure to acesulfame K in the EU.

Based on the information regarding the manufacturing process, the Panel identified 5-chloro-acesulfame as a possible impurity in E 950. Data on the occurrence of 5-chloro-acesulfame in E 950 were not available, leading to uncertainty about its actual presence in the food additive. An in silico QSAR analysis indicated genotoxicity alerts for 5-chloro-acesulfame (Section 3.5.3). In the absence of experimental toxicity data to dismiss these alerts, the Panel adopted a conservative approach to this potential impurity, using the TTC for potentially genotoxic compounds (0.0025 μg/kg bw per day). The Panel assumed the presence of 5-chloro-acesulfame in acesulfame K (E 950) to propose a maximum limit for this impurity in the EU specifications for E 950, aiming to minimise any potential concerns. The Panel noted that appropriate experimental data for 5-chloro-acesulfame would reduce uncertainties concerning the genotoxicity of this potential impurity in acesulfame K (E950).

The uncertainties concerning the animal and human studies were addressed in the WoE assessment or explained in the respective sections above (Sections 3.5.4, 3.5.5 and 3.7). The derivation of an ADI was based on absence of an effect at the

highest dose tested from a 2-year combined chronic toxicity and carcinogenicity study in rats (Documentation provided to EFSA No 17) previously assessed by SCF 2000 and JECFA 1991, and considered as study on which the ADI was based by JECFA (JECFA, 1991). The Panel considers that uncertainties associated with this study graded as tier 2 (moderate RoB; Annex D) did not influence the conclusions on the safety of acesulfame K (E 950).

Overall, the uncertainties addressed above were considered not to affect the conclusions on the safety of the food additive acesulfame K (E 950).

5 | CONCLUSIONS

Taking into account the available dataset, the Panel established an ADI of 15 mg/kg bw per day for acesulfame K based on the NOAEL of 1500 mg/kg bw per day, the highest dose tested and by applying an uncertainty factor of 100. Accordingly, this ADI replaces the ADI of 9 mg/kg bw per day established by SCF (2000).

The 95th percentile exposure estimates for acesulfame K were generally below the ADI in all population groups.

The Panel concluded that there is no safety concern at the reported uses and use levels for acesulfame K (E 950).

6 | RECOMMENDATIONS

The Panel recommended the European Commission to consider:

- Inserting a maximum limit of 0.1 mg/kg for 5-chloro-acesulfame in the EU specifications or alternatively request appropriate genotoxicity data for 5-chloro-acesulfame.
- Inserting a maximum limit of 1 mg/kg for acetylacetamide in the EU specifications.
- Lowering the limit of lead and mercury in the EU specifications.
- Including the CAS number 55589-62-3 in the EU specifications.

7 | DOCUMENTATION AS PROVIDED TO EFSA

1. International Sweeteners Association (ISA). Submission of data in response to the call for technical and toxicological data on sweeteners authorised as food additives in the EU (EFSA-Q-2017-00500). Data submitted on 14 June 2018.
2. International Sweeteners Association (ISA). Submission of data in response to the call for technical data on sweeteners authorised as food additives in the EU (EFSA-Q-2019-00318). Data submitted on 13 September 2019.
3. Association of the European Self-Medication Industry (AESGP). Submission of data on use levels of acesulfame K (E 950) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 2 October 2018.
4. Unione italiana Food (AIDEPI). Submission of data on use levels of acesulfame K (E 950) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 20 October 2018.
5. International Chewing Gum Association (ICGA). Submission of data on use levels of acesulfame K (E 950) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 20 October 2018.
6. Total Diet & Meal Replacements Europe (TDMR). Submission of data on use levels of acesulfame K (E 950) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 20 October 2018.
7. European Dairy Europe (EDA). Submission of data on use levels of acesulfame K (E 950) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 20 October 2018.
8. Cloetta. Submission of data on use levels of acesulfame K (E 950) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 2 October 2018.
9. Food Drink Europe (ISA). Submission of data on use levels of acesulfame K (E 950) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 20 October 2018.
10. Food Drink Europe (AIJN). Submission of data on use levels of acesulfame K (E 950) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 20 October 2018.
11. Food Drink Europe (PRODULCE). Submission of data on use levels of acesulfame K (E 950) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 20 October 2018.

12. Food Drink Europe (FSE). Submission of data on use levels of acesulfame K (E 950) in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 20 October 2018.
13. 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.
14. 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.
15. 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.
16. Call for genotoxicity data – Acesulfame K, Ref. EFSA-Q-2011-00721 – ISA submission of the requested data. (2022).
17. International Sweeteners Association (ISA). Submission of data in response to the call for technical on sweeteners authorised as food additives in the EU (EFSA-Q-2019-00318). Data submitted on September 2023. Cited in SCF 2000 and JECFA 1991.

ABBREVIATIONS

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
As	arsenic
AUC	area Under the Curve
BMDL	benchmark dose lower bound
Bw	bodyweight
CAS	Chemical Abstracts Service
Cd	cadmium
CE-C4D	capillary electrophoresis with contactless conductivity detector
CFU	colony forming units
CV	coefficient of variation
DAD	diode array detection
ESI/MS	electrospray ionisation mass spectrometry
FAF	EFSA Panel on Food Additives and Flavourings
FC	food category
FCC	Food Chemicals Codex
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GC	gas chromatography
GNPD	Global New Products Database
Hg	mercury
HOC	health outcome category
HPLC	high performance liquid chromatography
HPLC-UV	high performance liquid chromatography with ultraviolet detection
ICP-MS	inductively coupled plasma-mass spectrometry
IC	ion chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOEC	lowest observed effect concentrations
LOD	limit of detection
LOQ	limit of quantification
MOE	margin of exposure
MPL(s)	maximum permitted level(s)
MS	mass spectrometry
NOEC	no observed effect concentration
NOAEL	no observed adverse effect level
NTP	US National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
Pb	lead
PLS	partial least squares
P95	95th percentile
QSAR	quantitative structure activity relationship
QS	quantum satis
RoB	risk of bias
SCF	Scientific Committee on Food

SEM	scanning electron microscopy
SPE	solid-phase extraction
T1DM	type 1 diabetes mellitus
TLC	thin layer chromatography
TTC	threshold of toxicological concern
TWI	tolerable weekly intake
UHPLC	ultra-high performance liquid chromatography
WoE	weight of evidence

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SUPPORTING INFORMATION

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

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APPENDIX A

Tailored protocol for the hazard identification and characterisation of acesulfame K (E 950)

The assessment of acesulfame K 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 acesulfame K (E950) are outlined below.

Extensive literature search – acesulfame K (E950)

Methodology

For step 1.11, open-ended literature searches were conducted in the three selected databases with the search strings and criteria applied as listed below. The last update of the searches was performed on 2 December, 2024; the search included relevant literature published since the previous evaluation by the SCF (SCF 2000), allowing 1 year of overlap.

Web of Science (core collection[38])

Set	Query	Notes
#3	#2 and English (Languages)	Published in English
#2	PY = 1999–2100 AND #1	Published from 1999
#1	TS = ("acesulfame K" OR "acesulfame potassium" OR E950 OR "E 950" OR "55,589–62-3")	Acesulfame K

PubMed

Set	Query	Notes
#3	("1999/01/01"[Date - Publication]: "3000"[Date - Publication]) AND #1 Filters: English	Published in English
#2	("1999/01/01"[Date - Publication]: "3000"[Date - Publication]) AND #1	Published from 1999
#1	"acetosulfame" [Supplementary Concept] OR "acesulfame K"[All Fields] OR "acesulfame potassium"[All Fields] OR "E950"[All Fields] OR "E 950"[All Fields] OR "55,589–62-3"[All Fields]	Acesulfame K

SciFinder

Query
Substance search: 55589–62-3 - > References Filtering: Document: journal, review, clinical trial, commentary, preprint, report Language: English Publication Year: 1999–2024

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 1412.[39]

For step 1.11.1 (Screening of the studies for relevance), the general principles reported in the protocol applied. 1412 papers were included at the level of title and abstract screening, whereas 733 papers were excluded. From the 679 papers included for the full-text screening, 155 were considered as possibly relevant, whereas 524 were excluded at the level of full-text screening or preliminarily categorised into technical data, exposure data, environmental data or toxicological review and further screened for confirmation of their relevance. Following a confirmation or relevance screening, 35 studies were subjected to RoB assessment (see Figure A.1).

[38]Indexes: SCI-EXPANDED, CPCI-S, BKCI-S, ESCI, CCR-EXPANDED, IC.

[39]The final number of references includes primary studies which were identified from systematic reviews.

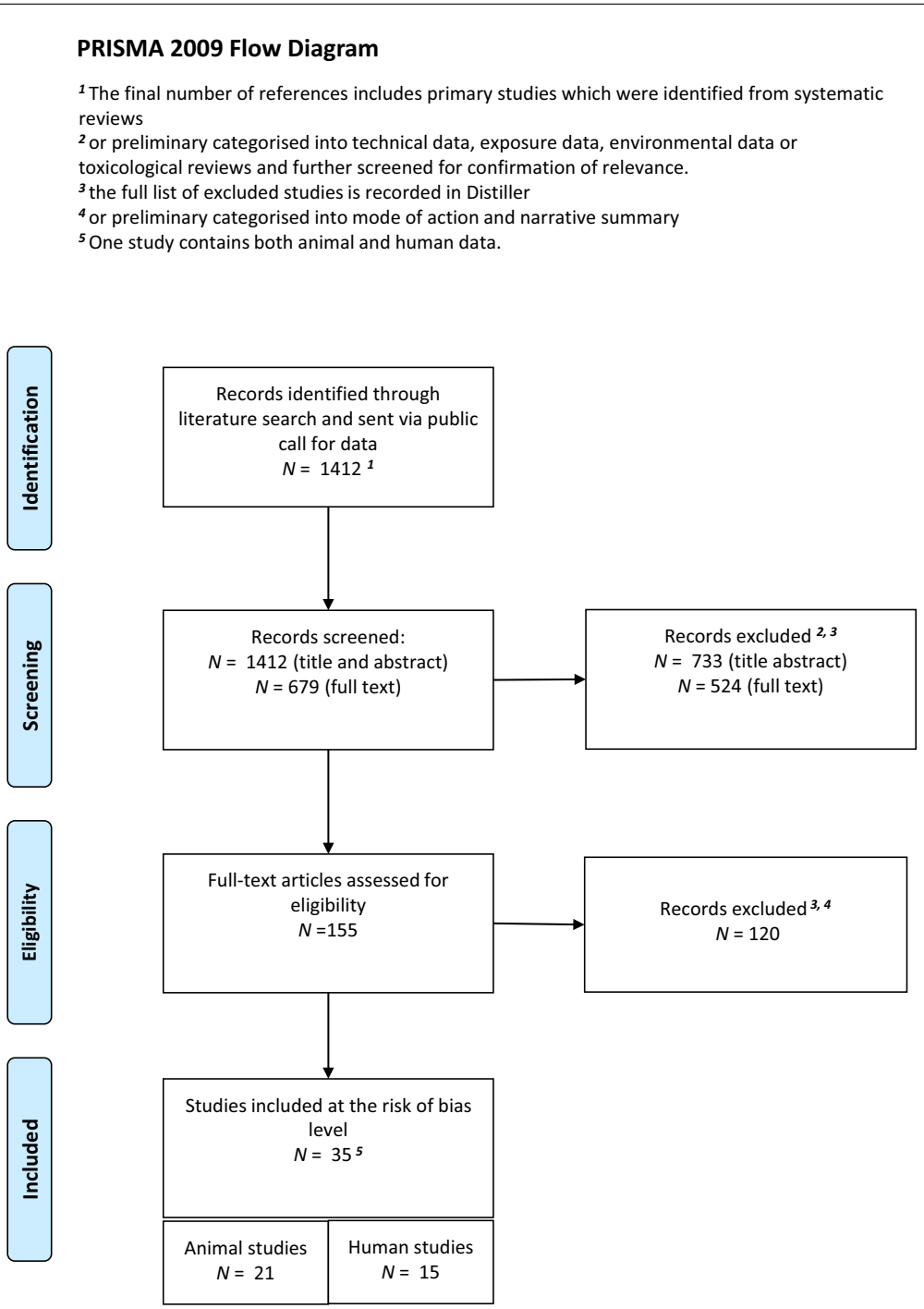


FIGURE A.1 PRISMA flow chart (adapted from Moher et al., 2009).

Evaluation of the risk of bias (RoB)

Methodology

For step 1.12, the criteria outlined in the revised protocol for the RoB evaluation of studies have been applied (NTP-OHAT, 2019), 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.

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, 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 the SCF, allowing 1 year of overlap. If studies that were previously considered as critical were evaluated as having moderate or low RoB, 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

Relevant studies retrieved from the literature, either in humans or in animals, were considered and evaluated for the RoB together with the pivotal study. The results of this assessment are reported in Annex D. Disagreements with the tier generated by the tool were identified by the reviewers and the tier was adjusted based on expert judgement, including the reasoning behind.

21 animal studies and 15 human studies were evaluated as having moderate or low RoB and therefore considered further in the assessment, while those having a high RoB were not included. One study contains animal and human data.

Data extraction

Methodology

For step 1.13, information and data from the assessed animals and human studies as well as from the included genotoxicity studies were extracted and reported in tabular form. The data extraction forms outlined in the revised protocol were used.

Results

The data extraction forms of the studies are reported in Annex B and Annex C of the current opinion.

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 the considered animal and human studies. The WoE analysis was performed using Excel tables. The detailed methodology is reported in the revised protocol.

Results

The result of the WoE analysis for acesulfame K (E950) is presented in Annex E1 and E2 and summarised in Section 3.5.4 on 'Synthesis of systematically appraised evidence and weight of evidence'.

Integration of animal and human evidence

Methodology

The integration of animal and human evidence was performed following the Figure A.2.

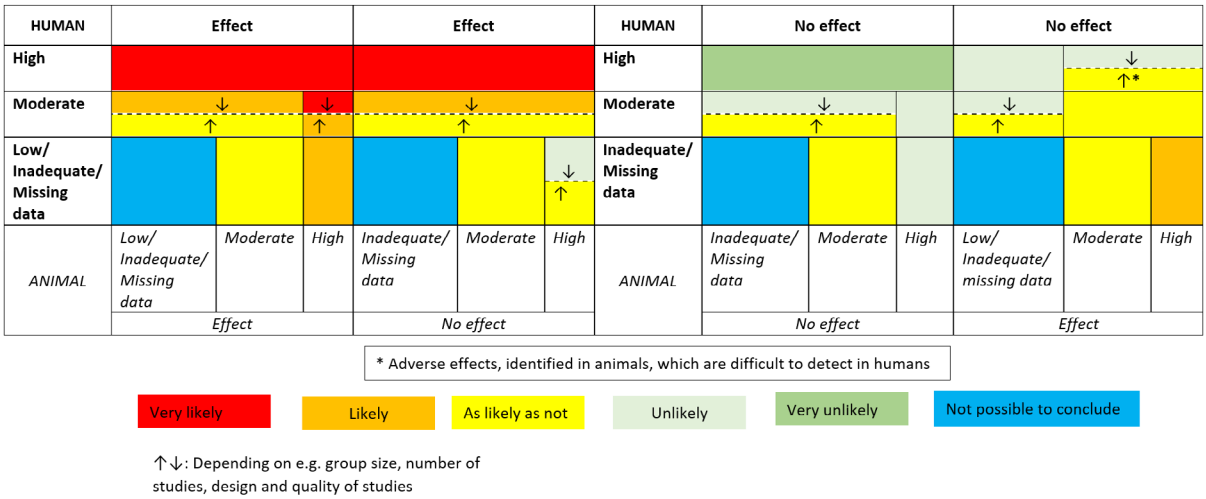


FIGURE A.2 Matrix for integration of animal and human body of evidence based on identification of effect and level of evidence.

The integration, as reported in the above-mentioned figure, was complemented by additional considerations from the Panel on the biological plausibility of each effect and MoA considerations. Consideration was given to the conclusions of previous evaluations (JECFA, 1991; SCF, 2000) to assess whether the new data support them. In the case of the HOCs with evidence available only from human data (birth outcomes (preterm) and development (precocious puberty)) or animal data (general toxicity (all but body weight), nephrotoxicity, liver toxicity, other organ toxicity (all but carcinogenicity) and haematotoxicity), the integration of evidence was done considering the option 'missing data' from Figure A.2.

Results

The results of the integration are reported in Section 3.5.4.3.

Literature search – acesulfame K (E950) degradation products and impurities

In addition to the extensive literature search for acesulfame K (E950), a targeted literature search was performed for (i) acetylacetamide, (ii) acetoacetamide-N-sulfonic acid and (iii) 5-chloro-acesulfame. The search focused on relevant literature reporting on toxicologically relevant endpoints, published since the previous evaluation by the SCF (SCF, 2000), allowing 1 year of overlap. The search was performed by an information specialists.

The final number of references that were screened, after removal of duplicates (based on title, year, author, journal, volume, issue and page numbers), was 366. 15 papers were included at the level of title and abstract screening, whereas 351 papers were excluded. At the full-text screening, no reference was considered as relevant, and 15 were excluded. The full list of excluded studies was recorded in the Distiller SR Tool.

APPENDIX B

Other studies

In this appendix, studies retrieved from the literature which do not directly contribute to the safety assessment of acesulfame K as a food additive are provided.

The publication of Chiang et al., 2022, which described a cohort study in 613 pregnant women and related the endpoint preterm birth to the consumption of acesulfame K (Table 13), had an experimental in vitro and ex-vivo part. The experiments were based on the assumption that preterm birth was due to premature uterine contractions elicited by hypersensitivity towards oxytocin. By critically reviewing the setting of the experiments it was remarkable that the concentrations of acesulfame K which were used in vitro and ex vivo were rather high, 10^{-2} M and 100 mM. From studies on the kinetics of acesulfame K in pigs (JECFA, 1991) it can be taken that an oral dose of roughly 4 mg/kg resulted in maximum concentrations between 0.35 and 0.72 μg/mL. When taking the mean of roughly 0.5 μg/mL for a concentration resulting from an oral dose of 4 mg/kg bw the oral dose to reach a concentration of 10^{-2} M (2012.4 mg/L or 2012.4 μg/mL) should be 16099.2 mg/kg bw in pigs. It is obvious that this dose is exceeding by far the 96th percentile upper bound in adults of 6.8 mg/kg bw per day, even when applying an interspecies adjustment factor of 10. The same holds true for the concentration of 100 μM used in another ex-vivo experiment in isolated uterine cells. The Panel noted that the results of the in vitro and ex-vivo experiments are not relevant for the exposure situation in pregnant women and cannot support the findings from the cohort study.

Three studies (Yu & Guo, 2022, Yu, Wang, Henderson, et al., 2022, Yu, Henderson, Guo, 2022) deal with four artificial sweeteners and are showing about the same effect of the four sweeteners analysed (acesulfame K, saccharin, sucralose, aspartame). The Panel noted that the listed papers are showing an effect of the sweeteners on the gene expression of bacteria, the cell envelope permeability and some other metabolic responses leading to a small increase in transformation and conjugative transfer of AMR genes between bacteria. The effects seen are very limited and it is questionable if they are of biological significance.

A total of of 11 animal studies (listed in Annex D) were evaluated for RoB as tier 3 (low internal validity) and were not considered in the WoE integration. Five additional animal studies identified (Bridge-Comer et al., 2022; Cong et al., 2013; Fujita et al., 2009; Lin et al., 2021; Tsan et al., 2022) addressing the impact of sweeteners on taste receptors, neurometabolic function, atherosclerosis, appetite responses, reproductive and metabolic health. The Panel noted that these studies, due to (i) the use of non-standard experimental designs and/or non-standard or disease animal models and/or (ii) the focus on non-apical mechanistic endpoints could not be considered in the WoE integration.

APPENDIX C

Additional human studies

In this appendix, studies retrieved from the literature which do not directly contribute to the safety assessment of acesulfame K as a food additive are provided.

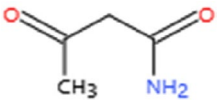
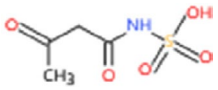
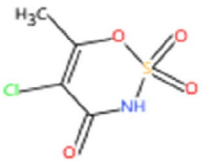
A total of 3 human studies (listed in Annex D) were evaluated for RoB as tier 3 (low internal validity) and were not considered in the WoE integration.

Several studies assessed the effects mixtures of sweeteners, including Sylvetsky et al. (2016), Solomi et al. (2019), Raben et al. (2002), Katsue et al. (2014), Fukuda et al. (2010), Brown et al. (2012), Bonnet et al. (2018) Andreatta et al. (2008), Sylvetsky et al. (2020), Fantino et al., 2018, Kim et al. (2020), Orku et al. (2023), Gonzalez-Dominguez et al. (2021), Almiron-Roig et al. (2023), Finassi et al. (2023) and Sylvetsky et al. (2023). The Panel noted that due to possible confounding effects and the resulting uncertainties associated with the combined exposure to multiple sweeteners a further consideration of these studies was not feasible.

A number of studies including, Frankenfeld et al. (2015), Gonzalez-Dominguez et al. (2021), Van den Abbeele et al. (2023) and Sylvetsky, Clement, et al. (2024) examined gastro intestinal tract (GIT) microbiota changes in response to oral exposure to acesulfame K. The Panel noted that there is currently no consensus on when changes in GIT microbiota should be considered adverse and on what the potential health consequences of changes in GIT microbiota would be. In this respect it is noted that EFSA has recently published the external report of an Art. 36 grant proposing a roadmap for action to (i) strengthen the nascent evidence relating to effects on/by human and animal gut microbiomes; and (ii) identify key knowledge gaps and provide guidance on how to experimentally approach these urgent research needs.

APPENDIX D

TABLE D.1 QSAR analysis of acesulfame K impurities/degradation products

Chemical name	CAS no.	Structure	QSAR toolbox
Acetylacetamide (acetoacetamide) (impurity/ degradation product)	5977-14-0		DNA binding OASIS: No alert found DNA binding by OECD: No alert found Protein binding OASIS: No alert found Protein binding by OECD: No alert found Toxic hazard classification by Cramer: High (Class III) DNA alerts for Ames, CA and MNT by OASIS: No alert found Carcinogenicity (genotox & non- genotox): No alert found Protein binding alerts for CA by OASIS: No alert found **VEGA** Prediction is NON-Mutagenic with a consensus score of 0.425, based on 4 models.
Acetoacetamide-N-sulfonic acid (degradation product)	99911-45-2		**QSAR Toolbox** DNA binding OASIS: No alert found DNA binding by OECD: No alert found Protein binding OASIS: No alert found Protein binding by OECD: No alert found Toxic hazard classification by Cramer: High (Class III) DNA alerts for Ames, CA and MNT by OASIS: No alert found Carcinogenicity (genotox & non- genotox): No alert found Protein binding alerts for CA by OASIS: No alert found **VEGA** Prediction is NON-Mutagenic with a consensus score of 0.35, based on 4 models.
5-chloro-acesulfame (impurity)	72827-08-8		**QSAR Toolbox** DNA binding OASIS: No alert found DNA binding by OECD: No alert found Protein binding OASIS: No alert found Protein binding by OECD: No alert found Toxic hazard classification by Cramer: High (Class III) DNA alerts for Ames, CA and MNT by OASIS: No alert found Carcinogenicity (genotox & non- genotox): alpha, beta-unsaturated carbonyls (Genotox) Protein binding alerts for CA by OASIS: No alert found **VEGA** Prediction is Mutagenic with a consensus score of 0.5, based on 4 models.

Abbreviations: CA, chromosomal aberration; MNT, micro nucleus test.

APPENDIX E

Exposure calculations to impurities from the use of acesulfame K as a food additive

The potential exposure to impurities from the use of E 950 was calculated by assuming that they are present in the food additive up to a certain limit value, and then by calculation pro-rata to the estimates of exposure to the food additive itself.

For the current assessment, the refined brand-loyal exposure scenario was considered as the most representative, the highest exposure levels for the mean and 95th percentile among the different population groups were considered, i.e. 5.2 and 15.7 mg/kg bw per day, for toddlers, respectively (Table 5).

The potential level of the impurities in the food additive combined with the estimated exposure levels to E 950, presented in Table 5, results in exposure estimates that can be compared with the following reference points (RP) or health-based guidance values (HBGV) (Table E.1) for the undesirable impurities. It is considered that any mercury in the food additive corresponds to the element in the inorganic form rather than organic form. Consequently, the HBGV for inorganic mercury was used for comparison (Table E.2).

TABLE E.1 Reference points/health-based guidance value for toxic elements potentially present in the food additive E 950.

Element/HBGV/RP	Basis/reference
Lead (Pb)/0.5 mg/kg bw per day ($BMDL_{01}$)	The reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. The EFSA CONTAM Panel mentioned that a 1-point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a 1-point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the $BMDL_{01}$ (MOE lower than 1). EFSA CONTAM Panel (2010)
Inorganic mercury (iHg)/4 mg/kg bw per week (tolerable weekly intake (TWI))	The HBGV was set using kidney weight changes in male rats as the pivotal effect. Based on the $BMDL_{10}$ of 0.06 mg/kg bw per day, expressed as mercury and an uncertainty factor of 100 to account for inter and intra species differences, with conversion to a weekly basis and rounding to one significant figure, a TWI for inorganic mercury of 4 µg/kg bw per week, expressed as mercury was established. EFSA CONTAM Panel (2012)

Inorganic impurities

The IBO provided a compilation of reporting data of the analysis of different toxic elements in samples of acesulfame K (E 950) being reported below a certain limit (Documentation provided to EFSA No 1) and without detailed information on the methodology of the analysis, apart from method applied. i.e. ICP–MS and AAS.

Additional results were provided for lead, mercury, cadmium, arsenic and fluoride in the certificate of analysis of six samples of E 950 (Documentation provided to EFSA No 1). Lead, cadmium and arsenic were analysed by ICP–MS, mercury by AAS and fluoride by IC. In the certificate of analyses the following limits were indicated: 0.02–0.1 mg/kg for lead, cadmium and arsenic; a maximum of 0.01 mg/kg for mercury; and 0.1–1 mg/kg for fluoride. In all analysed samples, the levels of lead, mercury, cadmium, arsenic and fluoride were reported as 'below LOD' without indicating the corresponding value.

No information on the lowest technologically achievable levels for the toxic elements was provided by the IBO.

The Panel noted that the reported results are substantially lower than the limits indicated in the EU specifications, where limit values for lead, mercury and fluoride are set.

Taking into account the data provided additionally for arsenic and cadmium and taking also into account that the production process is an organic synthesis with final crystallisation and recrystallisation steps and so systematic contamination by these inorganics is not anticipated, the Panel did not see a need to recommend additional specification limits for arsenic or cadmium. The Panel assessed the risk that would result if the toxic elements, lead and mercury, were present in E 950 at: (i) the existing limit in EU specifications; (ii) the reported limit values (0.1 mg/kg for lead and 0.01 for mercury) (Table E.2).

TABLE E.2 Toxic elements concentrations (mg/kg) in E 950 used for the calculation of their potential exposure from the use of the food additive.

Toxic elements concentrations in E 950	Lead	Mercury
(i) Current limits in the EU specifications (mg/kg)	1	1
(ii) The reported limit values (mg/kg)	0.1	0.01

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

TABLE E.3 Risk assessment for toxic elements from the use of E 950.

	(i) Considering the presence of toxic elements at the current limits of the EU specifications for E 950 (Commission Regulation (EU) No 231/2012)	
Exposure to E 950 (mg/kg bw per day)	**MOE for Pb at 1 mg/kg**	**% of the TWI for Hg at 1 mg/kg**
5.2[a]	96	0.9
15.7[b]	28	3.1
	(ii) Considering the presence of toxic elements at the reported limit values	
Exposure to E 950 (mg/kg bw per day)	**MOE for Pb at 0.1 mg/kg**	**% of the TWI for Hg at 0.01 mg/kg**
5.2[a]	961	< 0.1
15.7[b]	282	< 0.1

Abbreviations: MOE, margin of exposure; TWI, tolerable weekly intake.

[a]Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – mean (Table 5)).

[b]Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – 95th percentile (Table 5)).

The Panel recommended to lower the EU specification limit for lead and mercury taking into account (i) the results of the calculations performed by the Panel (Table E3), (ii) the fact that the food additive is not the only potential dietary source of toxic elements and that (iii) the maximum limits should be established based on actual levels in the commercial food additive. If the European Commission decides to revise the current limits in the EU specifications, the values in Table E3 could be considered.

The Panel also calculated the exposure to fluoride from the use of E 950 considering that it can be present in the food additive at the existing EU limit of 3 mg/kg (Table E.4). The resulting exposure (0.0002 mg/day and 0.0006 mg/day for the mean and 95th percentile in toddlers, respectively) was found to be four orders of magnitude below the tolerable upper intake level of 1.6 mg/day for children (1–3 years of age) proposed by the EFSA Scientific Committee (EFSA Scientific Committee, n.d.).

TABLE E.4 Potential exposure to fluoride from the use of E 950 considering its presence at the current limit (3 mg/kg) in Commission Regulation (EU) No 231/2012.

Exposure to E 950 (mg/kg bw per day)	Exposure to fluoride (µg/kg bw per day)	Exposure to fluoride (µg/day)
5.2[a]	0.016	0.2
15.7[b]	0.047	0.6

[a]Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – mean (Table 5)).

[b]Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – 95th percentile (Table 5)).

Organic impurities

In the six analysed samples, impurity A (acetylacetamide) was reported as 'below the LOD' without indicating the LOD value of the HPLC method used, but a limit value of 0.5–1 mg/kg was stated. The impurity B (5-chloro-acesulfame) was reported as below 2 mg/kg.

The Panel calculated the potential exposure to the identified impurities, considering the worst-case scenario that they are present at the reported limit values, i.e. 1 mg/kg acetylacetamide and 2 mg/kg for 5-chloro-acesulfame (Table E.5).

TABLE E.5 Potential exposure to organic impurities from the use of E 950.

Exposure to E 950 (mg/kg bw per day)	Exposure to acetylacetamide at a concentration of 1 mg/kg (µg/kg bw per day)	Exposure to 5-chloro-acesulfame at a concentration of 2 mg/kg (µg/kg bw per day)
5.2[a]	0.0052	0.010
15.7[b]	0.0157	0.031

[a]Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – mean (Table 5)).

[b]Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – 95th percentile (Table 5)).

For acetylacetymide no experimental data on toxicity including genotoxicity were retrieved in a systematic search of published literature (Appendix A).

The Panel noted that from a rat combined repeated dose toxicity study with the reproduction/developmental toxicity screening test according to the OECD Guideline 422 reported in the ECHA dossier publication[40] a NOAEL of 100 mg/kg bw per day, based on non-neoplastic effects is identified when acetylacetamide was administered by gavage. No genotoxicity concern was observed in the study reported in the REACH registration dossier and no genotoxicity alerts were identified in the QSAR analysis (section 3.4.3.1). The Panel considered that this NOAEL found in male animals after 28 days dosing should be corrected for the extrapolation from sub-acute to chronic dosing. From the range of factors presented in EFSA Scientific Committee (2012) (4.5–6) the Panel selected the factor of 5 which would result in a chronic NOAEL of 20 mg/kg bw per day and used this value as a reference point. When comparing the potential exposure to acetylacetamide from the use of E 950 (Table E4) with the chronic NOAEL of 20 mg/kg bw per day, the Panel noted that the MOE would be 10^6 and no safety concern is raised. The Panel noted that additional exposure to acetylacetamide is expected as degradation product of acesulfame K (no quatification data available) (Section 3.1.4). Considering the high MOE, the Panel considered no safety concern is raised for the presence of acetylacetamide as impurity and degradation product based on the available data.

QSAR analysis of 5-chloro-acesulfame triggered some genotoxicity alerts (both in VEGA and QSAR TB) (see section XX) and therefore a TTC of 0.15 μg/person per day or 0.0025 μg/kg bw per day is applicable when assessing the safety of the presence of this impurity in the food additive (EFSA Scientific Committee, 2019). The Panel noted that the potential exposure to this impurity considering that is present at the reported limit (Table E4) is above the TTC value.

The Panel noted that no quantification data on the presence of 5-chloro-acesulfame in E 950 are available, because this impurity was reported as simply below the limit of 2 mg/kg.

In order to ensure that the potential exposure to this impurity resulting from the use of E 950 would not raise a concern (less than the TTC value of 0.0025 μg/kg bw per day) the presence of this impurity should be less than 0.1 mg/kg in the food additive. This would be also valid considering the exposure estimated in the refined regulatory maximum level exposure assessment scenario (the highest exposure levels for the mean and 95th percentile among the different population groups were considered, i.e. 5.6 and 16.7 mg/kg bw per day, for toddlers).

[40] https://chem.echa.europa.eu/100.025.250/dossier-view/8724681f-86c0-4dcd-bf88-758cb88707bd/ec65bb2e-413f-4e99-8d0f-374294ab7787_9e1be6ad-081b-4921-bfe8-e4ed1d2af284?searchText=5977-14-0 [Accessed on 22 January 2025].

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
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