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SCIENTIFIC OPINION

Re-evaluation of saccharin and its sodium, potassium and calcium salts (E 954) as food additives

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Abstract

This opinion deals with the re-evaluation of saccharin and its sodium, potassium and calcium salts (E 954) as food additives. Saccharin is the chemically manufactured compound 1,2-benzisothiazol-3(2H)-one-1,1-dioxide. Along with its sodium (Na), potassium (K) and calcium (Ca) salts, they are authorised as sweeteners (E 954). E 954 can be produced by two manufacturing methods i.e. Remsen-Fahlberg and Maumee. No analytical data on potential impurities were provided for products manufactured with the Maumee process; therefore, the Panel could only evaluate saccharins (E 954) manufactured with the Remsen-Fahlberg process. The Panel concluded that the newly available studies do not raise a concern for genotoxicity of E 954 and the saccharins impurities associated with the Remsen-Fahlberg manufacturing process. For the potential impurities associated with the Maumee process, a concern for genotoxicity was identified. The data set evaluated consisted of animals and human studies. The Panel considered appropriate to set a numerical acceptable daily intake (ADI) and considered the decrease in body weight in animal studies as the relevant endpoint for the derivation of a reference point. An ADI of 9 mg/kg body weight (bw) per day, expressed as free imide, was derived for saccharins (E 954). This ADI replaces the ADI of 5 mg /kg bw per day (expressed as sodium saccharin, corresponding to 3.8 mg /kg bw per day saccharin as free imide) established by the Scientific Committee on Food. The Panel considered the refined brand-loyal exposure assessment scenario the most appropriate exposure scenario for the risk assessment. The Panel noted that the P95 exposure estimates for chronic exposure to saccharins (E 954) were below the ADI. The Panel recommended the European Commission to consider the revision of the EU specifications of saccharin and its sodium, potassium and calcium salts (E 954).

KEYWORDS

E 954, food additive, potassium and calcium salts, saccharin and its sodium, sweetener

CONTENTS

SUMMARY

The present opinion deals with the re-evaluation of saccharins (E 954) when used as food additives. Saccharins (E 954) are authorised as food additives in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and their specifications are defined in the Commission Regulation (EU) No 231/2012.

Saccharins (E 954) were previously evaluated by the Scientific Committee on Food (SCF). In its last opinion from 1995, the Committee reviewed the newly available information and concluded that saccharin is not a 'direct acting genotoxin'. The Committee concluded that it was appropriate to set a full ADI for sodium saccharin of 0–5 mg/kg bw (0–3.8 mg/kg bw expressed as the free acid) applying a 100-fold safety factor to the NOEL of 1% for bladder tumours in the rat (500 mg/kg bw).

In its latest assessment from 1993, JECFA concluded that the dose-related carcinogenic activity on the urinary bladder was specific to the male rat and that the exposure during the neonatal period was critical for the subsequent development of these tumours. A NOEL of 500 mg/ kg bw per day based on 'marked disturbance of homeostasis' was derived from the same study considered pivotal by SCF and an ADI of 5 mg/kg bw for the sodium salt of saccharin was derived.

The International Agency for Cancer (IARC) in its latest monograph classified saccharin and its salts as '*not classifiable as to their carcinogenicity to humans* '(Group 3).

Saccharins (E 954) can be manufactured by the Remsen-Fahlberg process or the Maumee process. No description of the manufacturing processes of E 954 is included in Commission Regulation (EU) No 231/2012. Since only IBOs manufacturing saccharins using the Remsen-Fahlberg process expressed an interest following the EFSA call for technical data, and no analytical data on potential impurities were provided for products manufactured with the Maumee process, the Panel could only evaluate saccharins (E 954) manufactured with the Remsen-Fahlberg process. Thus, the Panel proposed to add a definition in the EU specifications of these food additives, restricted to the manufacturing with the Remsen-Fahlberg process.

Considering the purity of saccharins (E 954) and of their impurities in the EU specifications and the JECFA specifications, the Panel recommended that, even though no analytical data were provided by the IBOs on the purity of calcium saccharin (E 954 (iii)), the assay could be modified to 'Not less than 99 % of $C_{14}H_8CaN_2O_6S_2$ on the anhydrous basis' in the EU specifications.

In the EU specifications, saccharin (E 954 (i)) is defined (according to the chemical name) as an anhydrous substance, whereas the sodium salt (E 954 (ii)) is defined as a dihydrate, the potassium salt (E 954 (iv)) as a monohydrate and the calcium salt (E 954 (iii)) as a hydrate with two saccharin units and 3.5-waters of crystallisation. However, the Panel noted that the EINECS numbers for E 954 (ii) and E 954 (iii) reported in the EU specifications refer to the anhydrous substances. The Panel also noted that the CAS numbers indicated in the JECFA specifications for the anhydrous substances E 954 (i), E 954 (ii) and E 954 (iv) are not included in the EU specifications.

The Panel noted that the current EU specifications for E 954 only include impurities derived from the Remsen-Fahlberg process. The Panel considered the TTC approach to conduct a risk assessment to organic impurities associated with the Remsen-Fahlberg process. Regarding those impurities included in the EU specifications, the Panel noted that the potential exposure to o-toluene sulfonamide, p-toluene sulfonamide and benzoic acid p-sulfonamide is below the Cramer Class III value of 1.5 μg/kg bw per day, and therefore does not raise a safety concern. The Panel noted that benzoic acid, another impurity of E 954, is an authorised food additive (E 210), with an ADI of 5 mg/kg bw per day (expressed as benzoic acid).

Considering that the purity of saccharin and its sodium, potassium and calcium salts is not less than 99% on the anhydrous basis, the maximum amount of salicylic acid, considering that other impurities are not present (worst-case scenario), would be 1%, resulting in a potential exposure to salicylic acid from the use of E 954 up to 77 μg/kg bw per day. When comparing with the lowest NOAEL for salicylic acid of 75 mg/kg bw per day, the Panel noted that the MOE would be at least 1000, and no safety concern was raised. In addition, the Panel is aware that an endocrine disruptor (ED) assessment is ongoing for this substance under the biocide regulatory framework. The Panel noted that if a HBGV will be established as an outcome of the ongoing assessment in the other regulatory frameworks, a numerical limit of salicylic acid for the EU specifications of E 954 could be considered.

The Panel noted that the parameter 'readily carbonisable substances' is unspecific, and therefore not needed in the EU specifications of E 954.

Regarding toxic elements, the Panel performed the risk assessment that would result if arsenic and lead were present in E 954 at the current maximum limits in the EU specifications and at the lowest reported LOD or reporting limit by the IBOs.

Considering the results of the exposure to the toxic element Pb, the Panel noted that its presence in E 954 at the current specification limit value would not give rise to concern. In the case of As, the Panel noted that its presence in E 954 at the current specification limit value would lead to an MOE around 3, which is considered insufficient. The Panel noted that the analytical data provided for Pb and As were reporting limits or below the LODs. The IBOs did not indicate the lowest technologically achievable levels for these toxic elements. The Panel considered that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. The Panel is of the view that the current EU specification limits for Pb and As should be lowered.

A current maximum limit for selenium of 'not more than 30 mg/kg' is set in the EU specifications of E 954. The Panel noted that E 954 may contribute to the total European dietary Se exposure. Considering the calculated by the Panel intakes of Se resulting from the use of E 954, the presence of Se in E 954 at the current specification limit would not be of concern.

In the absence of analytical data on the potential impurities associated with the Maumee process in the food additives, the exposure to the impurities attributed to the Maumee process could not be calculated and a risk assessment was, therefore, not performed.

Considering the microbiological data submitted by the IBOs, the Panel considered that a microbiological contamination is unlikely and, therefore, it is not necessary to recommend inclusion of microbiological criteria in the EU specifications for E 954.

The Panel noted that according to the literature data and the information provided, the solubility of sodium and calcium salt of saccharin ((E 954 (ii) and E 954 (iii), respectively) in water is higher than the threshold value of 33.3 g/L as a decision criterion for demonstrating that the material does not require specific assessment at the nanoscale. Regarding saccharin (E 954 (i)), the reported solubility values (3.2 g/L or 2 g/L (at 20°C)) are lower than the threshold value of 33.3 g/L. Taking into account the maximum reported use levels, the MPLs, the reported solubility values and the volume of gastric secretion (ranging from 215 mL within a single meal to 2000 mL daily), the Panel considered that full dissolution of E 954 (i) is to be expected in foods and/or in the gastrointestinal tract and that ingested particles (if any) would not persist. Considering the above, the Panel concluded that there is no concern with regard to the potential presence of small particles, including nanoparticles, in saccharin (E 954 (i)) and its sodium (E 954 (ii)) and calcium (E 954 (iii)) salts at the reported uses and use levels and considered that these food additives can be assessed following the conventional risk assessment, i.e. EFSA Guidance for submission for food additive evaluations.

The Panel noted that, based on the submitted information on the stability of saccharins, E 954 is expected to be stable in food under the normal conditions of use, in accordance with the authorised uses of E 954.

The biological and toxicological data set available to the Panel for the re-evaluation of saccharins (E 954) 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 were performed according to the principles outlined in the revised protocol on hazard identification and characterisation of sweeteners.

Regarding the absorption, distribution, metabolism and excretion, the Panel considered that the data on urinary excretion demonstrate that most, if not all, saccharin is absorbed when doses between 2 and 69 mg saccharin per person were administered orally. The Panel considered that saccharin is not metabolised, has a half-life of ~4 h and is primarily excreted into the urine. The Panel noted that, after administration, all the salts of saccharin will dissociate in biological fluids to saccharin (as free imide). Saccharin passes into breast milk and is capable of passing the placenta, as indicated by detection in amniotic fluid and cord blood samples, and entering the fetal circulation.

The Panel concluded that the newly available studies do not raise a concern for genotoxicity of saccharins (E 954), which concurs with the conclusion of the previous SCF opinion based on the database available at that time. Taking into account the available experimental and in silico data, the Panel concluded that saccharins impurities associated with the Remsen-Fahlberg process do not raise concern for genotoxicity. For the potential impurities associated with the Maumee process, a concern for genotoxicity was identified for benzamide, while the genotoxic potential of 2-chlorobenzamide could not be fully assessed.

An evaluation of the risk of bias (RoB) was performed and a weight of evidence (WoE) approach for the reliable studies was applied for each health outcome for both human and animal studies. Based on the outcome of WoE, the Panel considered it likely that the exposure to saccharins (E 954) at high doses is associated in animals with a decrease in body weight. The body weight decreases observed in animals were higher than 10% at doses equal to or higher than 4500 mg/kg bw per day. Generally, changes in body weight in laboratory rodents of this magnitude are considered adverse. These body weight changes did not appear to be clearly associated with a decrease in feed consumption. The Panel noted that reduced body weight was not observed in the included human studies; however, the exposure to equivalent high doses was not examined.

The Panel also noted that, at low doses of saccharins (2.5–730 mg/kg bw per day), most animal studies reported modest increases (less than 10%) in mean final body weight compared to the control. The highest increase was between 10% and 25%, in two studies. The Panel noted that this weight gain at low doses might be related to increased feed intake (data insufficient) and may reflect body weight changes which are well known from the use of sodium saccharin as fattening agent in farmed animals. Furthermore, in the absence of toxicological effects in this dose range, the Panel considered this effect not adverse. The two included human studies (one observational and one interventional), at normally consumed doses or at doses twice the ADI previously set by the SCF and JECFA, provided only limited support for these findings (i.e. low level of evidence for the observed effect). Overall, the Panel considered that the association between exposure to saccharins (E 954) and increase in body weight has not been convincingly demonstrated by the available studies (WoE analysis in accordance with the protocol: It is 'as likely as not' that saccharins (E 954) exposure in humans is associated with a small increase in body weight at doses up to twice the ADI of 5 mg/kg bw per day set by the SCF and JECFA).

Because of the possible health implications of increases in body weight, the Panel considered that further studies and research would be needed to understand any potential role of saccharins (E 954) in promoting this effect. The Panel is aware of existing evaluations from other bodies (WHO and BfR) on the association between exposure to non-nutritive sweeteners and body weight gain.

The Panel noted that the ADI of 5 mg/kg bw per day (expressed as sodium saccharin, corresponding to 3.8 mg/kg bw per day saccharin as free imide) established by the SCF in 1995 was derived from the NOEL of 500 mg sodium saccharin/kg bw for bladder tumours in male rat and by applying an uncertainty factor of 100. Based on the studies available at that time, the SCF noted that the mechanistic studies and the epidemiological studies strongly indicated that saccharin is not related to bladder cancer in humans but since it has not been possible to unequivocally demonstrate this, the Committee 'as a matter of prudence' decided to take these lesions into account in setting the ADI. The Panel noted that, according to

the current knowledge, the bladder tumours observed in male rats are not considered relevant to humans. The Panel also noted that the ADI of 5 mg/kg bw per day for sodium saccharin established by JECFA in 1993 was set considering the same pivotal study as in the SCF evaluation but identified a NOEL of 500 mg/kg bw per day based on a 'marked disturbance of the homeostasis' described as 'persistent dose-related decreases in body weight gain in the presence of increased food consumption probably related to the inhibitory effects of saccharin on carbohydrate and protein digestion'.

The Panel considered the decrease in body weight in animal studies as the relevant endpoint for the derivation of a reference point and considered it appropriate to set a numerical ADI. In the absence of an appropriate NOAEL and suitable data for a BMD modelling, a reference point was identified by the Panel as the LOAEL of 4500 mg sodium saccharin/kg bw per day (corresponding to a LOAEL of 3420 mg saccharin as free imide/kg bw per day) based on the observed body weight decrease (–15%) from an 8-week study in rats. In derivation of an ADI, the Panel considered that, in addition to the default uncertainty factor of 100, an extra factor of 2 for the extrapolation from the LOAEL to the NOAEL and another factor of 2 for the extrapolation to chronic exposure should be applied. Consequently, an ADI of 9 mg/kg bw per day, expressed as free imide, was derived for saccharin and its sodium, potassium and calcium salts (E 954).

One of the included studies reported information on the concentration of saccharin in breast milk following the intake of 20 mg saccharins by the nursing mothers (81.5 ng/mL). Using the maximum concentration measured in breast milk in this study, the Panel estimated the concentration of saccharin as free imide by linear extrapolation in breast milk for an intake of the mother corresponding to the ADI (9 mg/kg bw per day) and assuming a body weight of the mothers of 70 kg. On the basis of this estimation, the intake of saccharin for breast feeding infants was calculated using the default consumption values from the relevant EFSA Scientific Committee Guidance and resulted to be 510 μg/kg bw per day. If compared to the reference point of 3420 mg/kg bw per day, the MOE is more than 1000 for the infant exposure by nursing which the Panel considered as indicative for no health concern.

Currently, saccharins (E 954) are authorised food additives in the EU in 34 food categories (FCs) (corresponding to 46 authorised uses) with MPLs ranging from 50 to 3000 mg/kg and at quantum satis (QS) in three food categories (FC 11.4 Table Top Sweeteners in liquid, powder and tablet form). All MPLs for saccharin and its sodium, potassium and calcium salts (E 954) are concentrations expressed as the free imide. Dietary exposure to saccharins (E 954), expressed as free imide, was estimated according to different exposure scenarios based on consumers only. Currently, saccharins (E 954) are authorised food additives in the EU in 34 food categories, while IBOs provided EFSA with use levels for seven food categories and analytical data were available for 30 food categories.

The highest mean and P95 chronic exposure to saccharins (E 954), expressed as free imide, among consumers of one or more food categories containing saccharins (E 954) were estimated for the elderly at 2.1 and 7.8 mg/kg bw per day, respectively.

The Panel considered that the exposure to saccharins (E 954), expressed as free imide, from their use as food additives according to Annex II was overestimated in the regulatory maximum level exposure assessment scenario as well as in two refined exposure assessment scenarios (i.e. maximum and brand-loyal). This is mostly due to the fact that the exposure calculations were based on MPLs/maximum use levels/highest reliable percentiles of analytical data and these concentrations were considered applicable to all foods within each food category, while the percentage of foods in a subcategory labelled to contain saccharins (E 954) was maximally 20% in Mintel.

The Panel considered the refined brand-loyal exposure assessment scenario the most appropriate exposure scenario for the risk assessment of saccharins (E 954). The Panel noted that the P95 exposure estimates for chronic exposure to saccharins (E 954), expressed as free imide, were below the ADI of 9 mg/kg bw per day in all populations, indicating that there is no safety concern.

The Panel recommends the European Commission to consider:

- including a definition for saccharins (E 954) in the EU specifications, restricted to the manufacturing with the Remsen-Fahlberg process;
- including the CAS numbers 81-07-2 for saccharin (E 954 (i)), 128-44-9 for sodium saccharin (E 954 (ii)) and 10332-51-1 for potassium saccharin (E 954 (iv)) in the EU specifications, indicating that the CAS numbers for sodium saccharin and potassium saccharin refer to the anhydrous substances;
- modifying the purity of calcium saccharin (E 954 (iii)) to 'Not less than 99 % of $C_{14}H_8CaN_2O_6S_2$ on the anhydrous basis' in the EU specifications;
- removing the parameter 'readily carbonisable substances' from the EU specifications of saccharins (E 954);
- lowering the limit of lead and arsenic in the EU specifications of saccharins (E 954).

1 | INTRODUCTION

The present opinion deals with the re-evaluation of saccharin and its sodium, potassium and calcium salts (E 954) when used as food additives. The generic term 'saccharins' will be used in the body of this opinion unless more specific information is reported i.e. E number or tested material in biological and toxicological studies (i.e. saccharin (E 954 (i), sodium saccharin (E 954 (ii)), calcium saccharin (E 954 (iii)), potassium saccharin (E 954 (iv)).

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

Saccharin and its sodium, potassium and calcium salts (E 954) are authorised food additives in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives. Specifications for E 954 are laid down in Commission Regulation (EU) No 231/2012.[3]

Saccharin[4] was evaluated in 1977 by the Scientific Committee on Food (SCF) who, on the basis of the animal studies available at that time, endorsed the temporary ADI of 0–2.5 mg/kg bw for saccharin proposed by JECFA based on bladder cancer. The Committee noted that *'some members of the committee felt that saccharin should not be used in food for the general consumption'*. Epidemiological studies were available to the Committee who considered that *'these studies are not entirely satisfactory from the point of view of sample population and size. At the same time they are not solely related to sweeteners but deal also with other factors such as smoking. More recent epidemiological studies have produced conflicting results. In view of this the Committee recommends that prospective epidemiological studies should be carried out on the incidence of certain chronic state diseases in populations with a high intake of saccharin'*. Considering the evidence from animal and

[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]Term used in the SCF report (1977).

epidemiological studies available at the time of the assessment, the Committee also concluded that saccharin should not be used in food specially prepared for young children (up to 3 years) and that the intake by children and pregnant women should be limited (SCF, 1977).

In an additional opinion of saccharin[5] expressed 1988 by the SCF, the Committee considered additional studies which became available and concluded that the in utero exposure does not contribute to the incidence of bladder tumours and that, therefore, the special warning for pregnant women was no longer warranted. The Committee noted that the contribution of exposure during the lactation period and in early age remains unknown. However, the Committee noted that the ADI was based on a clear no-effect level in well-performed tests covering exposure at this stage and that it was a general policy to be very restrictive in the use of additives in food prepared for young children. Therefore, the Committee concluded that it was not necessary any more to warn against saccharin in this case. The temporary ADI was maintained (SCF, 1989).

In its last opinion on saccharin and its calcium, potassium and sodium salts[6] expressed in 1995 (SCF, 1995), the Committee reported that *'the SCF was informed that the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment had recently reviewed saccharin and recommended that it should be allocated a full ADI of 0–5 mg/kg bw. In September 1990 industry submitted further data and requested re-evaluation of the temporary ADI'*. The Committee reviewed the newly available information and concluded that saccharin is not a *'direct acting genotoxin'*. This conclusion was supported also by the fact that sodium saccharin has been shown to be a carcinogen, in one sex only of one species of animal, whereas according to the Committee, genotoxic carcinogens tend to be active at more than one site and/or in more than one sex or species. The Committee concluded that the difference between the male rat and other species (including humans) response was not based on any difference in metabolism but probably on a difference in local effect and response in the bladder walls. The available mechanistic studies combined with the information from the epidemiological studies strongly indicated that saccharin was not related to bladder cancer in humans. The Committee concluded that it was unlikely that the tumours in the male rat bladder are of relevance for humans, although it was not possible to unequivocally demonstrate this. Therefore, the Committee wished as a matter of prudence to take these lesions into account in setting an ADI. The Committee considered that in order to establish an ADI for this non-genotoxic, male rat bladder carcinogen, two considerations were relevant (i) the NOEL from the 'pivotal' two-generation, long-term rat study and (ii) the safety factor to be applied and considered reasonable to regard 1% sodium saccharin in the diet as a clear NOEL. The Committee noted that this was also the NOEL for other non-neoplastic effects of saccharin. The temporary ADI of 0–2.5 mg/kg bw set previously was based on a possible NOEL of 1% in the diet, equivalent to 500 mg/kg bw per day, using a safety factor of 200 because of the temporary nature of the ADI. Considering the newly available experimental information including the extensive epidemiological data and the additional information provided in response to the Committee's earlier questions, the Committee concluded that it is appropriate to set a full ADI for sodium saccharin of 0–5 mg/kg bw applying a 100-fold safety factor to the NOEL of 1% for bladder tumours in the rat (500 mg/kg bw). The Committee noted that it may also be necessary to express the ADI in terms of free acid, since sodium saccharin is not the only salt used. Taking account of the molecular weight (MW) difference between sodium saccharin (MW 241) and the free acid (MW 183), the Committee derived an ADI of 0–3.8 mg/kg bw (expressed as the free acid).

JECFA has evaluated saccharin[7] in several meetings (JECFA, 1968, 1974, 1978, 1980, 1982, 1984, 1993). At its twenty-first meeting (JECFA, 1978), the ADI of 0–5 mg/kg bw was changed to a temporary ADI of 0–2.5 mg/kg bw and the conditional ADI of 0–15 mg/kg bw for 'dietetic purposes only' was withdrawn based on studies indicating that excessive and long-term ingestion of saccharin might represent a carcinogenic hazard. In the following two evaluations (JECFA, 1980, 1982), the temporary ADI was extended pending the completion of some ongoing studies including a long-term study in rats and an epidemiological study. In 1984, the temporary ADI was further extended pending on the evaluation of further data including information to elucidate the mechanism at the basis of the bladder tumours. In its latest assessment (JECFA, 1993), the Committee concluded that the dose-related carcinogenic activity on the urinary bladder was specific to the male rat and that the exposure during the neonatal period was critical for the subsequent development of these tumours. The epidemiological studies did not show any evidence of a possible increase of the incidence of bladder cancer in humans. Rats have shown a 'marked disturbance of homeostasis' at levels of 3% in the diet. A NOEL of 500mg/ kg bw per day was derived from a long-term toxicity study in rats and in monkeys.[8] This was the basis to derive an ADI of 5 mg/kg bw for the sodium salt of saccharin. The Committee also considered the genotoxic potential of saccharin. The clastogenic activity observed in a number of in vivo and in vitro assays was attributed to ionic imbalances at the chromosomal level. The Committee also noted that clastogenic activity was also in disagreement with the results of the long-term studies and tumour initiation/promotion studies with sodium saccharin.

The International Agency for Cancer (IARC) published its monographs on saccharin and its salts[9] in 1980, 1987 and 1999 (IARC, 1980, 1987, 1999). In its latest monograph, it was concluded that *'sodium saccharin produces urothelial bladder tumours in rats by a non-DNA-reactive mechanism that involves the formation of a urinary calcium phosphate-containing precipitate, cytotoxicity and enhanced cell proliferation. This mechanism is not relevant to humans because of critical interspecies*

[5]Term used in the SCF report (1989).
[6]Term used in the SCF report (1995).
[7]Term used in the JECFA Report (1993).
[8]This study was not available to EFSA.
[9]As defined in IARC (1999).

differences in urine composition'. Saccharin and its salts were considered as *'not classifiable as to their carcinogenicity to humans* '(Group 3).

The EFSA AFC Panel (Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food) assessed the health implications of the presence of '1,2-benzisothiazolin-3-one (BIT) as an impurity in saccharin used as food additive' (EFSA AFC Panel, 2007). Regarding the genotoxicity of BIT, the Panel concluded that while BIT was clastogenic to CHO mammalian cells in vitro, two adequately performed in vivo tests in two different tissues provided no evidence for a genotoxic potential of BIT in vivo. The Panel has estimated the intake of BIT from consumption of saccharin at the acceptable daily intake (ADI) for sodium saccharin of 0–5 mg/kg bw assuming that saccharin contains BIT at the highest reported concentration (800 mg/kg). Using these assumptions, the Panel concluded that even the highest levels of BIT detected in some samples of saccharin do not represent a safety concern.

A risk assessment of the additive sodium saccharin,[10] for use as a nucleating agent up to 0.1% w/w in polyesters (Food Contact Materials) has been performed also by the EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) in 2012, that concluded that the use of the substance, as additive in polyesters, is not of safety concern for the consumer (EFSA CEF Panel, 2012).

The safety and efficacy of sodium saccharin when used as a feed and water flavour for piglets, pigs for fattening, calves for rearing and calves for fattening have been evaluated by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Panel (EFSA FEEDAP Panel, 2018a). The FEEDAP Panel concluded that no concern for the consumer would result from the use of sodium saccharin in feed and water for drinking at the dose considered safe for the target species. Sodium saccharin was considered to be potentially harmful for the user exposed by inhalation or by contact to skin and eyes.[11] Regarding the environmental risk assessment, the FEEDAP Panel concluded that the use of sodium saccharin at the dose considered safe for target species was unlikely to have detrimental effects on the terrestrial and freshwater compartments. The high mobility and relative persistence of saccharin and the high persistence of its transformation product 4-hydroxysaccharin indicate that groundwater contamination above 0.1 µg/L is likely to occur. During the discussion with the Member States at a meeting in the Standing Committee on Plants, Animals, Food and Feed (Animal Nutrition section), it was suggested to check the safety of the additive for workers and for the environment. The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinion. In 2023, the FEEDAP Panel assessed the newly available data from the applicant and the proposed new conditions of use and concluded that sodium saccharin is not a skin or eye irritant nor a dermal sensitiser. However, in the absence of data, the FEEDAP Panel could not conclude on the potential of the additive to be toxic by inhalation. As regards the safety of the additive for the environment, 1.13 mg sodium saccharin/kg feed could not be considered safe. The FEEDAP Panel estimated that the maximum use level that would result in a concentration in groundwater below the threshold of 0.1 µg/L is 0.022 mg sodium saccharin/kg feed. The available data did not allow to conclude on the potential effect of the transformation product 4-hydroxysaccharin in ground water (EFSA FEEDAP Panel, 2023a).

2 | DATA AND METHODOLOGIES

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

The draft protocol for the hazard identification and characterisation of sweeteners was published on EFSA's website for comments, and the online public consultation was made available until 19 September 2019. A technical report on the outcome of this public consultation with the overview of the comments received and the general responses from EFSA was published (EFSA, 2020a). During the implementation phase, some amendments and further elaborations to the original protocol were introduced. The changes introduced are documented in the revised version published in 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). A revised protocol was under finalisation at the time of the drafting of this scientific opinion.

[10]Referred to in the opinion as 1,2-benzisothiazol- 3(2H)-one 1,1-dioxide, sodium salt (saccharin, sodium salt).

[11]The feed additives authorisation process foresees the need for the assessment of the safety of an additive for people who may come into contact with it in the workplace. This definition is restricted to those workers who may be exposed to the additive while handling it, when incorporating it into premixtures, feed materials or compound feeds or using a premix or feeding stuff supplemented with the additive (see EFSA FEEDAP Panel, 2023b).

2.1 | Data

The Panel was not provided with a newly submitted dossier for the re-evaluation of saccharins (E 954). In accordance with Regulation (EU) No 257/2010, EFSA launched public calls for data[12,13,14] and contacted interested parties that had replied to the call for data to collect additional clarification or supplemental information (Documentation provided to EFSA n. 1–18).

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 February 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 saccharins (E 954) were derived from the EFSA Comprehensive European Food Consumption Database[15] (Comprehensive Database). The Mintel's Global New Products Database (GNPD) was checked to identify the uses of saccharins and its sodium, potassium and calcium salts (E 954) 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, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee. In line with these principles, this risk assessment was carried out based on structured protocols on hazard identification and characterisation of sweeteners (EFSA, 2020a; EFSA FAF Panel, 2023) and on exposure assessment (EFSA, 2020b).

The FAF Panel assessed the safety of saccharins (E 954) as food additives 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 Scientific Committee on Food (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 (risk of bias (RoB)) and the assessment of the body of evidence across all health outcomes, are described in the revised protocol and 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 weight of evidence (WoE) evaluation. The study previously evaluated by the SCF in its 1995 opinion and on which an ADI was derived was 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', 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

[12] https://www.efsa.europa.eu/en/data/call/170621.

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

[14] https://www.efsa.europa.eu/en/call/call-technical-data-saccharin-and-its-sodium-potassium-and-calcium-salts-e-954.

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

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 saccharins (E 954) 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 saccharins (E 954) from their use as food additives was estimated by combining food consumption data available within the EFSA Comprehensive Database with the maximum levels according to Annex II to Regulation (EC) No 1333/2008 and with reported use levels and analytical data submitted to EFSA following public calls for data. The exposure was calculated according to different scenarios (see Section 3.5).

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

3 | ASSESSMENT

3.1 | Technical data

3.1.1 | Identity of the substances and specifications

Saccharin is the chemically manufactured compound 1,2-benzisothiazol-3(2H)-one-1,1-dioxide. Along with its sodium (Na), potassium (K) and calcium (Ca) salts, they are authorised as sweeteners (E 954).

The chemical structures of saccharin and its sodium, potassium and calcium salts (E 954) are given in Figure 1.

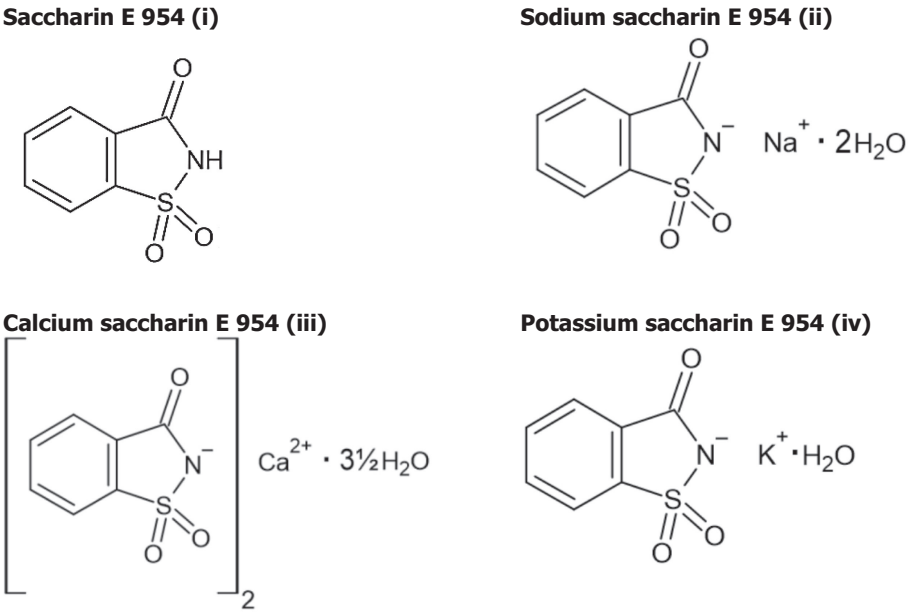


FIGURE 1 Chemical structure of saccharin and its sodium, calcium and potassium salts.

The specifications for saccharin (E 954 (i)) and its sodium (E 954 (ii)), calcium (E 954 (iii)) and potassium (E 954 (iv)) salts according to Commission Regulation (EU) No 231/2012 and proposed by JECFA (2006) are given in Table 1.

TABLE 1 Specifications for saccharin (E 954 (i)) and its sodium (E 954 (ii)), calcium (E 954 (iii)) and potassium (E 954 (iv)) salts according to Commission Regulation (EU) No 231/2012 and proposed by JECFA (2006).

	Commission Regulation No 231/2012	JECFA (2006)
	E 954 (i) Saccharin	
Synonyms		INS No. 954(i)
Definition		
EINECS	201-321-0	
Chemical name	3-Oxo-2,3dihydrobenzo(d) isothiazol-1,1-dioxide	1,2-Benzisothiazole-3(2H)-one-1,1-dioxide, 3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1-dioxide

(Continues)

TABLE 1 (Continued)

	Commission Regulation No 231/2012	JECFA (2006)
CAS number		81-07-2
Chemical formula	$C_7H_5NO_3S$	$C_7H_5NO_3S$
Molecular weight	183.18	183.18
Assay	Not less than 99% and not more than 101% of $C_7H_5NO_3S$ on the anhydrous basis	Not less than 99% and not more than 101.0% on the dried basis
Description	White crystals or a white crystalline powder, odourless or with a faint, aromatic odour. Approximately between 300 and 500 times as sweet as sucrose	White crystals or a white, crystalline powder, odourless or with a faint, aromatic odour
Identification		
Solubility	Slightly soluble in water, soluble in basic solutions, sparingly soluble in ethanol	Slightly soluble in water; soluble in basic solutions; sparingly soluble in ethanol
Acidity		A saturated aqueous solution is acidic
Derivation to salicylic acid		Dissolve about 0.1 g of the sample in 5 mL of 5% sodium hydroxide solution. Evaporate to dryness and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 mL of water, neutralize the solution with dilute hydrochloric acid TS and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet colour
Derivation to fluorescent substance		Mix 20 mg of the sample with 40 mg of resorcinol, add 10 drops of sulfuric acid and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 mL of water and an excess of sodium hydroxide TS. A fluorescent green liquid is produced
Purity		
Loss on drying	Not more than 1% (105°C, 2 h)	Not more than 1% (105°, 2 h)
Melting range	226–230°C	226–230°C
Sulfated ash	Not more than 0.2% (expressed on dry weight basis)	Not more than 0.2%
Benzoic and salicylic acid	To 10 mL of a 1 in 20 solution, previously acidified with five drops of acetic acid, add three drops of an approximately molar solution of ferric chloride in water. No precipitate or violet colour appears	Add ferric chloride TS dropwise to 10 mL of a hot, saturated solution of the sample. No precipitate or violet colour appears
o-Toluenesulphonamide	Not more than 10 mg/kg (expressed on dry weight basis)	
p-Toluenesulphonamide	Not more than 10 mg/kg (expressed on dry weight basis)	
Toluenesulphonamides		Not more than 25 mg/kg
Benzoic acid p-sulphonamide	Not more than 25 mg/kg (expressed on dry weight basis)	
Readily carbonisable substances	Absent	Dissolve 0.2 g of the sample in 5 mL of sulfuric acid TS. Keep at 48° to 50° for 10 min. The colour should not be darker than a very light brownish-yellow (Matching Fluid A)
Arsenic	Not more than 3 mg/kg (expressed on dry weight basis)	
Selenium	Not more than 30 mg/kg (expressed on dry weight basis)	Not more than 30 mg/kg
Lead	Not more than 1 mg/kg (expressed on dry weight basis)	Not more than 1 mg/kg
	E 954 (ii) Sodium Saccharin	
Synonyms	Saccharin; Sodium salt of saccharin	Soluble saccharin, INS No. 954(iv)
Definition		
EINECS	204-886-1	

TABLE 1 (Continued)

	Commission Regulation No 231/2012	JECFA (2006)
Chemical name	Sodium o-benzosulfimide; sodium salt of 2,3-dihydro-3-oxobenzisosulfonazole; oxobenzisosulfonazole; 1,2-benzisothia zolin-3- one-1, 1-dioxide sodium salt dehydrate	Sodium salt dihydrate of 1,2-Benzisothiazolin-3(2H)-one-1,1-dioxide, 3-oxo-2,3-dihydrobenzo[d]isothiazole-1,1-dioxide; sodium o-benzosulfimide
CAS number		128–44-9
Chemical formula	$C_7H_4NNaO_3S{\cdot}2H_2O$	$C_7H_4NNaO_3S{\cdot}2H_2O$
Molecular weight	241.19	241.19
Assay	Not less than 99% and not more than 101% of $C_7H_4NNaO_3S$ on the anhydrous basis	Not less than 99% and not more than 101% on the dried basis
Description	White crystals or a white crystalline efflorescent powder, odourless or with a faint odour. Approximately between 300 and 500 times as sweet as sucrose in dilute solutions	White crystals or a white, crystalline efflorescent powder, odourless or with a faint, aromatic odour
Identification		
Solubility	Freely soluble in water, sparingly soluble in ethanol	Freely soluble in water; sparingly soluble in ethanol
Melting range of saccharin derived from the sample		226–230° To 10 mL of a 1 in 10 solution add 1 mL of hydrochloric acid. A crystalline precipitate of saccharin is formed. Wash the precipitate well with cold water and dry at 105° for 2 h
Derivation to salicylic acid		Dissolve about 0.1 g of the sample in 5 mL of 5% sodium hydroxide solution. Evaporate to dryness and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 mL of water, neutralize the solution with dilute hydrochloric acid TS and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet colour
Derivation to fluorescent substance		Mix 20 mg of the sample with 40 mg of resorcinol, add 10 drops of sulfuric acid and heat the mixture in a liquid bath at 200o for 3 min. After cooling, add 10 mL of water and an excess of sodium hydroxide TS. A fluorescent green liquid is produced
Test for sodium		Passes test
Purity		
Loss on drying	Not more than 15% (120°C, 4 h)	Not more than 15% (120°C, 4 h)
Acidity and alkalinity		Dissolve 1 g of the sample in 10 mL of freshly boiled and cooled water. Add a drop of phenolphthalein TS. No pink colour should appear. Add a drop of 0.1 N sodium hydroxide. A pink colour should appear
Benzoic and salicylic acid	To 10 mL of a 1 in 20 solution, previously acidified with five drops of acetic acid, add three drops of an approximately molar solution of ferric chloride in water. No precipitate or violet colour appears	Add ferric chloride TS dropwise to 10 mL of a hot, saturated solution of the sample. No precipitate or violet colour appears
o-Toluenesulphonamide	Not more than 10 mg/kg (expressed on dry weight basis)	
p-Toluenesulphonamide	Not more than 10 mg/kg (expressed on dry weight basis)	
Toluenesulphonamides		Not more than 25 mg/kg
Benzoic acid p-sulphonamide	Not more than 25 mg/kg (expressed on dry weight basis)	Not more than 30 mg/kg
Readily carbonisable substances	Absent	Dissolve 0.2 g of the sample in 5 mL of sulfuric acid TS. Keep at 48° to 50° for 10 min. The colour should not be darker than a very light brownish-yellow (Matching Fluid A).

(Continues)

TABLE 1 (Continued)

	Commission Regulation No 231/2012	JECFA (2006)
Arsenic	Not more than 3 mg/kg (expressed on dry weight basis)	
Selenium	Not more than 30 mg/kg (expressed on dry weight basis)	Not more than 30 mg/kg
Lead	Not more than 1 mg/kg (expressed on dry weight basis)	Not more than 1 mg/kg
	E 954 (iii) Calcium Saccharin	
Synonyms	Saccharin; calcium salt of saccharin	INS No. 954(ii)
Definition		
EINECS	229-349-9	
Chemical name	Calcium o-benzosulfimide; calcium salt of 2,3-dihydro-3-oxobenzisosulfonazole; 1,2-benzisothiazolin-3-one-1,1-dioxide calcium salt hydrate (2:7)	Calcium salt hydrate (2:7) of 1,2-benzisothiazole-3-one-1,1-dioxide, 3-oxo2,3-dihydrobenzo[d]isothiazol-1,1-dioxide, 2,3-dihydro-3-oxobenzisosulfonazole; calcium o-benzosulfimide
Chemical formula	$C_{14}H_8CaN_2O_6S_2 \cdot 3½H_2O$	$C_{14}H_8CaN_2O_6S_2 \cdot 3½H_2O$
Molecular weight	467.48	467.48
Assay	Not less than 95% of $C_{14}H_8CaN_2O_6S_2$ on the anhydrous basis	Not less than 99% after drying
Description	White crystals or a white crystalline powder, odourless or with a faint odour. Approximately between 300 and 500 times as sweet as sucrose in dilute solutions	White crystals or a white, crystalline powder, odourless or with a faint, aromatic odour
Identification		
Solubility	Freely soluble in water, soluble in ethanol	Freely soluble in water, soluble in ethanol
Melting range of saccharin derived from the sample		Dissolve about 0.1 g of the sample in 5 mL of 5% sodium hydroxide solution. Evaporate to dryness and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 mL of water, neutralize the solution with dilute hydrochloric acid TS and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet colour
Derivation to fluorescent substance		Mix 20 mg of the sample with 40 mg of resorcinol, add 10 drops of sulfuric acid and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 mL of water and an excess of sodium hydroxide TS. A fluorescent green liquid is produced
Test for calcium		Passes test
Purity		
Loss on drying	Not more than 13.5% (120°C, 4 h)	Not more than 15% (120°, 4 h)
Benzoic and salicylic acid	To 10 mL of a 1 in 20 solution, previously acidified with five drops of acetic acid, add three drops of an approximately molar solution of ferric chloride in water. No precipitate or violet colour appears	Add ferric chloride TS dropwise to 10 mL of a hot, saturated solution of the sample. No precipitate or violet colour appears
o-Toluenesulphonamide	Not more than 10 mg/kg (expressed on dry weight basis)	
p-Toluenesulphonamide	Not more than 10 mg/kg (expressed on dry weight basis)	
Toluenesulphonamides		Not more than 25 mg/kg
Benzoic acid p-sulphonamide	Not more than 25 mg/kg (expressed on dry weight basis)	
Readily carbonisable substances	Absent	Dissolve 0.2 g of the sample in 5 mL of sulfuric acid TS. Keep at 48°–50° for 10 min. The colour should not be darker than a very light brownish-yellow (Matching Fluid A)
Arsenic	Not more than 3 mg/kg (expressed on dry weight basis)	

TABLE 1 (Continued)

	Commission Regulation No 231/2012	JECFA (2006)
Selenium	Not more than 30 mg/kg (expressed on dry weight basis)	Not more than 30 mg/kg
Lead	Not more than 1 mg/kg (expressed on dry weight basis)	Not more than 1 mg/kg
	E 954 (iv) Potassium Saccharin	
Synonyms	Saccharin; Potassium salt of saccharin	INS No. 954(iii)
Definition		
EINECS		
Chemical name	Potassium o-benzosulfimide; Potassium salt of 2,3-dihydro-3-oxobenziso sulfonazole; Potassium salt of 1,2-benzisothiazolin-3- one-1,1-dioxide monohydrate	Potassium salt of 1,2-benzisothiazole-3(2H)-one-1,1-dioxide monohydrate, 3-oxo-2,3-dihydrobenzo[d] isothiazol-1,1-dioxide monohydrate, 2,3-dihydro-3-oxobenziso-sulfonazole monohydrate; Potassium o-benzosulfimide
CAS number		10332–51-1
Chemical formula	$C_7H_4KNO_3S{\cdot}H_2O$	$C_7H_4KNO_3S{\cdot}H_2O$
Molecular weight	239.77	239.77
Assay	Not less than 99% and not more than 101% of $C_7H_4KNO_3S$ on the anhydrous basis	Not less than 99% and not more than 101% on the dried basis
Description	White crystals or a white crystalline powder, odourless or with a faint odour, having an intensely sweet taste, even in very dilute solutions. Approximately between 300 and 500 times as sweet as sucrose	White crystals or a white, crystalline powder, odourless or with a faint, aromatic odour
Identification		
Solubility	Freely soluble in water, sparingly soluble in ethanol	Freely soluble in water; sparingly soluble in ethanol
Melting range of saccharin derived from the sample		226–230° To 10 mL of a 1 in 10 solution add 1 mL of hydrochloric acid. A crystalline precipitate of saccharin is formed. Wash the precipitate well with cold water and dry at 105° for 2 h
Derivation to salicylic acid		Dissolve about 0.1 g of the sample in 5 mL of 5% sodium hydroxide solution. Evaporate to dryness and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 mL of water, neutralize the solution with dilute hydrochloric acid TS and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet colour
Derivation to fluorescent substance		Mix 20 mg of the sample with 40 mg of resorcinol, add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 mL of water and an excess of sodium hydroxide TS. A fluorescent green liquid is produced
Test for potassium		Passes test
Purity		
Loss on drying	Not more than 8% (120°C, 4 h)	Not more than 8% (120°, 4 h)
Acidity and alkalinity		Dissolve 1 g of the sample in 10 mL of freshly boiled and cooled water. Add a drop of phenolphthalein TS. No pink colour should appear. Add a drop of 0.1 N sodium hydroxide. A pink colour should appear
Benzoic and salicylic acid	To 10 mL of a 1 in 20 solution, previously acidified with five drops of acetic acid, add three drops of an approximately molar solution of ferric chloride in water. No precipitate or violet colour appears	To 10 mL of a 1 in 20 solution, previously acidified with 5 drops of acetic acid, add 3 drops of ferric chloride TS. No precipitate or violet colour appears
o-Toluenesulphonamide	Not more than 10 mg/kg (expressed on dry weight basis)	
p-Toluenesulphonamide	Not more than 10 mg/kg (expressed on dry weight basis)	

(Continues)

TABLE 1 (Continued)

	Commission Regulation No 231/2012	JECFA (2006)
Toluenesulphonamides		Not more than 25 mg/kg
Benzoic acid p-sulphonamide	Not more than 25 mg/kg (expressed on dry weight basis)	
Readily carbonisable substances	Absent	Dissolve 0.2 g of the sample in 5 mL of sulfuric acid TS. Keep at 48o to 50o for 10 min. The colour should not be darker than a very light brownish-yellow (Matching Fluid A)
Arsenic	Not more than 3 mg/kg (expressed on dry weight basis)	
Selenium	Not more than 30 mg/kg (expressed on dry weight basis)	Not more than 30 mg/kg
Lead	Not more than 1 mg/kg (expressed on dry weight basis)	Not more than 1 mg/kg

In the EU specifications (see Table 1), saccharin (E 954 (i)) is defined (according to the chemical name) as an anhydrous substance (molecular weight 183.18), whereas the sodium salt (E 954 (ii)) is defined as a dihydrate (molecular weight 241.19), the potassium salt (E 954 (iv)) as a monohydrate (molecular weight 239.77) and the calcium salt (E 954 (iii)) as a hydrate with two saccharin units and 3.5-waters of crystallisation (molecular weight 467.48). Considering the chemical formula of the salts of saccharin, the conversion factors to express the salts on a free imide basis (i.e. saccharin itself) are 0.76, 0.76 and 0.78 for the sodium, potassium and calcium salts, respectively.

The Panel noted that the EINECS numbers 204-886-1 for sodium saccharin (E 954 (ii)) and 229-349-9 for calcium saccharin (E 954 (iii)) reported in the EU specifications refer to the anhydrous substances. In addition, the Panel noted that no CAS numbers and no description of the manufacturing processes are indicated for saccharin and its sodium, potassium and calcium salts (E 954) in the EU specifications.

The chemical name of saccharin (E 954(i)) reported in the EU specifications is '3-oxo-2,3-dihydrobenzo(d)isothiazol-1,1-dioxide'. The Panel noted that another chemical name for saccharin (E 954(i)) is '1,2-benzisothiazole-3(2H)-one-1,1-dioxid e', as also reported in the JECFA specifications, and that the IUPAC name for saccharin (E 954(i)) is 2H-1λ^6,2-benzothiazol-1,1,3(2H)-trione. The Panel also noted that the IUPAC name for anhydrous sodium saccharin (E 954 (ii)) is sodium 1,1,3-trioxo-1,3-dihydro-1λ^6,2-benzothiazol-2-ide, for anhydrous calcium saccharin (E 954 (iii)) is calcium bis(1,1,3-trioxo-1,3-dihydro-1λ^6,2-benzothiazol-2-ide), and for anhydrous potassium saccharin (E 954 (iv)) is potassium 1,1,3-trioxo-1,3-dihydro-1λ^6,2-benzothiazol-2-ide.

The Panel also noted that the purity of calcium saccharin (E 954 (iii)) in the EU specifications is 'Not less than 95 % of $C_{14}H_8CaN_2O_6S_2$ on the anhydrous basis', while in the JECFA specifications it is 'Not less than 99 % after drying'. The purities of saccharin (E 954 (i)), sodium saccharin (E 954 (ii)) and potassium saccharin (E 954 (iv)) are reported as 'Not less than 99 % on the anhydrous basis' in the EU specifications.

3.1.2 | Manufacturing process

According to the information provided by four different interested business operators (IBOs), saccharins can be manufactured by the Remsen-Fahlberg process or the Maumee process (Documentation provided to EFSA n. 1, 2, 7, 8). Information was provided only for the manufacturing process of saccharin (E 954 (i)), sodium saccharin (E 954 (ii)) and calcium saccharin (E 954 (iii)), but not for potassium saccharin (E 954 (iv)).

The IBOs described both manufacturing processes. However, the two IBOs that manufacture saccharin (E 954 (i)) and sodium saccharin (E 954 (ii)) have confirmed that they only use the Remsen-Fahlberg process (Documentation provided to EFSA n. 4, 11, 12). No IBO manufacturing saccharins (E 954) using the Maumee process expressed an interest following the EFSA call for technical data on saccharin and its sodium, potassium and calcium salts (E 954),[16] requesting data and information on manufacturing process.

According to one IBO, in the Remsen-Fahlberg process (Figure 2), toluene reacts with chlorosulfonic acid, leading to a mixture of o- and p-toluene sulfochlorides. *o*-Toluene sulfochloride reacts with ammonia to form o-toluene sulfonamide, which is oxidised with the use of oxidising agents, such as potassium permanganate, dichromate salt or chromic acid, to benzoic acid o-sulfonamide and cyclised to saccharin (E 954 (i)) (Documentation provided to EFSA n. 7). The Panel noted that no description on the separation of the o-toluene sulfochloride and o-toluene sulfonamide from their p-isomers was reported by this IBO. Another IBO referred to the Remsen-Fahlberg process described in the Scientific Committee for Food as part of the original application for the approval of saccharin, which is in agreement with the description above (SCF, 1977) (Documentation provided to EFSA n. 2).

[16] https://www.efsa.europa.eu/en/call/call-technical-data-saccharin-and-its-sodium-potassium-and-calcium-salts-e-954.

Considering that chromic acid may be used as an oxidising agent in the Remsen-Fahlberg manufacturing process of E 954, EFSA requested analytical data on residues of Cr(VI) in commercial batches of the food additive, but no respective information was received. According to the EFSA CONTAM Scientific Opinion on the risks to public health related to the presence of chromium in food and drinking water (EFSA CONTAM Panel, 2014), Cr(VI) is able to cross cellular membranes and the interconversion of Cr(VI) to Cr(III) is of relevance for the risk assessment since, in general, Cr(VI) compounds are much more toxic than Cr(III) compounds. It is noted that food is generally a reducing medium that would likely determine soluble Cr(VI) to be converted to Cr(III), which is poorly bioavailable and presents low ability to enter cells, whereas no oxidation of Cr(III) to Cr(VI) is expected in such a medium. Despite no analytical data on Cr(VI) potentially present in E 954 were received, the exposure to Cr(VI) from the use of the food additive is considered unlikely.

According to another IBO, sodium saccharin (E 954 (ii)) can be manufactured with two different steps, meaning oxidation and purification. In this case, o-toluene sulfonamide may be used as the raw material, oxidised by sulfuric acid. The Panel noted that no information on the origin of o-toluene sulfonamide was reported by the IBO, while the same IBO mentioned that its purity may be variable. The 'crude insoluble saccharin slurry' is then isolated. In the following purification steps, sodium hydroxide and water are added to the 'crude insoluble saccharin slurry', and the mixture is adjusted to a specific pH value. After that, the mixture undergoes several crystallisation, filtration and separation steps to isolate the crystallised sodium saccharin (E 954 (ii)). During this process, no organic solvents are used, but only water. Saccharin (E 954 (i)) is then produced by dissolving the sodium saccharin (E 954 (ii)) in water and adding sulfuric acid to acidify it. The material is then collected by filtration (Documentation provided to EFSA n. 8).

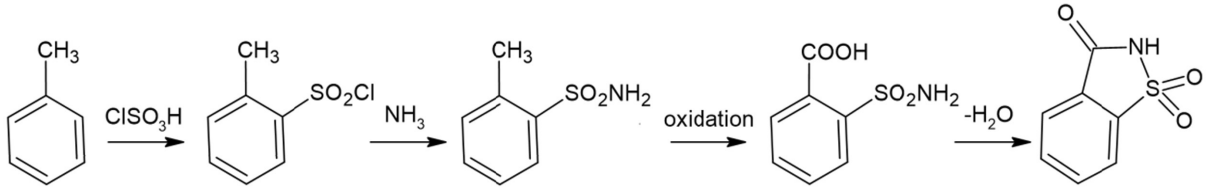


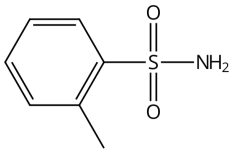
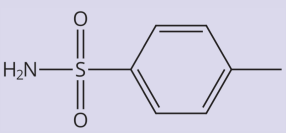
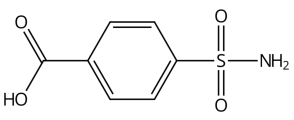
FIGURE 2 Reaction sequence resulting in the formation of E 954 (i), according to the Remsen-Fahlberg process.

Despite the fact that none of the IBOs uses the Maumee process to manufacture E 954, general information was provided for this manufacturing process by the IBOs. Two different starting materials may be used, i.e. phthalic anhydride or methyl anthranilate. Phthalic anhydride reacts with ammonia to form phthalimide, and by Hoffman degradation with bromine and sodium hydroxide, to form anthranilic acid. The acid reacts with methanol to form methyl anthranilate. Methyl anthranilate is diazotised to form o-carbomethoxybenzenediazonium chloride, by adding sodium nitrite under acidic conditions. Sulfonation followed by oxidation yields o-carbomethoxybenzenesulfonyl chloride. Amidation of this sulfonyl chloride, followed by acidification, forms insoluble acid saccharin. Subsequent addition of sodium hydroxide or calcium hydroxide produces the more soluble sodium or calcium salt, respectively (Documentation provided to EFSA n. 1, 7).

3.1.3 | Potential impurities and analytical data provided

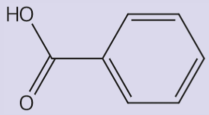
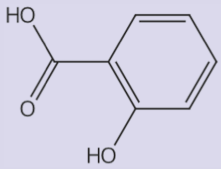
The potential impurities of saccharin and its sodium, potassium and calcium salts (E 954), reported in the EU regulation, are presented in Table 2, along with the analytical results provided by the IBOs.

TABLE 2 Chemical structures of potential impurities in E 954.

Chemical name	CAS no.	Structure	Analytical results provided by the IBOs	
			Sodium saccharin (E 954 (ii))	Saccharin (E 954 (i))
o-Toluenesulfonamide	88-19-7		1 batch: 6 mg/kg[a] 5 batches: < 0.3 (LOD) mg/kg in all[b] 5 batches: 2, 4, 5, 5, 5 mg/kg[c]	5 batches: 0.99, < 0.3 (LOD), < 0.3, 0.2, 2.83 mg/kg[b] 5 batches: < 0.1 (LOD) mg/kg in all[c]
p-Toluenesulfonamide	70-55-3		1 batch: 5 mg/kg[a] 5 batches: < 0.3 (LOD) mg/kg in all[b] 5 batches: < 0.1 (LOD) mg/kg in all[c]	5 batches: 0.08, < 0.3 (LOD), < 0.3, < 0.3, < 0.3 mg/kg[b] 5 batches: < 0.1 (LOD) mg/kg in all[c]
Benzoic acid p-sulfonamide	138-41-0		5 batches: < 25 (reporting limit) mg/kg in all[c]	5 batches: < 25 (reporting limit) mg/kg in all[c]

(Continues)

TABLE 2 (Continued)

Chemical name	CAS no.	Structure	Analytical results provided by the IBOs	
			Sodium saccharin (E 954 (ii))	Saccharin (E 954 (i))
Benzoic acid	65-85-0		1 batch: No precipitate or violet colour appears[a,d] 5 batches: No precipitate or violet colour appears[b,d] 5 batches: No precipitate or violet colour appears[c,d]	5 batches: No precipitate or violet colour appears[b,d] 5 batches: No precipitate or violet colour appears[c,d]
Salicylic acid	69-72-7			

[a]Documentation provided to EFSA n. 3.
[b]Documentation provided to EFSA n. 7.
[c]Documentation provided to EFSA n. 8 and 9.
[d]Analysed as benzoic and salicylic acid, according to the EU specifications.

Following the EFSA calls for data,[17,18,19] four IBOs provided data and information to support the re-evaluation of E 954 (Documentation provided to EFSA n. 1, 2, 3, 4, 5, 6, 7, 8, 9). EFSA requested analytical data on saccharin (E 954 (i)), sodium saccharin (E 954 (ii)), calcium saccharin (E 954 (iii)) and potassium saccharin (E 954 (iv)) in commercial batches of the food additive. Technical data on commercial batches of saccharin (E 954 (i)) and sodium saccharin (E 954 (ii)), manufactured with the Remsen-Fahlberg process supported by certificates of analysis, were provided by the IBOs. No analytical data for calcium saccharin (E 954 (iii)) and potassium saccharin (E 954 (iv)) were provided. In addition, no analytical data for E 954 manufactured with the Maumee process were provided by the IBOs.

The Panel noted that the current EU specifications only include impurities derived from the Remsen-Fahlberg process. When saccharins (E 954) are manufactured with the Maumee process, other potential impurities can be present (National Research Council/National Academy of Sciences, 1978; Riggin et al., 1978; Radford et al., 1985), such as methyl anthranilate, N-methyl saccharin and 2-chlorobenzamide mentioned by one IBO.

The Panel, also, noted that the two IBOs that manufacture E 954 have confirmed that they only use the Remsen-Fahlberg process (Documentation provided to EFSA n. 4, 11, 12). No IBO claimed to use the Maumee process for manufacturing commercially available saccharins following a specific EFSA call for technical data for E 954.

3.1.3.1 | *Organic impurities of E 954 associated with the Remsen-Fahlberg process*

One IBO provided analytical data on five batches of saccharin (E 954 (i)), in which the purity ranged from 99.88% to 99.91% and its impurities o-toluenesulfonamide (below the limit of detection (LOD) of 0.1 mg/kg in all batches), p-toluenesulfonamide (below the LOD of 0.1 mg/kg in all batches) and benzoic acid p-sulfonamide (below the reporting limit of 25 mg/kg in all batches). In addition, benzoic and salicylic acid and readily carbonisable substances were tested in the same five batches and were compliant with the EU specifications (Documentation provided to EFSA n. 8, 9). Another IBO provided analytical data on five batches of saccharin (E 954 (i)) in which the purity ranged from 99.0% to 100.5%, and its impurities o-toluenesulfonamide (up to 2.83 mg/kg) and p-toluenesulfonamide (was not detected at a LOD of 0.3 mg/kg in all batches). The Panel noted that in one batch, p-toluenesulfonamide was quantified as 0.08 mg/kg. The Panel, also, noted that the reported LOD and limit of quantification (LOQ) of o- and p-toluenesulfonamide are the same. In addition, benzoic and salicylic acid were tested in the same five batches and were compliant with the EU specifications (Documentation provided to EFSA n. 7).

One IBO provided analytical data on one batch of sodium saccharin (E 954 (ii)) with the purity of 99.49% and its impurities o-toluenesulfonamide (6 mg/kg) and p-toluenesulfonamide (5 mg/kg). In addition, benzoic and salicylic acid and readily carbonisable substances were tested in the same batch and were compliant with the EU specifications (Documentation provided to EFSA n. 3). Analytical data on five batches of sodium saccharin (E 954 (ii)) were provided by another IBO, in which the purity ranged from 99.4% to 99.7% and its impurities o-toluenesulfonamide (below the LOD of 0.3 mg/kg in all batches) and p-toluenesulfonamide (below the LOD of 0.3 mg/kg in all batches). In addition, benzoic and salicylic acid were tested in the same five batches and were compliant with the EU specifications (Documentation provided to EFSA n. 7). Another IBO provided analytical data on five batches of sodium saccharin (E 954 (ii)) in which the purity ranged from 99.82% to 100.07%, and its impurities o-toluenesulfonamide (5 mg/kg in three batches, 4 mg/kg in one batch and 2 mg/kg in one batch), p-toluenesulfonamide (below the LOD of 0.1 mg/kg in all batches) and benzoic acid p-sulfonamide (below the reporting

[17]https://www.efsa.europa.eu/en/data/call/170621.
[18]https://www.efsa.europa.eu/en/consultations/call/call-technical-data-sweeteners-authorised-food-additives-eu.
[19]https://www.efsa.europa.eu/en/call/call-technical-data-saccharin-and-its-sodium-potassium-and-calcium-salts-e-954.

limit of 25 mg/kg in all batches). In addition, benzoic and salicylic acid and readily carbonisable substances were tested in the same five batches and were compliant with the EU specifications (Documentation provided to EFSA n. 8, 9).

Considering the potential impurities in saccharins (E 954) manufactured with the Remsen-Fahlberg process, the Panel made the following observations.

The Panel noted that the analytical data provided for o-toluenesulfonamide, p-toluenesulfonamide and benzoic acid p-sulfonamide show that these impurities are below the limits indicated in the EU specifications (not more than 10 mg/kg expressed on dry weight basis for o- and p-toluenesulfonamide, and not more than 25 mg/kg expressed on dry weight basis for benzoic acid p-sulfonamide). The potential exposure to these impurities, considering the worst-case scenario that they are present at a concentration corresponding to their respective specification limits, resulting from the use of E 954 was assessed using the Threshold of Toxicological Concern (TTC) approach and no safety concern was raised (see Appendix E).

The Panel noted that in the EU regulation, the specification for benzoic and salicylic acid is based on the absence of precipitation or formation of colour using a prescribed test method of unknown sensitivity (see Table 1). No quantitative data were provided by the IBOs for those two substances. In this respect, the Panel noted that benzoic acid is an authorised food additive (E 210). The Panel also noted that salicylic acid is classified as Repr. 2 (H361d Suspected of damaging the unborn child). Considering that the purity of saccharin and its sodium, potassium and calcium salts (E 954) is not less than 99% on the anhydrous basis, the maximum amount of salicylic acid, considering that other impurities are not present (worst-case scenario), would be 1%, resulting in a potential exposure to salicylic acid from the use of E 954 up to 77 μg/kg bw per day. When comparing this value with the lowest no observed adverse effect level (NOAEL) for salicylic acid of 75 mg/kg bw per day (EFSA CEP Panel, 2020), the Panel noted that the margin of exposure (MOE) would be at least 1000, and no safety concern was raised. Even if the purity of calcium saccharin was not less than 95% on the anhydrous basis, as indicated currently in the EU specifications, the exposure to this impurity would not be of concern (see Appendix E).

The Panel noted that the specification for 'readily carbonisable substances' in the EU regulation is expressed as 'Absent'. The JECFA specifications (JECFA, 2006) for this parameter are based on formation of colour using a prescribed test method (see Table 1). The data were provided as 'passes test' by the IBOs in five batches of saccharin (E 954 (i)) and five batches of sodium saccharin (E 954 (ii)), without indicating the sensitivity of the method. The Panel noted that the parameter 'readily carbonisable substances' is unspecific, and therefore not needed in the EU specifications of E 954.

3.1.3.2 | *Organic impurities of E 954 associated with the Maumee process*

No analytical data on E 954 manufactured with the Maumee process have been submitted by the IBOs.

Analytical data on other potential impurities in saccharin (E 954 (i)) and sodium saccharin (E 954 (ii)), not expected to be derived from the Remsen-Fahlberg process, were provided by the IBOs, for samples manufactured with the Remsen-Fahlberg process (Appendix F). The Panel noted that some of those impurities have been reported to be derived from the Maumee process (National Research Council/National Academy of Sciences, 1978; Riggin et al., 1978).

One IBO provided analytical data on five batches of saccharin (E 954 (i)), in which the purity ranged from 99.88% to 99.91% and on the levels of potential impurities, such as 1,2-benzisotiazoline-3-one (BIT), methyl benzoate, methyl anthranilate, phthalates and their derivatives, benzamide, 2-chlorobenzamide, N-methyl saccharin, 2-chlorobenzene sulfonamide and dibutyl phthalate (all below the LOD of 0.1 mg/kg in all batches) (Documentation provided to EFSA n. 8, 9). Another IBO provided analytical data on five batches of saccharin (E 954 (i)), in which the purity ranged from 99.0% to 100.5% on the levels of the potential impurity 1,2-benzisotiazoline-3-one (below the LOD of 0.1 mg/kg in all batches) (Documentation provided to EFSA n. 7).

One IBO provided analytical data on one batch of sodium saccharin (E 954 (ii)), with purity 100% and on the potential impurities '2- and 4-methyl (saccharin) benzoate', which were not detected. The Panel noted that the LOD of the applied analytical method, liquid chromatography-mass spectrometry (HPLC–MS), was not provided by the IBO (Documentation provided to EFSA n. 4). Analytical data on five batches of sodium saccharin (E 954 (ii)) in which the purity ranged from 99.4% to 99.7% were provided by another IBO, on the levels of the potential impurity 1,2-benzisotiazoline-3-one (below the LOD of 0.1 mg/kg in all batches) (Documentation provided to EFSA n. 7). In addition, another IBO provided analytical data on five batches of sodium saccharin (E 954 (ii)) in which the purity ranged from 99.82% to 100.07%, and on the levels of potential impurities, such as 1,2-benzisotiazoline-3-one, methyl benzoate, methyl anthranilate, phthalates and their derivatives, benzamide, 2-chlorobenzamide, N-methyl saccharin, 2-chlorobenzene sulfonamide and dibutyl phthalate (all below the LOD of 0.1 mg/kg in all batches) (Documentation provided to EFSA n. 8, 9).

No data on those potential impurities were provided for products manufactured with the Maumee process, and thus, the Panel cannot confirm their presence in the food additive produced with the Maumee process.

In the absence of analytical data on the potential impurities associated with the Maumee process in the food additives, the exposure to the impurities attributed to the Maumee process could not be calculated and a risk assessment was, therefore, not performed. The Panel noted that potential genotoxic concern was identified for one impurity associated with the Maumee process (see Section 3.5.3.1).

Since only IBOs manufacturing saccharins (E 954) using the Remsen-Fahlberg process expressed an interest following the EFSA call for technical data,[20] and no analytical data on potential impurities were provided for products manufactured

[20] https://www.efsa.europa.eu/en/call/call-technical-data-saccharin-and-its-sodium-potassium-and-calcium-salts-e-954.

with the Maumee process, the Panel could only evaluate saccharins (E 954) manufactured with the Remsen-Fahlberg process. This is reflected in the proposal of the Panel for a definition of the food additives, restricted to the manufacturing with the Remsen-Fahlberg process (see Section 3.7).

3.1.3.3 | *Other purity parameters included in the EU specifications of E 954*

One IBO provided analytical data on five batches of saccharin (E 954 (i)) on the melting point (229.4–229.7°C) and sulfated ash (0.03%–0.10%), which were compliant with the EU specifications (Documentation provided to EFSA n. 8, 9). Another IBO provided analytical data on five batches of saccharin (E 954 (i)) on sulfated ash (0.03%–0.07%), which were compliant with the EU specifications (Documentation provided to EFSA n. 7).

One IBO provided analytical data on one batch of sodium saccharin (E 954 (ii)) on the melting point (228.5–229.6°C) and loss on drying (14.35%), which were compliant with the EU specifications (Documentation provided to EFSA n. 3). Another IBO provided analytical data on five batches of sodium saccharin (E 954 (ii)) on the melting point (229.5–229.6°C) and sulfated ash (0.03–0.10%), which were compliant with the EU specifications (Documentation provided to EFSA n. 8, 9).

3.1.3.4 | *Toxic elements*

With regard to toxic elements, the IBOs provided analytical data on the levels of lead (Pb) and arsenic (As) in commercial batches of saccharin (E 954 (i)) and sodium saccharin (E 954 (ii)), while one of these IBOs provided analytical data on the levels of cadmium (Cd) and mercury (Hg) in one batch of sodium saccharin (E 954 (ii)) (Documentation provided to EFSA n. 3, 4, 7, 8, 9). Details of the analytical data provided are available in Appendix G. The Panel noted that no information on the lowest technologically achievable levels for the toxic elements in E 954 was provided by the IBOs, as requested in the relevant call for data.

The Panel performed the risk assessment that would result if arsenic, and lead were present in E 954 at the current maximum limits in the EU specifications and at the lowest reported LOD or reporting limit by the IBOs.

The outcome of the risk assessment for this scenario is presented in Table G.1, Appendix G. Considering the results of the exposure to the toxic element Pb, the Panel noted that its presence in E 954 at the current specification limit value would not give rise to concern. In the case of As, the Panel noted that its presence in E 954 at the current specification limit value would lead to a MOE around 3, which indicates the need to lower the maximum limit for As in the EU specifications.

The Panel noted that the analytical data provided for Pb and As were reporting limits, rather than actual measured values, or below the LODs. Although, no quantitative data were provided, the Panel is of the view that the current EU specification limits should be lowered.

The Panel noted that the choice of maximum limits for toxic elements in the EU specifications is in the remit of risk management.

3.1.3.5 | *Microbiological parameters and residual solvents*

The Panel noted that, according to Commission Regulation (EU) No 231/2012, no microbiological specifications are currently set for E 954. Noting the nature of the food additive (E 954) and the various steps of the manufacturing process (see Section 3.1.2), the Panel considered that a microbiological contamination is unlikely. This was also supported by the microbiological data submitted by the IBOs (Documentation provided to EFSA no. 7, 8, 9). Hence, the Panel did not consider it necessary to recommend inclusion of microbiological criteria in the EU specifications for E 954.

One IBO provided analytical data on five batches of saccharin (E 954 (i)) and five batches of sodium saccharin (E 954 (ii)), in which residual solvents were not detected (Documentation provided to EFSA no. 8, 9). Another IBO provided analytical data on five batches of saccharin (E 954 (i)) and five batches of sodium saccharin (E 954 (ii)), in which residual solvents were also not detected (Documentation provided to EFSA no. 7). The Panel noted that neither the identity of the solvents nor the respective LODs and LOQs were provided by the IBOs.

3.1.4 | Solubility

One IBO provided a solubility curve of sodium saccharin (E 954 (ii)) at different temperatures (5–25°C). The solubility values varied from ~45% to 58% (Documentation provided to EFSA n. 3). Another IBO mentioned the solubility value of 1049 g/L for sodium saccharin (E 954 (ii)) and 3.2 g/L for saccharin (E 954 (i)) (Documentation provided to EFSA n. 5).

Data on the solubility or dissolution rate of saccharin and its sodium, potassium and calcium salts (E 954 (i-iv)), to address the safety of the fraction of small particles, including nanoparticles were further requested by EFSA, as proposed in the Guidance on Particle-TR (EFSA Scientific Committee, 2021), but no respective information was received.

The Panel noted, that according to the literature (Pearson, 2001), the solubility of saccharin (E 954 (i)), sodium saccharin (E 954 (ii)) and calcium saccharin (E 954 (iii)) is reported as 0.2, 100 and 37 g/100 g water, respectively, at 20°C; 0.4, 143, 82 g/100 g water, respectively, at 35°C, 0.7, 187 and 127 g/100 g water, respectively, at 50°C; and 1.3, 254 and 202 g/100 g water, respectively, at 75°C.

Noting the information above, along with other aspects (see Section 3.7), the Panel considered that there is no concern with regard to the potential presence of small particles, including nanoparticles, in saccharin (E 954 (i)) and its sodium (E 954 (ii)) and calcium (E 954 (iii)) salts at the reported uses and use levels and that these food additives can be assessed following the conventional risk assessment, i.e. EFSA Guidance for submission for food additive evaluations (EFSA ANS Panel, 2012).

3.1.5 | Particle size

One IBO provided a laser diffraction (LD) analysis of one batch of sodium saccharin (E 954 (ii)) and one batch of saccharin (E 954 (i)) (Documentation provided to EFSA n. 6).

The Panel noted that LD analysis is not considered a proper method to investigate the presence of nanosized particles as it does not provide information on the size of the constituent particles as required by the Guidance on Particle-TR and is prone to errors for polydisperse materials (Rauscher et al., 2019; Mech, Rauscher, Babick, et al., 2020; Mech, Rauscher, Rasmussen, et al., 2020).

EFSA further requested data on the particle size distribution of saccharin and its sodium, potassium and calcium salts (E 954 (i-iv)), to address the safety of the fraction of small particles, including nanoparticles, as proposed in the Guidance on Particle-TR (EFSA Scientific Committee, 2021), but no respective information was received.

3.1.6 | Methods of analysis in food

One IBO made reference to the analytical method mentioned in the JECFA Combined Compendium of Food Additive Specifications[21] for the impurities toluenesulfonamides in saccharin samples, in which gas chromatography with a flame-ionisation detector (GC-FID) is used (Documentation provided to EFSA n. 2). Another IBO proposed a high-performance liquid chromatography – mass spectrometry (HPLC–MS) analytical method to quantify the potential impurity 'methyl (saccharin) benzoate' in saccharin (E 954 (i)) samples (Documentation provided to EFSA n. 4). Another IBO referred to methods of analysis of different physicochemical parameters, such as melting point, derivation to fluorescent substances and to salicylic acid, appearance (clarity and colour) of the solution, acidity or alkalinity, and of other parameters, such moisture and possible impurities of saccharin and sodium saccharin, such as benzoic and salicylic acid, readily carbonisable substances, arsenic, selenium, lead, residual solvents, emphasising to the identification and quantification of toluenesulfonamides with GC-FID (Documentation provided to EFSA n. 8).

The Panel noted that, in all cases, the analytical methods refer to the identification and quantification of impurities of saccharins and other parameters and not of the saccharin and its salts per se.

Several publications on the development of analytical methods for saccharins (E 954) detection and determination, or simultaneous determination of different sweeteners in food and beverage matrices were retrieved by EFSA following a literature search (see Appendix A).

The main analytical method in the literature used for the determination of saccharins (E 954) is HPLC. HPLC-ELSD (evaporative light-scattering detection) was used to determine saccharins and other sweeteners in soft drinks and canned or bottled fruits (Buchgraber & Wasik, 2009), while HPLC-UV (ultraviolet) was used to separate different sweeteners, including saccharins, from each other and their degradation products (e.g. phenylalanine) (George et al., 2010), and to determine saccharins in vinegar samples (Cheng et al., 2020) and chocolate products (Petrova & Christova-Bagdassarian, 2020). Saccharins were also quantified using isotope dilution liquid chromatography mass spectrometry (ID-LC/MS) in tea drinks (Lee et al., 2011), HPLC-DAD (diode-array detection) in milk, dairy, white spirits, cola-type soft drinks and several other products (Trandafir et al., 2009; Song et al., 2010; Hou et al., 2015; Sezgin et al., 2021; Ma et al., 2020; Kim et al., 2020; Székelyhidi et al., 2023), liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS) in the fermented product kimchi (Kim et al., 2018), beverages (Krmela et al., 2020), wine (Neves et al., 2021), dairy products (Detry et al., 2022) and tabletop sweeteners (Nicoluci et al., 2023), UHPLC-CAD (charged aerosol detection technology) in sugar-free drinks (Cheng et al., 2024), HPTLC (high-performance thin layer chromatography) with densitometry and surface enhanced Raman spectroscopy in beverage samples (Chen et al., 2022) and pressurised liquid extraction (PLE) and liquid chromatography-high resolution mass spectrometry (LC-HRMS) in fish samples (Núñez et al., 2017). Aredes et al. (2022) compared the methods voltammetry, titrimetry, gravimetry and HPLC-DAD to detect saccharins in commercial samples. Other researchers separated saccharins from other sweetener mixtures and quantified them in sugar substitute tablets, soft drinks and confectionary products using capillary electrophoresis with contactless conductivity detector (CE-C^4D) (Bergamo et al., 2011; Stojkovic et al., 2013), and in sugar substitute products and fruit juice powders using UV-vis measurements and partial least squares (PLS) (Llamas et al., 2008).

[21] JECFA Combined Compendium of Food Additive Specifications (Volume 4), 2005.

3.1.7 | Stability of the substances and reaction and fate in food

One IBO provided literature data, derived from Pearson (2001), to support that saccharins (E 954) present high stability in aqueous solutions over a wide pH range (Documentation provided to EFSA n. 2). Degarmo et al. (1952) studied the hydrolytic stability of saccharin (E 954 (i)) solutions at four pH values (2, 3.3, 7, 8). The concentration of saccharin (E 954 (i)) in the solutions, measured with an UV absorption method, was essentially unchanged after heating for 1 h at 100, 125 and 150°C at pH 3.3, 7 and 8. However, at pH 2, the saccharin loss was 2.9% at 100°C, 8.5% at 125°C and 18.6% at 150°C. The Panel noted that such conditions do not reflect the normal conditions of use of saccharin (E 954 (i)). In another study mentioned by Pearson (2001), saccharin (E 954 (i)) was tested at various pH values (2, 3, 4, 5) and temperatures (20, 40, 80°C), for a time period of 5 months. According to the authors, only under severe experimental conditions of high temperature, high and low pH, over an extended period does saccharin hydrolyse to a measurable extent. The hydrolysis products detected were 2-sulfobenzoic acid and 2-sulfamoylbenzoic acid.

Another IBO studied the stability of saccharin (E 954 (i)) and sodium saccharin (E 954 (ii)) in powder form (17°C to 29°C, RH (relative humidity) % 58–71), at the time points of 0, 9, 12, 18, 24, 36, 48 and 60 months (5 years). The purity of saccharin (E 954 (i)) varied from 99.89% to 99.91%, while the purity of sodium saccharin (E 954 (ii)) varied from 99.89% to 99.94%. No degradation was observed in the tested time periods (Documentation provided to EFSA n. 6, 9).

Another IBO provided data on the stability of saccharin (E 954 (i)) in cola formulations (pH 3), stored at 40°C up to 1 year. Residual saccharin was analysed by HPLC (no information on the detector used was provided). The saccharin loss was 9% on the first month, 25% after 3 months and 68% after 1 year (Documentation provided to EFSA n. 13).

EFSA requested more data on the stability of saccharin and its sodium, potassium and calcium salts (E 954 (i-iv)) during storage as a powder and incorporated in different food types, but no respective information was received.

Following a literature search performed by EFSA, the Panel retrieved data on the stability of saccharin. According to DuBois (2012), who also reports the stability study of Degarmo et al. (1952), the stability of saccharin (E 954 (i)) was also studied at 120°C, at the pH values of 3.3, 7 and 9 and the time of 27 and 219 h (~ 1 and ~ 9 days, respectively). Only after 9 days, and especially in the lowest pH conditions (3.3), substantial degradation was observed (69%). The Panel noted that these conditions do not reflect the normal conditions of use of saccharin (E 954 (i)).

The Panel noted that, based on the submitted information on the stability of saccharins, E 954 is expected to be stable in food under the normal conditions of use, in accordance with the authorised uses of E 954.

3.2 | Authorised uses and use levels

Maximum levels of saccharins (E 954) have been defined in Annex II, Part E, to Regulation (EC) No 1333/2008[22] on food additives, as amended. In this opinion, these levels are called maximum permitted levels (MPLs).

Currently, saccharins (E 954) are authorised food additives in the EU in 34 food categories (FCs) (corresponding to 46 authorised uses) with MPLs ranging from 50 to 3000 mg/kg and at *quantum satis* (*QS*) in three food categories (FC 11.4 Table Top Sweeteners in liquid, powder and tablet form). All MPLs for saccharin and its sodium, potassium and calcium salts (E 954) are concentrations expressed as the free imide.

Table 3 lists the food categories with their restrictions/exceptions that are permitted to contain added saccharins and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

TABLE 3 MPLs of saccharins (E 954) in foods according to Annex II to Regulation (EC) No 1333/2008.

Food category number	Food category name	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	Only energy-reduced products or with no added sugar	100[a]
03	Edible ices	Only energy-reduced or with no added sugar	100[a]
04.2.2	Fruit and vegetables in vinegar, oil or brine	Only sweet–sour preserves of fruit and vegetables	160[a]
04.2.3	Canned or bottled fruit and vegetables	Only fruit energy-reduced or with no added sugar	200[a]
04.2.4.1	Fruit and vegetable preparations excluding compote	Only seaweed based fish roe analogues	50[a]
04.2.4.1	Fruit and vegetable preparations excluding compote	Only energy-reduced	200[a]
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EC	Only energy-reduced jams, jellies and marmalades	200[a]

[22]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	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	Only energy-reduced jams, jellies and *marmalades*	200[a]
04.2.5.3	Other similar fruit or vegetable spreads	Only energy-reduced fruit or vegetable spreads and dried-fruit-based sandwich spreads, energy-reduced or with no added sugar	200[a]
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	Only energy-reduced or with no added sugar	500[a]
05.2	Other confectionery including breath refreshening microsweets	Only cocoa, milk, dried fruit or fat based sandwich spreads, energy-reduced or with no added sugar	200[a]
05.2	Other confectionery including breath refreshening microsweets	Only starch based confectionery energy reduced or with no added sugar	300[a]
05.2	Other confectionery including breath refreshening microsweets	Only confectionery with no added sugar	500[a]
05.2	Other confectionery including breath refreshening microsweets	Only cocoa or dried fruit based, energy reduced or with no added sugar	500[a]
05.2	Other confectionery including breath refreshening microsweets	Only breath-freshening micro-sweets, with no added sugar	3000[a]
05.3	Chewing gum	Only with no added sugar	1200[a]
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only sauces	160[a]
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only starch-based confectionery energy reduced or with no added sugar	300[a]
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only cocoa or dried fruit based, energy reduced or with no added sugar	500[a]
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only confectionery with no added sugar	500[a]
06.3	Breakfast cereals	Only breakfast cereals with a fibre content of more than 15%, and containing at least 20% bran, energy reduced or with no added sugar	100[a]
07.2	Fine bakery wares	Only cornets and wafers, for ice-cream, with no added sugar	800[a]
07.2	Fine bakery wares	Only essoblaten – wafer paper	800[a]
09.2	Processed fish and fishery products including molluscs and crustaceans	Only sweet–sour preserves and semi-preserves of fish and marinades of fish, crustaceans and molluscs	160[a]
11.4.1	Table top sweeteners in liquid form		*quantum satis*
11.4.2	Table top sweeteners in powder form		*quantum satis*
11.4.3	Table top sweeteners in tablets		*quantum satis*
12.4	Mustard		320[a]
12.5	Soups and broths	Only energy-reduced soups	110[a]
12.6	Sauces		160[a]
12.7	Salads and savoury-based sandwich spreads	Only *Feinkostsalat*	160[a]
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		200[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)		240[a]
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Only energy-reduced or with no added sugar	80[a]
14.1.4	Flavoured drinks	Only energy reduced or with no added sugar	80[a]
14.1.4	Flavoured drinks	Only 'gaseosa' energy reduced or with no added sugar	100[a]

(Continues)

TABLE 3 (Continued)

Food category number	Food category name	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
14.2.1	Beer and malt beverages	Only alcohol-free beer or with an alcohol content not exceeding 1.2% vol; 'Bière de table/Tafelbier/Table beer' (original wort content less than 6%) except 'Obergäriges Einfachbier'; Beers with a minimum acidity of 30 milli-equivalents expressed as NaOH; Brown beers of the 'oud bruin' type	80[a]
14.2.3	Cider and perry		80[a]
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol		80[a]
15.1	Potato-, cereal-, flour- or starch-based snacks		100[a]
15.2	Processed nuts		100[a]
16	Desserts excluding products covered in category 1, 3 and 4	Only energy-reduced or with no added sugar	100[a]
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children		500[a]
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children	Only food supplements in chewable form	1200[a]
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children		80[a]
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children	Only food supplements in syrup form	1200[a]

Abbreviation: MPL, maximum permitted level.

[a]Maximum usable levels are expressed in free imide.

Saccharins (E 954) are not authorised according to Annex III of Regulation (EC) No 1333/2008.

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 realistic exposure assessment, especially for those food additives with an MPL at *QS* in one or more food categories.

To obtain concentration data, EFSA issued a public call for data[23,24] (use levels and/or analytical data) on saccharins (E 954) 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.

In response to this public call, information on use levels of saccharins (E 954) in foods and beverages was made available to EFSA by four industry stakeholders by 2 October 2018 through the batch 7 call for data (Documentation provided to EFSA n. 15–18).

Analytical data on saccharins (E 954) in foods and beverages submitted to EFSA by 17 Member States, the United Kingdom and one non-EU European country, and extracted in September 2023 were available for the present exposure assessment.

Reported use levels in foods of saccharins (E 954)

Industry provided EFSA with 20 use levels of saccharins (E 954) in foods and beverages for seven out of the 46 authorised uses, corresponding to seven out of the 34 authorised food categories[25] according to Annex II to Regulation (EC) No 1333/2008 (Table 3).

The use levels of saccharins (E 954) were provided by European Dairy Association (EDA), Food Drink Europe (FDE), European Fruit Juice Association (AIJN) and Food Supplement Europe (FSE) (Documentation provided to EFSA n. 15–18).

[23]https://wayback.archive-it.org/12090/20180625074455/http://www.efsa.europa.eu/sites/default/files/engage/180122.pdf.

[24]https://www.efsa.europa.eu/en/call/open-call-food-additive-occurrence-data-food-and-beverages-intended-human-consumption-2.

[25]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.

Most of the use levels (14 of the 20) were expressed as sodium saccharin and therefore the concentrations were converted by the Panel to free imide by applying a conversion factor of 0.76 (see Section 3.1.1). The remaining six use levels were expressed as the free imide.

The Panel noted that industry indicated that three use levels for the FC 15.1 'Potato-, cereal-, flour- or starch-based snacks' referred to niche products. Since analytical levels were also available for this food category, the Panel did not use these reported use levels in the exposure assessment, but used the analytical data instead (EFSA, 2020b).

The data set available for the exposure assessment comprised 17 use levels for six authorised food categories.

Annex A, Table A1 summarises the reported use levels of saccharins (E 954) in foods and beverages.

Analytical results of saccharins (E 954) provided by Member States

In total, 44,749 analytical results of saccharins (E 954) were reported to EFSA by 17 EU Member States, the United Kingdom and one non-EU European country (Montenegro) (Figure 3). The major contributor to the collection of analytical results was Germany which reported 74% of data.

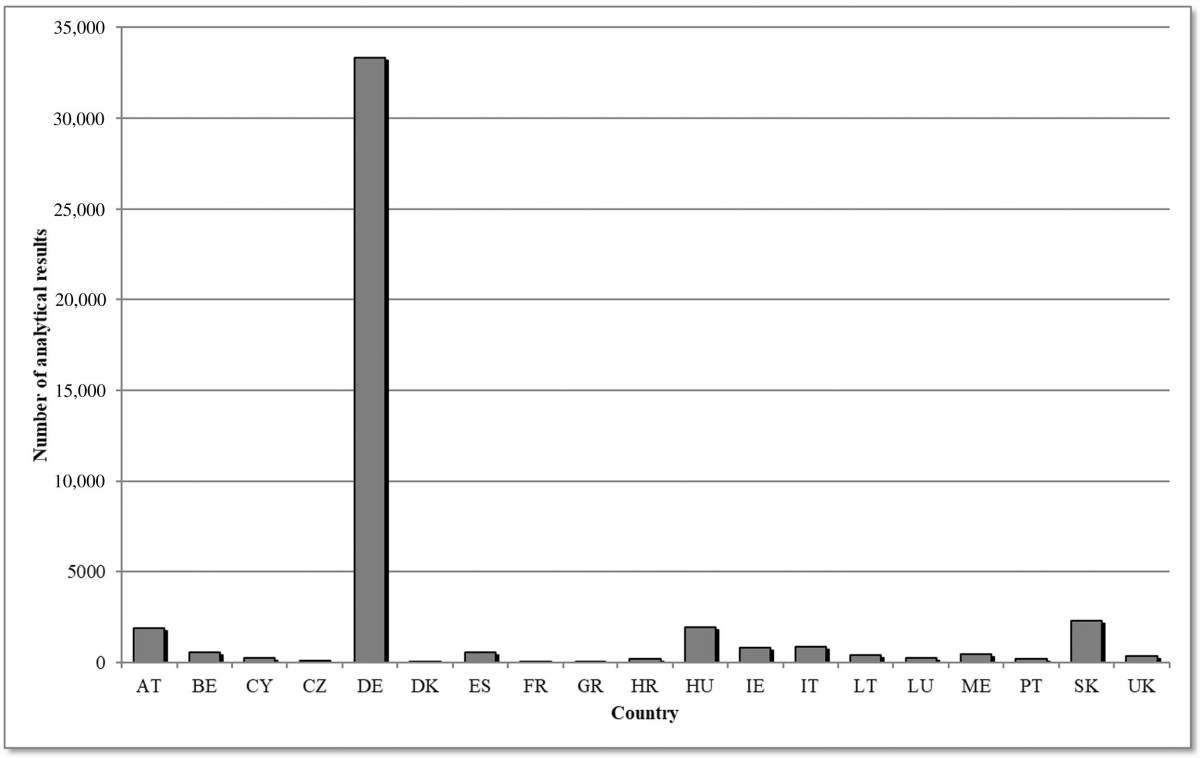


FIGURE 3 Distribution of analytical results reported for saccharins (E 954) across different European countries (initial data set, not cleaned). AT: Austria; BE: Belgium; CY: Cyprus; CZ: Czechia; DE: Germany; DK: Denmark; ES: Spain; FR: France; GR: Greece; HR: Croatia; HU: Hungary; IE: Ireland; IT: Italy; LT: Lithuania; LU: Luxembourg; ME: Montenegro; PT: Portugal; SK: Slovakia; UK: the United Kingdom.[26]

These data were mainly for FC 12.7 'Salads and savoury based sandwich spreads' and FC 14.1.4 'Flavoured drinks'. In total, 1406 analytical results referred to drinking water (well water and bottled water; FC 14.1.1) with almost all results ($n = 1389$) being left-censored. Foods and beverages were sampled between 2004 and 2022.

Most of the reported concentrations were expressed as free imide. The concentrations expressed as sodium saccharin or calcium saccharin were converted to free imide by applying a conversion factor of 0.76 or 0.78, respectively (see Section 3.1.1). For the data with no information on how the concentration was expressed, it was assumed that the analytical result was expressed as free imide since this is the conservative assumption and (as described above) this was also the most frequent reporting basis when the basis was known.

A total of 33,608 analytical results were either reported as non-detected or non-quantified. These results were not considered in the exposure assessment (EFSA, 2020b).

The Panel considered that data for foods and beverages sampled before 2013 ($n = 3262$) are outdated and were therefore excluded from the assessment.

A number of the remaining data did not fulfil the inclusion criteria and were not further considered, including analytical results reported with no further clarification on chemical structure ($n = 77$), data identified by the data provider as potential

[26] Analytical data included in the assessment were submitted to EFSA when the UK was a member of the EU.

errors ($n = 23$), data reported by a non-EU country ($n = 8$), duplicated data ($n = 4$), data excluded due to inconsistency between the values reported and the LOQs claimed ($n = 32$) and data obtained through suspect sampling ($n = 143$)[27] (EFSA).

Analytical results with either unspecified classification of the food (i.e. processed food and vegetables, $n = 75$) or with unclear physical form (i.e. table top sweeteners and food supplements, $n = 192$) were also excluded.

The Panel noted that 344 quantified analytical results were reported in food categories in which saccharins (E 954) are not authorised for direct addition according to Annex II of Regulation (EC) No 1333/2008, including unflavoured pasteurised cream (FC 01.6.1), other creams (FC 01.6.3), unripened cheese (FC 01.7.1), meat products (FC 08.3), heat–treated meat products (FC 08.3.2), fish roe (FC 09.3), seasoning and condiments (FC 12.2.2), water (FC 14.1.1), wine (FC 14.2.2), aromatised wine-based products (FC 14.2.7) and processed foods not covered by categories 1–17, excluding foods for infants (FC 18). These results were not considered in the exposure assessment.

Also, 463 analytical results for authorised food categories were above the MPL and were not considered. These results were for fruit and vegetables in vinegar, oil or brine (FC 04.2.2), jam, jellies and marmalades and sweetened chestnut puree (FC 04.2.5.2), cocoa and chocolate products (FC 05.1), fine bakery wares (FC 07.2), processed fish and fishery products (FC 09.2), sauces (FC 12.6), salads and savoury based sandwich spreads (FC 12.7), dietary foods for special medical purposes (FC 13.2), dietary foods for weight control diets intended to replace total daily food intake or an individual meal (FC 13.3), fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products (FC 14.1.3), flavoured drinks (FC 14.1.4), desserts (FC 16) and food supplements supplied in solid and liquid form (FC 17.1 and 17.2). The Panel assumed that the majority of these results were for food products in powder/concentrated forms, which should be diluted before consumption. This was specifically the case for FCs 13.2, 13.3, 14.1.4 and 17.1. Since it is likely that, in these cases, no MPLs are exceeded after dilution, an additional exposure scenario considering the analytical data above the MPL was not performed.

In addition, six analytical results (including data on FC 05.2 Other confectionery including breath freshening microsweets and FC 14.1.4 Flavoured drinks) below the MPL but referring to very specific restrictions that are not referenced in the EFSA Comprehensive database were also not included in the exposure assessment.

Overall, 6512 analytical results for saccharins (E 954) in foods and beverages were available for the exposure assessment corresponding to 30 food categories out of the 34 in which saccharins (E 954) are authorised as a food additive (Annex II to Regulation No 1333/2008).

Details on the analytical results 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

Mintel's Global New Products Database (GNPD) is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 3.8 million food and beverage products of which more than 1200,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 out of its 27 member countries and Norway presented in the Mintel GNPD.[28]

For this opinion, Mintel's GNPD[29] was used for checking the labelling of food and beverage products and food supplements for saccharins (E 954) within the EU's food market as the database contains the required ingredient information on the label.

According to Mintel's GNPD, saccharins (E 954) were labelled on 5461 products between January 2018 and November 2023. These products belong mainly to 'Nuts' ($n = 763$), 'Vitamins & Dietary Supplements' ($n = 569$), 'Fruit Snacks' ($n = 517$) and 'Carbonated Soft Drinks' ($n = 472$).

Annex A, Table A3 lists the percentages of the food products labelled to contain saccharins (E 954) out of the total number of food products per food subcategory according to Mintel's GNPD food classification. The percentages ranged from less than 0.1% in many food subcategories to 20.1% in Mintel's GNPD food subcategory 'Artificial Sweeteners', followed by 'Beverage Mixes' with 3.9%. The overall percentage of foods labelled to contain saccharins (E 954) was 0.42%. However, these percentages do not consider the market share of the products listed per food subcategory.

Annex A, Table A3 also contains the list of corresponding food categories according to Annex II to Regulation (EC) No 1333/2008. The information from Mintel's GNPD indicated use of saccharins (E 954) in two authorised food categories (i.e. FC 06.3 'Breakfast cereals' via subcategories 'Hot cereals' and 'Cold cereals' and FC 15.2 'Processed nuts' via sub-category 'Nuts'), for which no use levels/analytical data were reported to EFSA. The Panel considered that the impact of not including these two food categories into the exposure assessment is low; foods belonging to FC 06.3 'Breakfast cereals' are widely consumed but only 13 food products contain saccharins (E 954) according to Mintel's GNPD, and for FC 15.2 'Processed nuts', more than 700 food products were indicated to contain the sweetener, but foods belonging to this food category are not widely consumed.

The Panel noted that for a few food categories in which the use of saccharin and its sodium, potassium and calcium salts (E 954) is not authorised, foods were found labelled to contain this sweetener (e.g. bread and rolls (FC 07.1), meat products (FC 08.3) and seasonings and condiments (FC 14.2.2)). However, the number of non-authorised food products labelled to contain the sweetener was low (up to 34 out of 54,087 food items for meat products). As a one-to-one linkage between

[27]Suspect sampling' is defined as a selection of an individual product or establishment in order to confirm or reject a suspicion of non-conformity (EFSA).

[28]Missing Cyprus, Luxembourg and Malta.

[29]http://www.gnpd.com/sinatra/home/.

Mintel's GNPD food subcategories and the food categories according to Annex II to Regulation No 1333/2008 was not possible, these results should be considered indicative.

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.[30] 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 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 4). Not all Member States provided consumption information for all population groups, and in some cases, food consumption data from more than one consumption survey of one country were available. In most cases, when, for one country and age class, different dietary surveys were available, 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 saccharin and its sodium, potassium and calcium salts (E 954).

Population	Age range	EU member states with food consumption surveys covering more than one 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, 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, 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, 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, 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, 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 are codified according to the FoodEx2 classification system (EFSA, 2015b). 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 and 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 saccharins (E 954) 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).

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

Food categories considered for the exposure assessment of saccharins (E 954)

Food categories for which concentration data of saccharins (E 954) 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, 2015b).

Facets were used to identify eating events referring to foods reported to contain sweeteners (i.e. energy reduced or with no added sugar) and to foods related to the specific restrictions/exceptions as defined in the legislation for the use of saccharins (E 954) (see details in Annex A, Table A5). Facets were not used to identify relevant eating events for FCs 11.4 Table-top sweeteners and 05.3 Chewing gum, for gum drops in FC 05.2 Other confectionery including breath refreshening microsweets, for energy drinks in FC 14.1.4 Flavoured drinks, and for vitamin and mineral supplements in FC 17 Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children (EFSA, 2020b).

As FC 17 Food supplements does not consider food supplements for infants and toddlers as defined in the legislation, the exposure estimates of saccharins (E 954) for these two population groups do not include the exposure via food supplements.

Eating occasions belonging to FCs 13.2 Dietary foods for special medical purposes and 13.3 Dietary foods for weight control diets intended to replace total daily food intake or an individual meal were reclassified under food categories in accordance with their main component (e.g. gluten-free pasta reclassified as pasta).

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 in overestimation/underestimation of the exposure via eight 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, 06.3 Breakfast cereals, 12.7 Salads and savoury based sandwich spreads, 14.1.4 Flavoured drinks, 14.2.1 Beer and malt beverages, 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 saccharins (E 954) for consumers of 'gaseosa', falling within the FC 14.1.4. 'Gaseosa' is a specific type of flavoured drink that is not referenced in the EFSA Comprehensive database. Due to this, the MPL for other flavoured drinks within FC 14.1.4, which is lower than the one set for 'gaseosa', was considered in the exposure assessment. In addition, FC 04.2.5.1 Extra jam and extra jelly as defined by Directive 2001/113/EC cannot be distinguished from FC 04.2.5.2 Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC in the Comprehensive Database. Therefore, consumption of foods belonging to these food categories was considered in the exposure assessment under the general category of jam.

Overall, out of the 34 food categories in which saccharins (E 954) is authorised, 31 food categories were included in the *regulatory maximum level exposure scenario* (28 food categories with an MPL and the highest reliable percentile of reported analytical level for FCs 11.4.1, 11.4.2 and 11.4.3 ('Table-top sweeteners')). For the refined scenarios (i.e. *refined regulatory maximum level exposure assessment scenario* and *refined brand-loyal exposure assessment scenario*), 29 food categories were included. Compared to the *regulatory maximum level exposure scenario*, FC 06.3 Breakfast cereals and FC 15.2 Processed nuts were not included in the refined exposure scenarios due to lack of concentration data.

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

3.4 | Exposure estimates

The Panel considered appropriate, in the remit of the re-evaluation of sweeteners, to estimate the chronic exposure to saccharins (E 954) (EFSA, 2020b). As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011), dietary surveys with only one day per subject were not considered as they are not adequate to assess repeated exposure. Similarly, subjects who participated only one 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 saccharins (E 954), 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, 2020b).

Chronic dietary exposure was calculated by combining MPLs/concentration levels of saccharins (E 954) in each food with the average daily consumption for 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 estimates based on dietary surveys/population groups with less than six consumers and P95 estimates with less than 60 observations are not presented (EFSA, 2011).

In this evaluation, as stated in section 5.2.3 in the protocol (EFSA, 2020b), the dietary exposure was assessed for consumers only of at least one food category that could contain saccharins (E 954)[31] for all scenarios. 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.

Additionally, the exposure to saccharins (E 954) 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 and more explanation is given in Section 3.4.1.

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 saccharins (E 954), the MPLs used in the assessment are listed in Table A5 of Annex A. For the three food categories of 11.4 Table-top sweetener in which saccharins (E 954) is 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 saccharins (E 954) 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 the panel. In this refined scenario, it was assumed that a consumer is exposed long-term to saccharins (E 954) 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, 2020b).

Annex A, Table A5 summarises the concentration levels of (E 954) 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 is different ($n = 31$ and 29, respectively) with different facets considered (see EFSA, 2020b), the underlying populations of consumers only are not the same. For this reason, the Panel considered it appropriate to also perform a *refined regulatory maximum level exposure assessment scenario* based on the same population group as included in the *refined brand-loyal exposure assessment scenario*.

As in the *refined brand-loyal exposure assessment scenario,* the *refined regulatory maximum level exposure assessment scenario* considers only those food categories for which use levels or analytical data were provided to the Panel (Annex A, Table A5). In this scenario, it is assumed that a consumer is exposed long term to saccharins (E 954) present at the MPL/highest reliable percentile for these food categories, instead of at use level/analytical level as in the *refined brand-loyal exposure assessment scenario*.

3.4.1 | Dietary exposure to saccharins expressed as free imide

Table 5 summarises the estimated dietary exposure to saccharins (E 954), expressed as saccharin free imide, from its use as food additives in six population groups (Table 2) according to three exposure scenarios among consumers only of at least one food category containing saccharins (E 954).

[31]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.

TABLE 5 Summary of chronic dietary exposure to saccharins (E 954), expressed as free imide, from their use 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 saccharins (E 954) (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]	0.1–0.5 (11)	0.1–1.1 (15)	0.1–0.9 (19)	0.1–0.5 (21)	0.1–0.1 (22)	0.03–0.9 (23)
• 95th percentile[c]	0.3–1.3 (3)	0.3–3.6 (14)	0.4–2.5 (19)	0.2–1.5 (20)	0.3–3.7 (22)	0.2–4.6 (22)
Refined regulatory maximum level exposure assessment scenario						
• Mean[b]	0.06–1.1 (5)	0.2–1.3 (13)	0.1–0.9 (19)	0.1–0.7 (21)	0.1–1.5 (22)	0.03–2.1 (21)
• 95th percentile[c]	0.4 (1)	0.6–4.1 (4)	0.7–2.9 (15)	0.4–2.3 (14)	0.2–7.5 (18)	0.2–7.8 (13)
Refined brand-loyal exposure assessment scenario						
• Mean[b]	0.03–1.03 (5)	0.1–1.2 (13)	0.02–0.7 (19)	0.01–0.7 (21)	0.01–1.4 (22)	0.02–2.1 (21)
• 95th percentile[c]	0.2 (1)	0.3–3.6 (4)	0.3–2.2 (15)	0.4–2.2 (14)	0.2–7.4 (18)	0.2–7.7 (13)

[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 classes up to and including 5 consumers may not represent the population group and are thus not included in this table.

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

For the *regulatory maximum level exposure assessment scenario*, the highest mean exposure to saccharins (E 954) as free imide was estimated for toddlers (1.1 mg/kg bw per day) and the highest P95 for the elderly (4.6 mg/kg bw per day).

In the *refined regulatory maximum level* and *brand-loyal exposure assessment scenario*, the highest mean exposure to saccharins (E 954) as free imide was estimated for the elderly (2.1 mg/kg bw per day) and the highest P95 for the elderly (7.8 and 7.7 mg/kg bw per day, respectively). The Panel noted that these highest P95 exposure levels in the refined scenarios were observed for two dietary surveys for adults and the elderly due to a wide consumption of table-top sweeteners in tablet form.

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

Main food categories contributing to the exposure to saccharins (E 954) as free imide

In the *regulatory maximum level exposure assessment scenario*, the main contributing food category to the total mean exposure estimates was FC 14.1.1 Flavoured drinks for all population groups. In addition, FC 9.2 Processed fish and fishery products including molluscs and crustaceans and FC 12.6 Sauces contributed considerably for infants, toddlers and other children and FC 11.4.3 Table-top sweeteners in tablet form for adults and the elderly.

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 in tablet form and FC 17.1 Food supplements in solid form were important contributors to the exposure. A similar pattern was observed for the *refined brand-loyal exposure assessment scenario*.

For details on the contribution of each food category to the exposure to saccharins (E 954) as free imide in the three scenarios, see Tables A8, A9 and A10 in Annex A.

Dietary exposure for consumers of a single food category containing saccharins (E 954) as free imide

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, 2020b) is based on the population of consumers of any food category containing the sweetener, estimated for the mean and the 95th percentile. The consumers of specifically one food category (e.g. table-top sweeteners) are a subpopulation of the population of interest (i.e. consumers of any sweetened foods). For some food categories, the mean and high consumers (defined as the mean and the P95 estimates of exposure for that population) will fall outside (above) the mean and P95 limits of interest and these estimates should not be considered in isolation. The additional estimates are calculated to support the interpretation of the dietary exposure data and they are not 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, mean exposure for consumers-only of one food category was comparable to those for consumers-only of at least one food category (less than twice as high), 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 powder could exceed the mean dietary exposure for consumers of at least one food category by a factor of 2.7, and for FC 11.4.3. Table top sweeteners in tablets by a factor up to 4.6 (the exposure estimates up to 4.9 mg/kg bw day for saccharins (E 954) as free imide (Table A11 of Annex A). This twofold difference is also seen at the P95 exposure with the maximum level being 14.7 mg/kg bw day for saccharins (E 954) as free imide.

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 dietary exposure estimate.

Sources of uncertainties	Direction[a]
Consumption data	
Different methodologies/representativeness/underreporting/misreporting/no portion size standard/only a few days	+/–
Underreporting of food descriptors (facets) concerning the presence or potential presence of sweeteners	–[b]
Not considering some of the restrictions specified in the legislation	+
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	+/–
Limited occurrence data for majority of European countries and extrapolation of occurrence data to whole Europe	+/–
Uncertainty in possible national differences in use levels of food categories	+/–
For the analytical data with no information on how the concentration is expressed, it was assumed that the analytical result is expressed as the free imide	+
Refined regulatory maximum level exposure assessment and *brand-loyal scenario*: 29 food categories out of the 34 authorised to contain saccharins (E 954) were considered in the exposure assessment	–
Refined regulatory maximum level exposure assessment and *brand-loyal exposure assessment scenario*: 154 out of 157 food subcategories in Mintel, representing 86% of the products labelled with saccharins (E 954) were included in the current exposure assessment	–
Concentration levels/MPLs considered applicable to all foods for the majority of food categories, while the percentage of foods out of the total number of foods in a corresponding food subcategory were labelled to contain saccharins (E 954) in Mintel was maximally 20% (FCs 11.4)	+
Methodology	
Regulatory and *refined maximum level exposure assessment scenario*: – exposure calculations based on the MPL according to Annex II to Regulation (EC) No 1333/2008 and highest reliable percentile 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 survey covering only a few days to estimate high percentile (95th) of long-term (chronic) exposure	+

[a]+, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.
[b]Direction of the uncertainty is based on the assumption that the underlying population of consumers does not change.

The dietary exposure to saccharin as free imide estimated for the *refined regulatory maximum level exposure assessment* and *brand-loyal exposure assessment scenario* covers the majority of food categories in which the sweetener is authorised (29 out of the 34 authorised food categories and 154 out of 157 food subcategories in recorded in Mintel).

Even though not all possible uses may have been considered, the Panel acknowledged that the assumption that all foods within a food category for which concentrations were available contain saccharins (E 954) has resulted in an overestimation of the exposure to this sweetener. This observation is supported by the fact that a maximum of 20% of foods out of the total number of foods in a corresponding food subcategory were labelled to contain saccharins in Mintel.

Overall, the Panel considered the dietary exposure to saccharins (E 954) expressed as free imide, from their use as food additives, to be overestimated by the *regulatory maximum* and the two *refined exposure assessment scenarios* (i.e. *maximum* and *brand-loyal*).

The Panel considered the *refined brand-loyal exposure assessment scenario* the most appropriate exposure scenario for the risk assessment of saccharin as free imide.

3.4.3 | Concentrations of and dietary exposure to saccharin and its sodium, potassium and calcium salt (E 954) in the literature

A literature search was carried out to collect data on the levels of saccharins (E 954) in food and beverages, as well as to gather information on the dietary exposure estimates to this sweetener in Europe.

Occurrence data

Several European studies have analysed saccharin in food and beverages from regional markets. The results are discussed below. The literature overview showed that the form of saccharin mentioned was its sodium salt. Therefore, saccharin within this section refers to sodium saccharin. The reported mean concentrations are expressed based on quantified values.

Leclercq et al. (1999) showed that only a few foods contained saccharin: jam (1/1 sample), beverages (1/4 samples) and table-top sweeteners from the Italian market.

Arcella et al. (2004) reported that, in foods from the Italian market, saccharin was present almost exclusively in table-top sweeteners. In addition, the only sugar-free jam consumed during the survey contained saccharin.

Leth et al. (2008) reported saccharin in non-alcoholic beverages from the Danish market at a mean level of 24 mg/L in carbonated light drinks ($n = 27$) and 31 mg/L in non-carbonated light drinks ($n = 12$).

A study conducted by Huvaere et al. (2012) found saccharin concentrations in non-alcoholic drinks, table-top sweeteners, beers, marmalade and canned fruits from the Belgian market. The mean concentrations of saccharin in three tablet-top sweetener formulations were 103 g/kg as pellets ($n = 10$), 4 g/kg as powders ($n = 14$) and 6 g/kg as liquid ($n = 5$). In non-alcoholic drinks ($n = 46$), the mean saccharin level was reported at 31 mg/L, in beers ($n = 16$) at 13 mg/L, in marmalade at 135 mg/kg ($n = 3$) and in canned fruits at 168 mg/kg (n = 4).

In four Portuguese studies, saccharin was reported in non-alcoholic beverages (Lino & Pena, 2010; Diogo et al., 2013; Basílio et al., 2020; Silva et al., 2021). Lino and Pena (2010) analysed 25 soft drinks, 13 soft drinks based on mineral water and 10 nectars from the Portugal market and found saccharin in 24% of soft drinks at a mean level of 75 mg/L with a range from 55 to 89 mg/L ($n = 6$ quantified samples out of the 25 samples). The level of saccharin exceeded the MPL of 80 mg/L in one soft drink sample. Diogo et al. (2013) analysed four beverage types ($n = 78$), including 59 traditional soft drinks and drinks based on mineral waters, three energy drinks and 16 nectars. The concentration range in the four beverage types was from 3.2 to 80.5 mg/L. In Basílio et al. (2020), a total of 56 samples were analysed including 27 soft drinks, 10 soft drinks based on tea extracts, four soft drinks based on mineral waters, six sport/energy drinks and nine nectars. The range of concentrations of saccharin was from < LOQ – 101.8 mg/L. The level of saccharin exceeded the MPL of 80 mg/L in one nectar sample reported at 93.7 mg/L, in three soft drinks based on tea extracts, reported as 80.5, 82.7 and 101.8 mg/L and in at least one traditional soft drink at 85.4 mg/L. Silva et al. (2021) analysed soft drinks ($n = 68$) and grouped them as colas ($n = 16$), juice drinks ($n = 28$), iced teas ($n = 13$) and lemon-flavoured drinks ($n = 11$). Saccharin was found at concentrations ranging from 16 to 69 mg/L in all soft drink samples.

The concentration of saccharin 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 ($n = 54$). In four flavoured drinks, saccharin was found at a mean concentration of 40 mg/L, and in five jams at a mean concentration of 42 mg/kg. Of 14 samples of table-top sweetener formulations, saccharin was found in 50% of the samples at a mean concentration of 61 g/kg. Saccharin was found in 10 solid food supplements at a mean concentration of 1525 mg/kg which exceeded the MPL of 1200 mg/kg.

Thirty carbonated drink samples from the Romanian market were analysed (Oroian et al., 2013). The concentrations of saccharin ranged from 0.1 to 83.75 mg/L ($n = 16$). One sample exceeded the MPL of 80 mg/L.

In summary, the occurrence levels of saccharin in food and beverages reported in the above mentioned studies in Europe are comparable to the analytical data reported to EFSA (see Section 3.3.1).

Dietary Exposure

Exposure assessment described in the different studies below were performed according to Tier 2 and 3. Tier 1 is the theoretical approach using theoretical food consumption data and MPLs. Tier 2 and 3 are assessments at the individual member state level which combine national food consumption data with MPLs (Tier 2) and with actual use levels/analytical levels (Tier 3) (European Commission, 2001).

Saccharin exposure among 241 Italian teenagers aged 13–19 was assessed using 14-day dietary survey data collected from high school students in Rome in 1996, i.e. similar to Tier 2 and 3 (Leclercq et al., 1999). The mean dietary exposure to saccharin was 0.21 mg/kg bw per day among nine consumers, almost entirely from consumption of table-top sweeteners.

Saccharin exposure of French, insulin-dependent children was assessed using 5-day survey data in sugar-free foods and table-top sweeteners (Garnier-Sagne et al., 2001). The survey was conducted by members of the Aid for Young Diabetics Association. In total, 227 diabetic children responded to a randomly selected mailed questionnaire (112 girls and 115 boys). More than 76% of the population consumed products that could contain saccharin. The main contributor to saccharin exposure was non-alcoholic diet beverages. For the ages 2–6 years, 7–10 years, 11–14 years, 15–17 years and 18–20 years, the mean exposure to saccharin was up to 1.2 mg/kg bw per day with children aged 2–6 years having the highest exposure (Tier 2).

Arcella et al. (2004) estimated dietary exposure to high intense sweeteners including saccharin among Italian female teenagers living in Rome. The 12-day survey data were collected from students in 10 randomly selected high schools in the District of Rome (125 boys and 108 girls). The consumers-only mean dietary exposure to saccharin was 0.026 mg/kg bw per day and 95th percentile was 1.5 mg/kg bw per day. Among high consumers of sugar free soft drinks and table-top sweeteners, the mean exposure to saccharin was 0.037 and 0.125 mg/kg bw per day, respectively (Tier 3).

Based on measured saccharin concentrations in non-alcoholic beverages in the Danish market and data from the 7-day Danish Dietary Survey, dietary exposure to saccharin was estimated considering only soft drinks. The 90th percentiles of dietary exposure to saccharin in 3098 subjects aged 1–80 years, considering all samples of soft drinks or only the samples containing the sweetener, were 0.10 and 0.17 mg/kg bw per day, respectively (Leth et al., 2008) (Tier 3).

A Tier 3 assessment of saccharin exposure in the Belgian population (15 years and older) was performed by Huvaere et al. (2012), using concentrations in food and table-top sweeteners in the Belgian market and percentage of foods containing saccharin according to labelling. The 95th percentiles of exposure to saccharin of consumers only was 1.14 and 0.97 mg/kg bw per day for total population and diabetics, respectively.

In the Diogo et al. (2013), the highest estimated dietary exposure to saccharin was found to be from traditional soft drinks at 0.024 mg/kg bw per day.

Carvalho et al. (2022) estimated the exposure to saccharin 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 saccharins ranged from 0.17 to 1.11 mg/kg bw per day and from 0.46 to 2.26 mg/kg bw per day at the 95th percentile between the elderly and children, respectively. Considering MPLs only for food categories where saccharin (E 954) is labelled, exposure estimates were 0.01 mg/kg bw per day at the mean for all population groups and ranged from 0.03 mg/kg bw per day for children up to 0.08 mg/kg bw per day for adults at the 95th percentile. Using analytical data only for food categories where saccharin (E 954) is labelled, exposure estimates were 0.01 mg/kg bw per day at the mean for all population groups and ranged from 0.02 mg/kg bw per day for adolescents to 0.05 mg/kg bw per day for adults and the elderly at the 95th percentile.

Conclusion

It is not possible to directly compare dietary exposure estimates from the literature to those reported in this opinion. There are several reasons for this; in the current assessment, more food categories are considered, the approach is different (consumers only approach vs. whole population), maximum concentration data are used across all European countries and/or the population groups are different. Nonetheless, estimates from the current opinion tend to be in the same order of magnitude compared with the exposure estimates from literature.

3.5 | Biological and toxicological data

The biological and toxicological studies that were assessed as relevant and reliable 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 from the literature.

An evaluation of the risk of bias (RoB) was performed (Annex E1 and E2) and a weight of evidence (WoE) approach for the reliable studies was applied for each health outcome for both human and animal studies (Appendix A, Annexes E1 and E2). The animal and human studies are summarised in detail in Annex B. A narrative synthesis of the WoE analysis is reported in Section 3.5.4.

Studies on absorption, distribution, metabolism and excretion (ADME) were not subject to an RoB assessment but were evaluated independently by two experts. Information from mechanistic studies and studies considered not directly relevant for the derivation of a reference point available in the literature were included and described narratively, briefly discussing the outcomes (Appendix B).

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 the ADME studies provided by one IBO (Documentation provided to EFSA n. 2) were already considered and evaluated by the SCF or were among the publications retrieved in the systematic literature search (see Appendix A). In its evaluation of saccharin from 1977 (SCF, 1977), the Committee reported that several studies were performed investigating the metabolism of saccharin[32] in humans. These studies, dated as far back as 1905, showed that saccharin is rapidly excreted unchanged mainly in the urine. In additional

[32]The Panel noted that, after administration, all the salts of saccharin will dissociate in biological fluids to saccharin (as free imide), therefore, in this section the term 'saccharin' is used.

studies in monkeys, rats and several other species, metabolites were not identified. A study in rats was available to the SCF showing that in animals on a 'normal' diet fed both 1% and 5% labelled saccharin for up to 12 months, 'some' 90% of the dose was excreted within 24 h, the majority in urine (up to 80%) and the remaining 20% in faeces. Metabolites were not detected in the excreta. The same publication showed that no metabolites were detected in humans after the ingestion of 1 gram of saccharin daily for 21 days (SCF, 1977). In its evaluations from 1989 and 1995, the SCF did not address specifically the ADME of saccharin.

The ADME of saccharin has been evaluated also by JECFA (JECFA, 1993). In its evaluation, the Committee reported that the disposition of saccharin is influenced by its acidic properties (pKa of 2.2). Saccharin is more readily absorbed from the stomach of species with low pH (guinea pig – pH 1.4; rabbit – pH 1.9) than from those with a higher pH (rat – pH 4.2) (Ball, 1973; Minegishi et al., 1972). In the higher pH of the intestines, it is slowly absorbed. It is then rapidly eliminated in the urine. In the rat, the percentage of the administered dose recovered in the faeces ranged from 3% to 39% and in humans between 1% and 8%. Urine was identified as the principal route of elimination for saccharin after both oral and parenteral dosing. A gastrointestinal absorption of orally administered saccharin of 85% in humans was reported. JECFA concluded that according to the information available in a number of experiments in different species, including humans, saccharin is not metabolised.

The most relevant information on ADME of saccharin from studies previously evaluated is reported below.

Ball et al. (1977) reported that in pregnant rats radiolabelled saccharin (3-14C saccharin) was excreted unchanged in the urine following ingestion of 1 gram saccharin within 24 h. No change in the excretion pattern was observed when the radiolabelled saccharin was given for 21 days and studied on day 21. No metabolites could be found in the urine. Roughly 0.6% of the dose reached the fetus. Saccharin was not metabolised in vitro by liver microsomal preparations from rats.

Sweatman et al. (1981) performed a study in healthy male volunteers who were given an intravenous bolus of 10 mg/kg bw sodium saccharin. The terminal half-life was 70 min and the amount of saccharin recovered in the urine as the parent compound was 101.1 ± 3.6% of the dose. The results indicate that saccharin is not metabolised and is excreted by the kidneys. The renal clearance, being 310 ± 120 mL/min without probenecid, was reduced to 180 ± 70 mL/min by co-administration of probenecid, a blocker of tubular secretion. Thus, the mechanism of renal excretion is by glomerular filtration and tubular excretion. The volunteers also received 2 g of sodium saccharin by oral administration; 85% of the dose was absorbed, as determined by the recovery rate in the urine and by comparing the areas under the curve (AUCs) after oral and intravenous administration.

Cohen-Addad et al. (1986) measured the post-partum concentration of saccharin in the blood and urine of six women who had consumed saccharin in the last months of pregnancy. The concentration of saccharin was also determined in cord blood. Saccharin was found in the cord blood of all newborns indicating transplacental passage of saccharin (quantitative data not provided).

The data above show that saccharin is well absorbed in animal and man, it is not metabolised and its urinary excretion is fast. Saccharin can cross the placenta and the fetus can be exposed to the substance.

New relevant studies in humans identified in the literature are summarised below.

In the study from Zyba et al. (2021), 23 healthy non-pregnant women 18–45 years of age (body mass index (BMI) between 20 and 25 kg/m^2) were administered a single 20 gram oral dose of a peanut-based dietary supplement paste[33] with 10 mg (equal to 48 μmol) sodium saccharin (reported as food grade; 15% hydrated, BENEO GmbH). Participants were provided with a list of foods and products that contained saccharin and were asked to refrain from consuming any foods or products on the list for 3 days before and after the administration. Two participants left the study, for which the authors provided a plausible reason. On the day before and for 2 days following saccharin administration, 24 h urine samples were collected in 3 h fractions over the day and one 12-h fraction overnight. Urinary concentrations of saccharin were measured by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC/MS/MS).

Prior to administration of saccharin, urinary saccharin concentrations were determined to be 0.1 μmol/L (95% CI: 0.1–0.1 μmol/L). The mean urinary saccharin peak concentration was 33.7 μmol/L (95% CI: −7.5 - 65.0 μmol/L) between 0 and 4 h after administration. Thereafter, mean urinary saccharin concentrations declined and were close to baseline concentrations by 24 h. The mean cumulative urinary excretion of saccharin was 34.5 μmol (95% CI: −8.8 to 41.3 μmol) 48 h after consumption of saccharin, with 98.3% of this excreted within the first 24 h. The mean percentage dose of saccharin recovered in urine was 67.5% (95% CI: 56.4%–80.8%).

In Weinborn et al. (2021),[34] three children (12–24 months old) completed a small proof-of-concept study with the administration of saccharin and resveratrol, both evaluated as adherence markers for the consumption of the peanut-based dietary supplement paste. Because only spot urine samples were collected, the results are not informative for the kinetics of saccharin.

In the study from Sylvetsky et al. (2015), 20 participating women provided one sample of milk, without information on food consumption, and time point of obtaining the milk sample. One of the women declared that she was consuming

[33]Nutriset S.A.S., France; containing energy, protein, essential fatty acids, vitamins and minerals – provided to pregnant women, lactating women and young children in low-income countries to prevent undernutrition.

[34]Limited data from the study above (Zyba et al., 2021) appear to be included in Weinborn et al. (2021) (from the same group) but appear to contradict the data given in Zyba et al. (2021) in that baseline urine saccharin concentrations are reported to be 1909 ± 1325.63 nmol/L with the first 4 h collection saccharin concentrations reported to be 54,812.04 ± 43,587.44 nmol/L.

saccharin. Saccharin was measured with a specific method (UPLC/MS). In this publication data on LOQ, LOD and precision are not given. The authors report that five milk samples had a concentration of 0 ng/mL; 11 samples had a concentration below the LOQ, two of 0.01 ng/mL, 1 of 0.02 ng/mL and the sample from the woman consuming saccharin had a concentration of 1.42 ng/mL. The Panel considered that saccharin is transferred to breast milk in humans without further information on the milk/plasma ratio.

The study from Wilson et al. (1999) had two parts. In part 1, 22 volunteers (12 males, 10 females) were given doses of saccharin between 2.2 mg and 69.3 mg after saccharin-free food consumption for 3 days before and during exposure. Urine was collected and compliance was monitored by co-administration of para-amino-benzoic acid and measurement of this marker which is fully excreted in the urine. The full dose of saccharin was excreted in the urine within 24 h and excretion was linear with dose. The Panel considered that this study demonstrates that absorption of saccharin is not dose-dependent in the examined dose range (2.2–69.3 mg); that the half-life is low (about 4 h) and that saccharin is not metabolised.

In the second part of the study, urine was collected over 24 h from 188 volunteers (97 males, 91 females) who completed a dietary questionnaire covering the last 48 h. Compliance of urine sampling was monitored by co-administration of *para*-amino-benzoic acid. The reported intake by the questionnaire matched well with the urinary excretion according to the authors; however, the variability was higher than in the controlled part of the study (part 1, see above).

In Halasa et al. (2023), amniotic fluid was obtained from 12 pregnant women, eight samples in women undergoing caesarean section and in four women when amniocentesis was performed because of a medical indication. The gestational age was 20 weeks in one case and 33, 34 and 37 weeks in the other cases with amniocentesis. Saccharin concentration was measured with a specific and sensitive method (UPLC/MS) with an LOQ of 1 ng/mL. Saccharin concentrations between 0.7 and 55.9 ng/mL were measured in 8 of the 12 samples, in the remaining samples the concentrations were declared as undetectable. Separately, infant cord blood was collected from 15 cases; no information is available on the gestational week when the infant was born and on further anthropometric data. In 12 of the 15 samples, saccharin was detected in concentrations ranging from 0.9 to 2.7 ng/mL.

The Panel considered that the data show that saccharin passes through the placenta into the fetus. Given the absence of blood concentrations in the mothers, no transfer ratios can be calculated.

Stampe et al. (2022) investigated the concentration–time profile of saccharin 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 hypoglycaemic 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 saccharin, the plasma peak concentration (C_{max}) was 350.7 ng/mL and the time to reach the peak concentration (t_{max}) was 30 min. The milk C_{max} was 81.5 ng/mL and the milk t_{max} was 240 min. The ratio of AUCs (AUC milk/AUC plasma) was 38.91%.

The Panel noted both mean saccharin 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 t_{max} in plasma and milk were markedly different (0.5 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 saccharin in plasma and milk were independent of participant BMI or T1DM in mothers.

Based on their data, the authors calculated the number of saccharin-containing drinks needed for mothers to be exposed at a level of 5 mg/kg bw per day (the ADI set by SCF, 1995). They also calculated the number of saccharin-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

saccharin concentration was 101.8 ng/mL (95% CI: 71.8–131.7 ng/mL), and the mean fetal cord saccharin plasma concentration was 61.2 ng/mL (95% CI: 43.5–79.0 ng/mL) which gave a cord/maternal plasma concentration ratio of 0.65 (0.56–0.73). Foetal cord and maternal saccharin plasma concentrations were correlated (correlation coefficient of 0.56, range 0.38–0.73). The amniotic fluid concentration was 61.3 ng/mL (95% CI: 42.0–80.6 ng/mL) The mean cord plasma/amniotic concentration ratio was 1.09 but varied widely (95% CI: 0.62–1.57) and lacked any discernible correlation (95% CI: −0.37 to 0.67).

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

Summary and conclusion on ADME of saccharin in humans

The majority of the newly available data from humans were on fractionated urinary excretion. No data describing the concentration time profile in blood was obtained. The Panel considered that the data on urinary excretion demonstrate that most, if not all saccharin is absorbed when doses between 2.2 and 69.3 mg saccharin per person were administered orally. The Panel considered that saccharin is not metabolised, has a half-life of approximately 4 h and is primarily excreted into the urine. Saccharin also passes into breast milk and is capable of passing the placenta, as indicated by detection in amniotic fluid and cord blood samples and entering the foetal circulation.

3.5.2 | Animal toxicity

No data on acute toxicity were received from the interested parties and 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

Saccharins (E 954) were previously evaluated by the Scientific Committee on Food in 1977, 1985 and 1995 (SCF, 1977, 1985, 1995).

Concerning genotoxicity, in the last opinion, the Committee noted that sodium saccharin was found weakly positive in several in vitro studies for induction of chromosomal aberrations. However, these responses were only seen at high concentrations, and it was considered probable that they were attributable to non-specific effects such as ionic imbalances. The Committee also noted that there were conflicting data from in vivo studies, but the interpretation of these findings was uncertain due to the possible presence of impurities or contaminants from the manufacture in test material (SCF, 1995).

Based on these findings, the Committee concluded that '*Considering the weight of evidence from all the genotoxicity studies, the Committee considers that these indicate saccharin is not a direct acting genotoxin. Support for this view comes also from the fact that it has been shown to be a carcinogen at only one site in only one sex of one species of animal, whereas genotoxic carcinogens tend to be active at more than one site and/or in more than one sex or species*.'

The Panel noted that, when the approach outlined in the revised protocol for the appraisal of the genotoxicity studies is considered (EFSA, 2020a, 2020b; EFSA FAF Panel, 2023), all the genotoxicity studies previously evaluated by the SCF would be considered of low relevance because of their insufficient reliability and/or because of low relevance of the test system.

A systematic literature search covering the period after the last SCF opinion (timeframe: 01/01/1994–02/02/2024) identified 23 in vitro and 5 in vivo studies on saccharins (E 954) genotoxicity. These studies are described in detail in Annex C. No new studies were provided through the public call for data. The main findings from studies with sufficient (high or limited) relevance are summarised below (Tables 7 and 8). Studies evaluated as of low relevance because of important flaws or limitations are not considered in the WoE evaluation and excluded from Tables 7 and 8.

TABLE 7 Summary table of the in vitro genotoxicity studies on saccharins (E 954).

Test system/test object	Exposure conditions (concentration/testing conditions)	Information on the characteristics of the test substance	Result	Reliability/ comments[35]	Relevance of the test system/ relevance of the result[36]	Reference
Salmonella/ microsome (Ames) test with TA97a and TA100 strains	Plate-incorporation assay in Salmonella Typhimurium TA 97a and TA 100 strains both in the absence and presence of the S9 mix. Concentrations: 10, 100, 250, 500, 1000 and 10,000 µg/plate.	Saccharin was purchased from Sigma Chemical Co.; purity not stated	Negative	Reliable with restrictions	High/ Limited	Bandyopadhyay et al. (2008)
Salmonella/ microsome (Ames) test with TA98 and TA100 strains	Concentrations 2500, 5000, 10,000, 20,000, 40,000 µg/plate, with and without metabolic activation	Saccharin was purchased from Harmann Pharmaceuticals Pvt. Ltd., India; purity not stated	Negative	Reliable with restrictions	High/ Limited	Najam et al. (2017)
Rat hepatocyte DNA repair analysis in vitro. Hepatocytes from male F344 and SD rats	Hepatocytes were exposed 20 h to four saccharin concentrations: 2.5×10^{-2} M; 5×10^{-2} M, 1×10^{-1} M, 2×10^{-1} M DNA repair was evaluated by autoradiography in 50 nuclei randomly selected and counted per slide, and three slides per dose level.	Saccharin was purchased from Sigma Chemical Co., purity not stated	Negative	Reliable with restrictions	Limited/ Limited	Jeffrey and Williams (2000)
In vitro comet assay in HepG2, CHL/IU and TK6 cell lines	4 h treatment Concentrations were based on toxicity measured with the MTT assay. The IC_{20} was applied as the high dose, together with two lower doses obtained by serial 2-fold dilution. Final concentrations tested were: HepG2 2.3, 4.6, 9.2 mM; CHL/IU 0.7, 1.3, 2.6 mM; TK6 1.2, 2.4, 4.8 mM	Saccharin was purchased from Sigma Chemical Co.; purity not stated	Negative	Reliable without restrictions	Limited/ Limited	Hong et al. (2018)
In vitro micronucleus test in Chinese hamster lung cell line (CHL) and p53-competent TK6 human lymphoblastoid cells	Treatments: 3 h with a 21 h recovery phase (with and without S9); 24 h treatment in the absence of S9. Concentration up to 10 mM.	Purchased from Sigma, UK (purity not stated)	Negative	Reliable with restrictions	High/ Limited	Fowler et al., 2012
High Throughput Flow Cytometric In Vitro Micronucleus Assay in TK6 Cells	Cells were treated with saccharin up to the maximum dose of 1 mM for 3 h with S9 and 24 h without S9. Micronuclei were scored by flow cytometry in 10,000–12,000 cells per concentration.	Saccharin was purchased from Sigma-Aldrich; purity not stated.	Negative	Reliable with restrictions	Limited/ Limited	Thougaard et al. (2014)

(Continues)

[35]See Annex C for comments.

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

TABLE 7 (Continued)

Test system/test object	Exposure conditions (concentration/testing conditions)	Information on the characteristics of the test substance	Result	Reliability/ comments[35]	Relevance of the test system/ relevance of the result[36]	Reference
Forward mutation assay based on 5-fluorouracil (FU) resistance in Salmonella typhimurium TA100	Exposure for 2 h in liquid medium, with and without metabolic activation. Concentration range 10–5000 µg/mL.	Saccharin was purchased from Sigma Chemical Co.; purity not stated	Negative	Reliable with restrictions	Limited/ Limited	Miller et al. (2019)
The test method GreenScreen monitors genotoxin induced transcription of the *GADD45a* gene human lymphoblastoid cell line TK6 using a reporter gene encoding the Green Fluorescent Protein.	Saccharin was tested with and without metabolic activation up to the concentration 10 mM (2052 µg/L).	'Sourced at the highest purity available in the UK INo other information provided	Negative	Reliable with restrictions	Limited/ Limited	Birrell et al. (2010)
The test system is a novel high throughput genotoxicity screening assay based on the use of the human colon carcinoma cell line HCT116 expressing three reporter genes under transcriptional control of promoters of DNA damage inducible genes (p21, GADD153 and p53)	Maximum dose 1 mM, with and without metabolic activation	Identity, batch, purity: not stated	Negative	Reliable with restrictions	Limited/ Limited	Rajakrishna et al. (2014)
High-throughput luciferase gene reporter expression assay in the human U2OS cell line, detecting the activation of the p53 pathway (p53 CALUX) for genotoxicity and the Nrf2 pathway (Nrf2 CALUX) for oxidative stress	Cells were incubated for 24 h. Saccharin was applied in 9 serial dilutions in the range of 3×10^{-7} – 10^{-3} M.	Identity, batch, purity: not stated	Negative, both for p53 (with and without S9) and Nrf2 activation (only assayed without S9).	Reliable with restrictions	Limited/ Limited	van der Linden et al. (2014)
The test is based on the quantification of the phosphorylated histone H2AX (γH2AX) in HepG2 cells using a high-throughput technique based on Infrared Imaging Scanning	24 h treatment in the absence of metabolic activation. Maximum tested concentration 1 mM (no other details provided)	Saccharin from Sigma-Aldrich (purity not stated)	Negative	Reliable with restrictions	Limited/ Limited	Khoury et al. (2013)

TABLE 7 (Continued)

Test system/test object	Exposure conditions (concentration/testing conditions)	Information on the characteristics of the test substance	Result	Reliability/ comments[35]	Relevance of the test system/ relevance of the result[36]	Reference
Detection of phosphorylated γ-H2AX histone and cell cycle arrest in HepG2 cells using a high content screening method based on automated fluorescence imaging	1 h and 24 h treatment to 10 concentrations, up to 40 mM	Saccharin was purchased from Sigma-Aldrich; purity not stated	Negative	Reliable with restrictions	Limited/ Limited	Ando et al. (2014)
In vitro micronucleus assay in TK6 cell lines	0.625–10 mM tested in triplicate for 4 h without metabolic activation	Sodium saccharin purchased from Sigma–Aldrich Chemical Co.; purity not stated	Negative	Reliable with restrictions	High/ Limited	Allemang et al. (2021)
Gene expression analysis (Affymetrix) TGx DDI (DNA Damage Inducing) response	0.625–10 mM tested in triplicate for 4 h without metabolic activation	Sodium saccharin purchased from Sigma–Aldrich Chemical Co.; purity not stated	Negative	Reliable with restrictions	Limited/ Limited	Allemang et al. (2021)

TABLE 8 Summary table of the in vivo genotoxicity studies on saccharins (E 954).

Test system/test object	Exposure conditions (dose/testing conditions) (administration)	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of the test system/ relevance of the result	Reference
Micronucleus analysis in rat peripheral blood cells (reticulocytes). Male SD rats	Sodium saccharin administered at 0, 2500, 5000, 7500 mg/kg bw per day. Oral dosing by gavage. Blood samples were collected from the tail vein before and on five continuous days after treatments; all of which were analysed for micronuclei presence by both the manual (Giemsa staining) and FCM methods.	Sodium saccharin (purity not specified) from Macklin Biochemical Co., Ltd. (Shanghai, China)	Negative	Reliable without restrictions/	High/High	Chen et al. (2020)
Mutagenicity in *lac*I transgenic Big Blue™ rat. DNA from liver and bladder was examined. 200,000 plaque forming units were analysed from Liver (Bladder not specified)	Mutagenicity was assessed 14 days after the last of 10 daily exposures to saccharin. 4-aminobiphenyl (20 mg/kg) was used as a positive control. 10 rats per group fed sodium saccharin in the diet at 5%.	Sodium saccharin purchased from Sigma, Poole, UK; purity not stated	Negative in liver ($n = 7$ samples) or in the bladder (pooled samples)	Reliable with restrictions	High/ Limited	Turner et al. (2001)

(Continues)

TABLE 8 (Continued)

Test system/test object	Exposure conditions (dose/testing conditions) (administration)	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of the test system/ relevance of the result	Reference
In vivo comet assay in mice	Groups of 4 male ddY mice were administered once orally with either saccharin (given in olive oil) or sodium saccharin (given in saline) at 100, 1000 or 2000 mg/kg bw (limit dose). Mice were sacrificed 3 or 24 h after administration, and comet assays performed with 8 organs (stomach, colon, liver, kidney, bladder, lung, brain, bone marrow) using an in-house developed procedure based on the isolation of nuclei from tissue homogenates. 50 nuclei for each organ were examined for DNA damage, measured as DNA migration calculated as difference between whole comet length and comet head diameter.	Saccharin 98% purity and Sodium saccharin 99% purity were both tested. Obtained from Kanto Chemical Co. Inc., Tokyo, Japan	Saccharin: Positive in liver Negative in other tissues analysed. Sodium saccharin: Positive in stomach and colon (3 h). Negative in other tissues analysed	Reliable with restrictions	High/ Limited	Sasaki et al. (2002)

Summary and conclusion on genotoxicity

In the genotoxicity studies published after the last SCF opinion, saccharins (E 954) were tested with negative results in the following in vitro studies, all evaluated of limited relevance: Ames test with strains TA97a and TA100 (Bandyopadhyay et al., 2008) and with strains TA98 and TA100 (Najam et al., 2017); unscheduled DNA synthesis (UDS) in primary rat hepatocytes (Jeffrey & Williams, 2000); comet assays in rodent and human cell lines (Hong et al., 2018). In Allemang et al. (2021), an in vitro micronucleus assay in TK6 cell lines and gene expression analysis (Affymetrix) TGx DDI (DNA Damage Inducing) response on sodium saccharin were reported (high and limited relevance, respectively). Sodium saccharin was also included as reference non-genotoxic carcinogen[37] in the set of model compounds evaluated in several validation studies on newly developed, non-standard methods. In these validation exercises, sodium saccharin was uniformly evaluated as negative. Additionally available studies on saccharins included: a forward mutation assay in Salmonella (Miller et al., 2019), high throughput in vitro micronucleus assays (Fowler et al., 2012; Thougaard et al., 2014), gene reporter-based assays for the expression of DNA damage and/or oxidative stress pathways (Birrell et al., 2010; Rajakrishna et al., 2014; van der Linden et al., 2014) and screening methods for histone H2AX phosphorylation as marker of DNA *dsb* (Khoury et al., 2013; Ando et al., 2014).

In vivo studies published after the last SCF opinion, provided a negative micronucleus response in rat peripheral reticulocytes (Chen et al., 2020) and a negative response in the rat Big Blue mutagenicity assay (Turner et al., 2001): the Panel noted that this assay was in the liver and bladder, the available evidence on urinary excretion (see Section 3.5.1) provides sufficient evidence of exposure to these tissues. There was limited evidence of positive responses in the stomach and colon of mice in the comet assay (Sasaki et al., 2002). Other studies were of low relevance.

The Panel identified several limitations in the Sasaki et al. study (see Annex C), as noted in previous EFSA opinions (EFSA ANS Panel, 2009, 2013). Moreover, comet assay results are considered of lower relevance compared to tests detecting apical genetic endpoints (OECD, 2017). Based on the consistently negative results obtained in all other in vitro and in vivo genotoxicity studies, including robust in vivo micronucleus and transgenic rodent mutation assays, the Panel concluded that the newly available studies do not raise a concern for genotoxicity of saccharins (E 954), which concurs with the conclusion of the previous SCF opinion.

[37] In group 3 iii (*non-genotoxic carcinogen or carcinogenic by irrelevant (for humans) mechanism*) in the list of chemicals recommended by the European Centre for Validation of Alternative Methods (ECVAM) for validation of in vitro genotoxicity assays (Kirkland et al., 2008).

3.5.3.1 | *Genotoxicity assessment of saccharins (E 954) impurities*

As reported in Section 3.1.3, only IBOs manufacturing saccharins (E 954) using the Remsen-Fahlberg process expressed an interest following the EFSA call for technical data, and no analytical data on potential impurities were provided for products manufactured with the Maumee process. Considering that (i), in 1995, the SCF noted that there were conflicting reports from in vivo genotoxicity studies, but the interpretation of these findings was uncertain due to the possible presence of impurities or contaminants from the manufacture of test material, and (ii) that there is no information on the manufacturing process used in the production of the saccharins (E 954) tested in the available studies, the Panel decided to perform a genotoxicity assessment for potential impurities of saccharins (E 954) produced with either the Maumee or Remsen-Fahlberg processes. Among chemically identified impurities of saccharins (E 954) produced with either the Maumee or Remsen-Fahlberg processes, a few are anticipated not to raise safety concern for genotoxicity as approved for use as food additive (benzoic acid; EFSA ANS Panel, 2016) and food contact material (salicylic acid; EFSA CEP Panel, 2020). In addition, the Panel noted that dibutyl phthalate (DBP), reported as potential impurity of the Maumee process only, is part of the phthalates assessed by the Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel) for which a group TDI on a temporary basis was established (EFSA CEP Panel, 2019). The CEP Panel did not identify any concern for genotoxicity for this substance.

For the remaining impurities, genotoxicity data retrieved in the scientific literature were used as primary source for safety assessment. All studies were preliminary evaluated for their reliability and relevance of results, according to the EFSA evaluation criteria. The results evaluated of sufficient (i.e. high or limited) relevance are summarised in Table 9.

Negative results were reported in a limited bacterial reversion assay (Ames tests) and a limited micronucleus test in mouse bone marrow with o-toluene sulfonamide and p-toluene sulfonamide, and with o-sulfamoylbenzoic acid and benzoic acid p-sulfonamide (Eckhardt et al., 1980). Genotoxicity studies on benzamide, an inhibitor of the DNA repair enzyme, poly(ADP ribose) polymerase, indicate co-mutagenic and genotoxic potential, with the enhancement and delayed repair of MMS-induced single strand breaks, the enhancement of MNNG-induced UDS in vitro (Park et al., 1983), the induction of sister chromatid exchange (SCE) in rodent cells in vitro (Natarajan et al., 1981; Lindhal-Kiessing & Shall, 1987) and the formation of micronuclei in erythropoietic cells of mice treated orally (Chieli et al., 1987). For methyl anthranilate, no genotoxic activity was shown in a bacterial reversion (Ames) test (Mortekmans et al., 1986) and an in vitro UDS assay (Yoshimi et al., 1988). Methyl anthranilate was also previously evaluated by the EFSA AFC Panel in the framework of the evaluation of flavouring substances (FGE 84) and was considered to present 'no significant genotoxic potential' (EFSA AFC Panel, 2008).

An in silico assessment of the potential genotoxicity of saccharins (E 954) impurities was also performed using the QSAR Toolbox resource (version 4.4.1), see Appendix D. A panel of profilers for DNA binding and DNA damaging activity associated with mutagenicity endpoints (reverse mutation in the Ames test, in vitro chromosomal aberrations and micronuclei) and non-DNA binding (in vivo micronucleus by ISS and protein binding alert for chromosomal aberrations by OASIS) was applied.

The application of the OECD QSAR ToolBox did not identify alerts for DNA damage and in vitro genotoxicity in any of the chemical structures analysed. An alert for in vivo genotoxicity (H-acceptor-path3-Hacceptor) was identified by one profiler (in vivo MN by ISS) for N-methyl saccharin (CAS 15448-99-4) and 2- and 4-methy(saccharin)benzoate (no CAS available). This alert is related to the capacity to form non-covalent binding with DNA and/or proteins as the result of the presence of two bonded atoms connecting two hydrogen bond acceptors. However, the same alert was identified in the non-genotoxic saccharins, and the analysis of a large genotoxicity data set (> 9000 substances) indicated the absence of positive predictivity for this alert (PPV, positive predictive value 43%) (Pradeep et al., 2021), which therefore, was not taken in further consideration.

Another alert, related to the chromosome damaging activity secondary to protein acylation, was identified by the OASIS profiler for N-methyl saccharin, 2- and 4- methyl (saccharin) benzoates and 2-chlorobenzamide (CAS 609-66-5). The Panel considered that such interaction would support indirect mechanisms of genotoxicity, based on the interaction with targets other than DNA, which are thresholded and not of concern in case of impurities for which very low exposure levels are foreseen. Moreover, the Panel noted that predictions of genotoxicity using computational models should not be based on the use of a single model alone (EFSA PPR Panel, 2016) and that, in case of methyl saccharins, the predicted chromosome damaging activity is not supported by read across with the structurally related saccharin, which also bears the same alert. However, for 2-chlorobenzamide, the Panel also noted that this alert (chromosome damage via protein binding) is identified by the OASIS profiler in the structurally related benzamide, for which experimental evidence of chromosome damaging activity was available.

Based on the available experimental and in silico data, the Panel concluded that saccharins (E 954) impurities associated with the Remsen-Fahlberg process do not raise concern for genotoxicity. For the potential impurities associated with the Maumee process, a concern for genotoxicity was identified for benzamide, while the genotoxic potential of 2-chlorobenzamide could not be fully assessed.

TABLE 9 Genotoxicity studies on saccharins (E 954) impurities.

Test system	Exposure conditions	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of test system/result	Reference
Bacterial reverse mutation assay (Ames test) with Salmonella Typhimurium TA1535, TA1537, TA1538, TA98, TA100	Plate incorporation method; 10 doses from 270 to 18,000 µg/plate using standard (Vogel-Bonner) and modified (ZLM) medium, with and without S9	Sodium saccharin from Hoesch AG; OTS (o-toluene sulfonamide) from Priem & Co.; PTS (p-toluene sulfonamide) from Merck-Schuchardt AG; PSBA (p-sulfamoylbenzoic acid) from EGA-Chemie; OSBA (o-sulfamoylbenzoic acid) prepared in house	**Negative** In all assays The borderline increase in revertant colonies observed in strain TA98 only with a modified culture medium and at doses above the maximum recommended is not considered biologically relevant	Reliable with restrictions Limited protocol (set of tester strains) and reporting (only data with strain TA 98 reported in detail)	High/limited	Eckhardt et al. (1980)
Micronucleus test in mice	Two i.p. or p.o. administrations 24 h apart, with sacrifice 6 h after the second administration Dosing: saccharin 205, 410, 1025 mg/kg by i.p. and 1025 mg/kg p.o.; PTS 428 and 855 mg/kg by i.p. and 855 mg/kg p.o.; OSBA and PSBA 400 and 1000 mg/kg by i.p. and 1000 mg/kg p.o.	Sodium saccharin from Hoesch AG; OTS (o-toluene sulfonamide) from Priem & Co.; PTS (p-toluene sulfonamide) from Merck-Schuchardt AG; PSBA (p-sulfamoylbenzoic acid) from EGA-Chemie; OSBA (o-sulfamoylbenzoic acid) prepared in house		Reliable with restrictions Limited protocol (4 animals/group, treatment regimen no more recommended in TG 474)	High/limited	Eckhardt et al. (1980)
Sister chromatid exchange, unscheduled DNA synthesis (UDS) assay by autoradiography in Chinese hamster ovary cells DNA ssb by alkaline elution in HeLa S_3 cells	In the UDS assay, cells were treated for 24 h with 2–10^{-2}–2–10^{-4} M benzamide (BA), with or without 1 µM MNNG. For the SCE assay, cells were treated for 28–30 h with 10^{-3} –10^{-5} M BA with or without 10^{-8} M MNNG In the alkaline elution assay BA 5 mM was added 30 min after incubation with 0.3 mM MMS and ssb rejoining evaluated during the subsequent 9 h	Benzamide from Sigma	BA alone slightly increased UDS and significantly enhanced MNNG-induced UDS BA increased SCE at 10^{-4}–10^{-3} M BA enhanced the formation of ssb by MMS and delayed ssb rejoining	Reliable with restrictions Guidelines for the in vitro SCE and UDS assays (OECD TG 479 and 482) were not available at the date of the study, and no TG exists for the alkaline elution assay. However, this study is considered acceptable for the mechanistic information provided	Limited/limited	Park et al. (1983)
Sister chromatid exchange in Chinese hamster ovary cells	Benzamide was applied at 3 mM during either the first or second cell cycle or both, in the absence of metabolic activation. For sister chromatid differentiation cells were grown for one or two cell cycles in the presence of BrdUrd	Benzamide from Aldrich Europe	**Positive** At the single dose applied, benzamide increased 3–4 fold the baseline SCE rate observed in cells treated with BrdUrd	Reliable with restrictions The OECD TG for in vitro SCE (TG 479, adopted in 1986 and deleted in 2013) was not available at the date of the study, which is however considered acceptable	Limited/limited	Natarajan et al. (1981)

TABLE 9 (Continued)

Test system	Exposure conditions	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of test system/result	Reference
Gene mutation assay (hprt locus) in Chinese hamster ovary cells	Cells were treated for 27 h with 10 mM benzamide in presence and absence of BrdUrd (5 μM) in the medium No metabolic activation	Benzamide from Aldrich Europe	**Negative** No increase in hprt mutant frequency was observed. Cloning efficiency was only slightly decreased (87%). In the same conditions a 10-fold increase in SCE was observed	Reliable with restrictions The OECD TG for in vitro gene mutation in mammalian cells (TG 476, adopted in 1984) was not available at the date of the study, which is however considered acceptable	High/limited	Natarajan et al. (1981)
Sister chromatid exchange assay in mouse lymphoma LI210 cells	Benzamide (1 mM) was applied for 24 in normal medium or deficient of nicotinamide with BrdUrd (20 μM) for chromatid differentiation. No metabolic activation was used	Benzamide. No information on supplier and purity provided	**Positive** Benzamide induced a 3-fold increase in the SCE/cell rate. The effect was enhanced in nicotinamide-free medium	Reliable with restrictions The study is shortly described but adequately performed and acceptable	Limited/limited	Lindhal-Kiessing and Shall (1987)
Micronucleus test in bone marrow cells (PCE) of C57B1/6 female mice	Benzamide suspended in albuminated water was administered orally 30 and 6 h before sacrifice at 1.65 mmoles/kg bw (200 mg/kg bw) Micronuclei scored in 1000 PCE per animal (3 treated and 5 vehicle control) No positive control	Benzamide. Purchased by Aldrich Co. (USA) and recrystallised before use	**Positive** Mean micronucleus frequency increased (~3-fold) in treated mice compared to control	Reliable with restrictions The study used a very limited protocol, with less animal and scored cells than recommended	High/limited	Chieli et al. (1987)
Bacterial reversion assay (Ames test) with *S. typhimurium* strains TA1535, TA1537, TA98 and TA100	Preincubation assay with 33, 100, 333, 1000 and 1800 μg/plate with and without S9 Mix from Aroclor induced rats and Chinese hamsters. Strain and S9 specific positive controls included. Triplicate plating.	Methyl anthranilate Aldrich 99% pure Solvent DMSO	**Negative** No increase in revertant colonies in any strain, with or without S9	Reliable with restrictions The study was conducted overall in line with OECD TG 471 (except that a strain sensitive to oxidising agents (TA102/E.coli WP2) was not used as not recommended in the TG at the date of the study). This limitation is not considered to affect the relevance of the negative result, as mutagenic aromatic amines are efficiently detected with strains TA98 and TA100[38]	High/High	Mortekmans et al. (1986)

(Continues)

[38]Cross, K. p., & DeMarini, D. M. (2023). Analysis of the chemical structures and mutations detected by Salmonella TA98 and TA100. Mutation Research, 827, 111838.

TABLE 9 (Continued)

Test system	Exposure conditions	Information on the characteristics of the test substance	Result	Reliability/ comments	Relevance of test system/result	Reference
DNA repair test (UDS) in primary cultured rat hepatocytes	Concentration range 10e-6 to 10e-3 M–S9 Treatment 20 h 150 cells/concentration analysed by autoradiography in two experiments. 2-acetylaminofluorene positive control	Anthranilic acid methyl ester (methyl anthranilate) from Tokyo Kasei Chem. Co., purity not specified. Solvent DMSO	**Negative** No increase in grains/ nucleus or % UDS positive cells	Reliable with restrictions The study was conducted overall in line with OECD TG 482, which was withdrawn in 2013	Limited/limited The UDS assay detects DNA repair of bulky adducts by excision repair. This genotoxic MoA is relevant for primary aromatic amines, including the test item.	Yoshimi et al. (1988)

3.5.4 | Synthesis of systematically appraised evidence and weight of evidence

A total of 8629 references were screened based on title and abstract. These references included studies retrieved from the literature (timeframe: 01/01/1994 to 02/02/2024) as well as the pivotal study on which the derivation of the current ADI was based (full study report [Documentation provided to EFSA n. 14] in addition to an associated publication from Schoenig et al., 1985). No new studies were submitted by the IBOs. 1183 papers were included for screening at the level of title and abstract and in a second step at the full-text level, resulting in 334 animal and human studies for inclusion (Appendix A, Figure A1).

According to the protocol (EFSA, 2020a; EFSA FAF Panel, 2023), the previously considered pivotal study on which the derivation of the current ADI was based was evaluated for risk of bias (RoB) together with relevant literature published since the previous evaluation by the SCF, allowing 1 year of overlap (cut-off date: 1994). The toxicity study previously considered as pivotal in the original evaluation (SCF, 1995) was allocated to Tier 1 (low RoB). Thus, in line with the approach described in the protocol (EFSA FAF Panel, 2023), the systematic approach for the appraisal of the evidence was applied only to the new evidence which (i) became available since the last evaluation of saccharins (E 954) from the SCF (1995) and (ii) was judged to be of low or moderate risk of bias (i.e. Tier 1 and 2) in addition to the previously identified pivotal study on which the derivation of the current ADI was based.

3.5.4.1 | *Animal studies*

The studies included in the assessment encompassed 18 animal studies. Among these studies, eight were allocated to tier 1 and 9 to tier 2 following a RoB evaluation. One study was allocated to tier 1 or tier 2 depending on the measured endpoint. The Panel noted that in several included studies, only one dose was tested, see Table 10.

TABLE 10 Animal studies included in the assessment.

Authors	Study type	Species/strain	No of animals	Exposure duration	Dose level	Dose level in mg/kg bw per day	RoB tier
Glendinning et al. (2019)	Subacute	Mice, C57BL/6 (B6)	Experiment 1: 5 animals/sex/control group; 13 animals/sex/Sac group Experiment 2: 5 animals/sex/control group; 4 animals/sex/sac group	Experiment 1: 28 days Experiment 2: 29 days	0, 30 mM (0.61%) in drinking water	Equivalent to: Experiment 1: 0, 1900 mg/kg bw per day NaSac Experiment 2: 0, 2500 mg/kg bw per day NaSac	1
Okamura et al. (1994)	Repeated dose study (up to 70 days)	Mice, BALB/c and rats, F344	Exp 1: 30M rats/group Exp 2: 20 rats or mice/sex/group Exp 3 and 4: 5M rats/group	Experiment 1: 4, 7 and 10 weeks. Experiment 2, 3 and 4: 4 weeks	Experiment 1: 0, 3, 5 or 7.5% NaSac in the diet. Experiment 2: 0 or 7.5% NaSac (male and female rats, both rats and mice) or 6.3% acid saccharin (male rats only) in the diet Experiment 3: 0, 7.5% NaSac in the diet Experiment 4: 0, 7.5% NaSac in the diet	Equivalent to: Experiment 1: 0, 3600, 6000, 9000 mg/kg bw per day NaSac for 4-week exposure and 2700, 4500, 6750 mg/kg bw per day NaSac for 7- and 10-week exposure. Experiment 2: 0, 15,000 mg/kg bw per day NaSac for mice, 0, 9000 mg/kg bw per day NaSac for rats and 7560 mg/kg bw per day acid saccharin in male rats only. Experiments 3 & 4: 0, 9000 mg/kg bw per day in male rats only	1
Cohen et al. (1996)	Subchronic	Rats, F344	40 M, 10/group	10 weeks	0 or 7.5% NaSac in the diet	Equivalent to 0, 6750 mg/kg bw per day	1
Pinto et al. (2017)	Sub-chronic	Rats, Wistar	12M/group	17 weeks	0.3% NaSac in the diet (20 mL yogurt and 15 mL water to adjust viscosity) Compared to a 20% sucrose group	Equivalent to 270 mg/kg bw per day NaSac Compared to a sucrose control	1
Shi et al. (2019)	Subchronic	Mice, ICR	10 M/group	12 weeks	0, 1.0 g L^{-1} saccharin in drinking water	Equivalent to 0, 144 mg/kg bw per day	1
Schoenig et al. (1985)	One generation	Rats, Charles River CD	52–700 animals/sex/group (number of animals varied, being greater in the lower concentration groups in order to increase the statistical power)	F0 Bioassay: 4 months (62 days prior to mating, gestation and lactation) F1 Bioassay: 29 months F1 phase gestation only: 4 days prior to mating until end of gestation F1 phase following gestation: from lactation until 29 months	0, 1, 3, 4, 5, 6.25 and 7.50% NaSac, 5% NaSac through gestation, 5% NaSac from lactation until month 29 in the diet	F0 Bioassay: equivalent to 0, 900, 2700, 3600, 4500, 5625 and 6750 mg/kg bw per day NaSac F1 Bioassay: equivalent to 0, 500, 1500, 2000, 2500, 3125 and 3750 mg/kg bw per day NaSac F1 phase (gestation only): equivalent to 6000 mg/kg bw per day NaSac F1 phase (29 months): equivalent to 2500 mg/kg bw per day NaSac (doses of 500 and 1500 mg/kg bw per day used for weeks 1 and 2, respectively)	1

(Continues)

TABLE 10 (Continued)

Authors	Study type	Species/strain	No of animals	Exposure duration	Dose level	Dose level in mg/kg bw per day	RoB tier
Cohen, Cano, et al. (1995)	One-generation	Rats, Sprague–Dawley/Fischer 344	Exp 1: group 1 + 1a and 2 20M and 40F (SD), groups 3 + 3a + 3b 55Fand 28M, group 4, 5, 6 16F and 8M (F344). Exp2: 20F and 20M (SD)	2 weeks before mating (males and females), through gestation and lactation (F0 females) and for 10 weeks after weaning (F1 males)	0 or 5% NaSac or 5% acid saccharin in the diet	Experiment 1: Equivalent to 0, 2500-4050 mg/kg bw per day (depending on the group/strainstrain[b])) Experiment 2: Equivalent to 0, 2500-mg/kg bw per day	1
Cohen, Garland, et al. (1995)	One-generation	Rats, F344	F0: 4M and 7F/group F1: 4 animals/sex/group	F0 females: at least 7–8 weeks, F0 males: at least 4–5 weeks (until mating), F1 males: 105 days. (F1 females culled after weaning)	0, 5 or 7.5% NaSac in the diet	Equivalent to 0, 4500, 6750 mg/kg bw per day NaSac	1
Jiang et al. (2018)	Reproductive toxicity	Rats, Sprague Dawley	6F/group	48 days (6/7 weeks)	0, 1.5 mM, 7.5 mM NaSac in drinking water	Equal to 0, 140 or 730 mg/kg bw per day NaSac	1/2[a]
Uwagawa et al. (1994)	Repeated dose toxicity study (8 weeks)	Rats, NCI-Black-Reiter (NBR) and F344	Treatment group: 6M (NBR or F344) Control group: 10M NBR and 5M F344	8 weeks	5% NaSac in the diet	Equivalent to 0, 4500 mg/kg bw per day NaSac	2
Shi et al. (2021)	Subchronic	Mice, C57BL/6	10F/group	11 weeks	0, 0.1 mg/mL saccharin in drinking water	Equivalent to 0, 15 mg/kg bw per day saccharin	2
Feijó et al. (2013)	Repeated dose toxicity study (12 weeks)	Rats, Wistar	10 animal/group	12 weeks	0.3% NaSac in the diet (20 mL plain yogurt and 10 mL water to adjust viscosity) (available for ~ 22 h, 5 days per week) Compared to a 20% sucrose group	Equivalent to 270 mg/kg bw per day NaSac Compared to a sucrose control	2
Boakes et al. (2016)	Repeated dose toxicity studies (7 weeks) and subchronic	Rats, Sprague–Dawley	Experiment 1: 10M/group Experiment 2: 14-15M/group	Experiment 1: 15 weeks Experiment 2: 7 weeks	0 or 0.3% NaSac in the diet (fat-free yogurt)	Equivalent to: Experiment 1: 0, 360 mg/kg bw per day NaSac Experiment 2: 0, 360 mg/kg bw per day NaSac	2
Foletto et al. (2016)	Subchronic	Rats, Wistar	8M/group	14 weeks	0, 0.3% NaSac in the diet (20 mL plain yogurt and 10 mL pure water to adjust viscosity)	Equivalent to 0, 270 mg/kg bw per day NaSac	2
Azeez et al. (2019)	Subchronic	Rats, Wistar	10M/group	120 days	0, 2.5, 5 or 10 mg/kg bw in water by gavage	0, 2.5, 5 or 10 mg/kg bw per day NaSac	2

TABLE 10 (Continued)

Authors	Study type	Species/strain	No of animals	Exposure duration	Dose level	Dose level in mg/kg bw per day	RoB tier
Bian et al. (2017)	Repeated dose toxicity (6 months)	Mice, C57BL/6J WT	10M/group	6 months	0, 0.3 mg/mL in drinking water	Equivalent to 0, 45 mg/kg bw per day saccharin	2
Takayama et al. (1998)	Chronic	Non-human primates (monkeys: African green (*Cercopithecus aethiops*), rhesus (*Macaca mulatta*), cynomolgus (*Macaca fascicularis*), and one hybrid animal (rhesus male and cynomolgus female parentage)	Control group: 10M and 6F Treatment group: 9M and 11F	24 years	0 or 25 mg/kg bw NaSac, for 5 days a week in the diet.	0, 25 mg/kg bw per day NaSac	2
Li, Geng, et al. (2019), Li, Ren, et al. (2019)	Reproductive toxicity	Guinea pigs	6 animals/group	28 days	0 or 1.5 mM, 7.5 mM NaSac solutions	Equal to 0, 95 and 662 mg/kg bw per day	2

[a]Tier 2 for the endpoint 'progesterone level' considering the low number of animals used for hormonal measurement.

[b]Equivalent to 4050 mg/kg bw per day sodium saccharin only for group 3B (Control during gestation and lactation → 10 wks. 5 % NaSac). For group 1 A the dose conversion was not included since exposure is stopped interrupted after lactation and this makes the calculation difficult.

The animal studies included in the weight of evidence (WoE) assessment are described in detail in Annex E1. This annex shows each WoE step for each health outcome category (HOC). Each HOC consists of groups of endpoints (see Table 11), each endpoint being addressed in one or more of the included animal studies.

TABLE 11 Health outcome categories and related endpoints of the appraised animal studies subjected to WoE evaluation.

Health outcome categories (HOCs)	Endpoints
General toxicity	Clinical signs/survival/faecal occult blood; body weight; body weight gain; body fat mass and percentage
Additional clinical chemistry[a]	Serum cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides
Haematotoxicity	Red blood cells (RBC), white blood cells (WBC), platelet count, bone marrow histopathology
Liver toxicity	Liver weight (absolute); histopathology of liver; oxidative stress (catalase activity), hepatic proinflammatory cytokines (IL-6 mRNA and TNF-alpha mRNA); liver - inflammation (iNOS, TNF-α, IL-1β, IL-6 gene expression on mRNA level); hepatic triglycerides; untargeted 1H nuclear magnetic resonance (NMR)-based metabolomics in liver; long chain fatty acids composition in liver; serum aspartate transaminase (AST); serum alkaline phosphatase (ALP), serum alanine transaminase (ALT); serum total bile acid (TBA), serum and urinary bilirubin, urinary urobilinogen, serum direct bilirubin (d-BIL), serum albumin
Nephrotoxicity	Kidney weight (absolute); kidney weight (absolute and relative); kidney histopathology; serum and urinary creatinine, urinary urea, serum urea nitrogen, serum uric acid levels
Other organ toxicity	Pancreas, stomach, spleen, pulmonary, heart, brain, pituitary, salivary gland, thyroid, tongue, cheek pouches, trachea, oesophagus, aorta, gallbladder, duodenum, jejunum, ileum, large intestine, lymph nodes breast and skin histopathology caecum weight and enlargement and duodenum (carbohydrate absorption)
Glucose/insulin homeostasis	Blood glucose levels, fasting blood glucose level, blood insulin level and insulin sensitivity
Reproductive and developmental toxicity	Litter size, health status of pups/malformations; growth (F1); puberty onset, oestrous cycle, ovaries, uterus, testes/seminal vesicles, prostate, ureters and urethra histopathology, clinical chemistry (progesterone and oestradiol levels)

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

In addition to the apical and related endpoints reported in Table 11, 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 12. 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 were evaluated across all endpoints within a specific HOC, and this rating of the 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 FAF Panel, 2023[39]) and in Section 2.2.

[39]https://zenodo.org/records/7788969.

TABLE 12 Summary table of rating confidence in the body of evidence for each health outcome category: Animal studies (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 ($n=17$)	Not serious	Not serious	Not serious	Not serious	No	Large	Yes	Yes	N.A.	High	Effect
Additional clinical chemistry	High ($n=2$)	Serious	Not serious	Not serious	Not serious	Yes	Large	No	No	No	Moderate	No effect
Haematotoxicity	High ($n=3$)	Not serious	Not serious	Not serious	Not serious	No	Large	N.A.	Yes	No	High	No effect
Liver toxicity	High ($n=6$)	Serious	Not serious	Not serious	Not serious	Yes	Not large	No	No	No	Moderate	No effect
Nephrotoxicity	High ($n=5$)	Serious	Not serious	Not serious	Not serious	Yes	Large	Yes	No	No	Moderate	No effect
Other organs toxicity	High ($n=5$)	Serious	Not serious	Not serious	Not serious	Yes	Large	Yes	No	No	Moderate	No effect
Reproductive and developmental toxicity	High ($n=6$)	Serious	Not serious	Not serious	Not serious	Yes	Large	No	No	No	Moderate	Effect
Glucose/insulin homeostasis	High ($n=7$)	Serious	Serious	Not serious	Not serious	Yes	Large	N.A.	No	No	Moderate	No effect

Abbreviation: N.A., not applicable.

[a]The total number of studies assessed was 18. The number in parentheses refers to studies considered under the specific HOC.

[b]Please refer to Appendix A and Annexes E1 and E2 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.

In this section, the confidence and level of evidence for each HOC are presented. Further consideration on the overall conclusion will be presented in Sections 3.5.4.3 and 3.6.

General toxicity

Adverse effects related to general toxicity (body weight, body fat mass and composition and clinical signs) were evaluated in 17 studies of different duration and design (e.g. route of administration, control group) and in three different species (rat, mice and guinea pig) (see Table 10).

Body weight

In order to assess the potential effects of saccharins (E 954) on body weight, the Panel considered the mean final body weight (i.e. at the end of each study) as % of the control group mean final body weight in that study. When this endpoint was not reported by the study authors, an estimation was done by the Panel.

Mean final body weights as % of the control group in their respective study are plotted against the doses of saccharins in Figure 4 and for dose range 2.5–730 mg/kg bw per day in Figure 5. It is noted that, for the available one-generation studies (Cohen, Cano, et al., 1995; Cohen, Garland, et al., 1995; Schoenig et al., 1985), Figures 4 and 5 include only data from the F0 generation, because it was not possible to quantify offspring exposure during gestation and lactation.

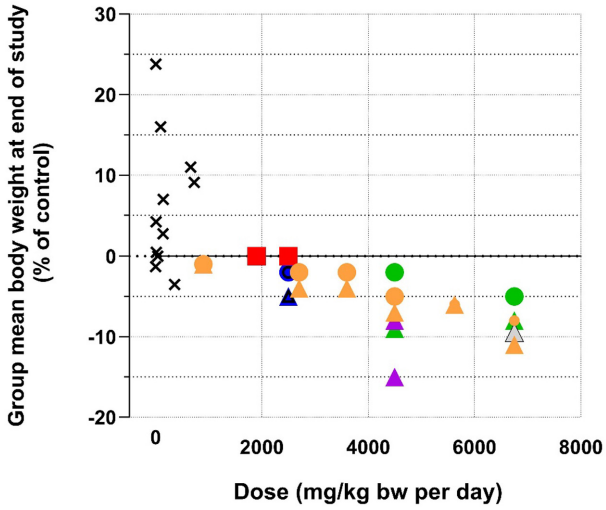


FIGURE 4 Mean final body weight as % of control in animals after exposure to saccharins doses in the range 900–6750 mg/kg bw per day (from six references: Cohen et al. (1996), Cohen, Cano, et al. (1995), Cohen, Garland, et al. (1995), Uwagawa et al. (1994), Glendinning et al. (2019); Schoenig et al. (1985)). F1 data from one-generation studies were excluded as well as parental data during gestation and lactation periods. In studies with more than one time point measurements, only the values at the end of the study were included. Each colour represents data from one reference. Symbols: Triangles: M; Dots: F; Squares: M/F combined. Studies = colour: Cohen, Cano, et al. (1995) experiment 1 = blue; Cohen, Garland, et al. (1995) = green; Cohen et al. (1996) = grey with black border; Uwagawa et al. (1994) = purple (NBR and F344 rats); Glendinning et al. (2019) = red; Schoenig et al., 1985= orange. Black crosses indicate values after exposure to doses in the range 2.5–730 mg/kg bw per day, which are shown also in detail in Figure 4.

Not included in Figure 4 are two studies which had sucrose as control group (Feijó et al., 2013; Pinto et al., 2017). The aim of these studies was to compare the long-term energy expenditure at rest and the effects on weight gain and the caloric intake between rats with diets supplemented with sodium saccharin or sucrose. The results of such studies are difficult to interpret in the absence of a control group without added sucrose.

Reduced body weight was observed at doses above 900 mg/kg bw per day, the reduction was more than 10% at high doses (above 4500 mg/kg bw per day) in two studies (Schoenig et al., 1985, Uwagawa et al., 1994).

The Panel considered that a possible explanation for these effects might be reduced palatability (Petrov et al., 2004; Tordoff et al., 2008; see Appendix B) and/or osmotic diarrhoea (Schoenig et al., 1985; Uwagawa et al., 1994).

It is noted that, at low doses of saccharins (2.5–730 mg/kg bw per day; refs. 4892, 6031, 6674, 7684, 6365, 6069, 7094, 7014), most studies reported modest increases in mean final body weight (Figure 4). Only in two studies (Azeez et al., 2019; Li, Geng, et al., 2019; Li, Ren, et al., 2019) the increase was between 10% and 25%.

The Panel noted that only 5 out of 13 of the included studies measuring body weight changes tested more than one dose.

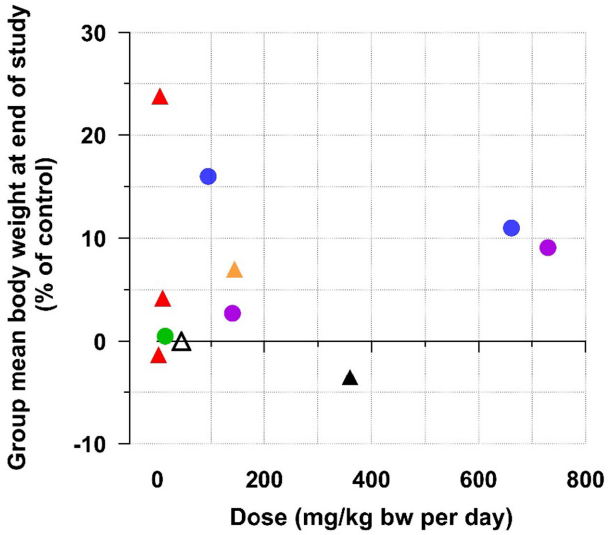


FIGURE 5 Mean final body weight as % of control in animals after exposure to saccharins doses in the range 2.5–730 mg/kg bw per day (from eight references) shown with coloured symbols, each colour representing values from one reference. In studies with more than one time point, the values at the end of the study are included. Symbols: Triangles: M; Dots: F. Studies = colour: Boakes et al. (2016) = black; Azeez et al. (2019) = Red; Bian et al. (2017) = white with black border; Jiang et al. (2018) = purple; Shi et al. (2019) = orange; Li et al. (2019) = blue; Shi et al. (2021) = green.

The Panel noted a weight gain at low doses which might be related to increased feed intake (data insufficient). In the absence of effects on other toxicological effects in this dose range, the Panel considered this effect as not adverse.

Body fat mass and percentage

Only two included studies measured changes in body fat mass (retroperitoneal, visceral and epididymal fat pads) and in body composition (body fat mass %) in rats and mice following the exposure to sodium saccharin (Boakes et al., 2016; Glendinning et al., 2019). In these two studies, saccharins had no consistent effects on body fat mass and percentage. It is noted that no effect on final body weight was observed in these studies (Figures 4 and 5, respectively).

Clinical signs of toxicity

At doses above 3600 mg sodium saccharin/kg bw per day dose-dependent increases in clinical signs of toxicity including faecal occult blood and death were observed (Cohen, Garland, et al., 1995; Uwagawa et al., 1994; Schoenig et al., 1985; Okamura et al., 1994).

Considering the final rating of confidence in the body of evidence for the HOC 'General toxicity' as 'high' and identification of adverse effects (see Tables 12 and 13), the Panel considered that there is high confidence in the body of evidence that the exposure to saccharins (E 954) is associated with general toxicity effects, i.e. decrease in body weight and increases in clinical signs of toxicity.

Additional clinical chemistry

Changes in clinical chemistry were evaluated in two studies of different duration and design in mice (see Table 10). In Shi et al. (2021), serum levels of cholesterol, LDL and HDL were measured while in Glendinning et al. (2019), only serum triglycerides levels were measured. Saccharins had no effect on serum cholesterol, triglycerides or LDL levels but resulted in a significant decrease in serum HDL levels (–25%).

Considering the final rating of confidence in the body of evidence for the HOC 'additional clinical chemistry' as 'moderate' and the absence of any toxicologically significant changes (see Tables 12 and 13), the Panel considered that there is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with adverse changes in clinical chemistry parameters.

Haematotoxicity

A chronic study in non-human primates (Takayama et al., 1998), a one generation study in rats (Schoenig et al., 1985) and a sub-chronic study in rats and mice (Okamura et al., 1994) addressed endpoints relevant for the assessment of haematotoxicity (see Table 10). High dose dietary saccharins (> 2500 mg/kg bw per day) had no direct adverse haematological effects in male rats (except for secondary anaemia due to marked glandular stomach haemorrhage in young rats; see below 'other organ toxicity'). Lifelong dietary exposure of non-human primates to 25 mg saccharin/kg body weight, daily for 5 days a week, had no adverse effects on haematology (Takayama et al., 1998).

Considering the final rating of confidence in the body of evidence for the HOC 'haematotoxicity' as 'high' and the absence of adverse effects (see Tables 12 and 13), the Panel considered that there is high confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with adverse haematological effects.

Liver toxicity

Liver toxicity was addressed in six studies of different duration and design and in three different species, rat, mice and non-human primates (Azeez et al., 2019; Bian et al., 2017; Cohen, Cano, et al., 1995; Cohen, Garland, et al., 1995; Schoenig et al., 1985; Shi et al., 2021; Takayama et al., 1998; see Table 10). Saccharins had no effects on serum ALT, serum AST or serum ALP levels, serum bile acid or serum and urinary bilirubin concentrations. A decrease in serum albumin levels was observed in one study (Azeez et al., 2019). There were no consistent effects on liver weight (Cohen, Garland, et al., 1995) or selected cytokine levels in the liver (Shi et al., 2021) and no histopathological changes (Cohen, Garland, et al., 1995; Shi et al., 2021; Takayama et al., 1998). No changes in additional non apical endpoints including markers of oxidative stress were observed (see Annex E1).

Considering the final rating of confidence in the body of evidence for the HOC 'liver toxicity' as 'moderate' and the absence of adverse effects (see Tables 12 and 13), the Panel considered that there is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with adverse effects on liver.

Nephrotoxicity

Nephrotoxicity was addressed in five studies of different duration and design in rats, non-human primates and mice (Cohen, Garland, et al., 1995; Schoenig et al., 1985; Takayama et al., 1998; Azeez et al., 2019; Shi et al., 2021). Saccharins had no effect on serum and urinary creatinine concentrations (Cohen, Garland, et al., 1995; Takayama et al. 1998; Azeez et al., 2019; Shi et al., 2021), but a statistically significant increase in uric acid concentration in a dose-responsive manner was measured and observed in one study (Azeez et al., 2019). There were no effects on absolute or relative kidney weights (Cohen, Garland, et al., 1995; Schoenig et al., 1985) and no histopathological changes in the kidney (Cohen, Garland, et al., 1995; Schoenig et al., 1985; Takayama et al., 1998).

Considering the final rating of confidence in the body of evidence for the HOC 'nephrotoxicity' as 'moderate' and the absence of adverse effects (see Tables 12 and 13), the Panel considered that there is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with nephrotoxicity.

Other organ toxicity

Five studies of different duration and design addressing adverse effects on organs other than liver and kidney were included in the assessment (Cohen, Garland, et al., 1995; Cohen et al., 1996; Takayama et al., 1998; Okamura et al., 1994; Shi et al., 2019; see Table 10). Three different species were tested (mice, rats and non-human primates). According to the available scientific knowledge (see Hildebrand et al., 1997), bladder tumours in male rat are not considered to be human-relevant; the bladder effect seen only in male rats is alpha2-microglobulin-dependent, a protein which is rat-male specific (Hildebrand et al., 1997). Therefore, such effects were excluded from the WoE.

Bladder effects were not observed in a study on non-human primates (control group: 10 males and 6 females; Treatment group: 9 males and 11 females) exposed to sodium saccharin at 25 mg/kg bw per day for 24 years (Takayama et al., 1998). High dietary dose of sodium saccharin (≥ 2500 mg/kg bw per day) produced haemorrhage of the glandular stomach in young rats (Okamura et al., 1994) and mild bladder urothelial regenerative hyperplasia in adult male rats following 10 weeks of exposure (Cohen et al., 1996: bladder tumours are seen after lifelong exposure; Schoenig et al., 1985). Such effects were not seen in primates at 10-fold lower doses (25 mg sodium saccharin/kg bw per day; Takayama et al., 1998). These effects are considered to be due to high local concentrations of saccharin which are not consumer relevant. The enlargement of the caecum in rats observed after high oral doses (Cohen, Garland, et al., 1995) is considered adaptive and of no toxicological relevance. No adverse effects were observed in other organs.

Considering the final rating of confidence in the body of evidence for the HOC 'other organ toxicity' as 'moderate' and the absence of adverse effects (see Tables 12 and 13), the Panel considered that there is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with (other) organ toxicity.

Reproductive and developmental toxicity

Six studies of different duration and design measuring endpoints relevant for assessment of reproductive and developmental toxicity were included (see Table 10). The species tested were rat, guinea pig and non-human primates.

Reproductive effects

In a 48-day repeated dose toxicity study in weanling rats (Jiang et al., 2018), doses equal to 140 or 730 mg sodium saccharin/kg bw per day in drinking water had no significant treatment-related effect on time of puberty onset but showed dose-related increase in percentage of abnormal oestrous cycles and serum progesterone. In the same study, quantitative

histopathology of the ovary was performed (number of ovarian cysts and corpora lutea), at both doses. However, due to shortcomings in the performance of ovarian histological examination, i.e. no blinding for quantitative histopathology, the corpora lutea and cyst count endpoints were not considered.

In a study in guinea pigs (Li et al., 2020), saccharin at doses equal to 95 and 662 mg sodium saccharin/kg body weight per day for 28 days had no effect on day of vaginal opening (puberty onset), oestrous cycle (the smear checks indicated that the oestrous stage of guinea pigs was all in luteal phase at day 28). Due to shortcomings in the ovarian and uterine histological examination and hormonal measurements, these endpoints were not considered further. The lack of clear effects on oestrous cyclicity contrasts with the effects reported in rats (Jiang et al., 2018; increased serum progesterone, abnormal oestrous cycles). This could be due to a species difference between rat and guinea pig, given the major difference in oestrus cycle length between the two species (4–5 days in rat versus 15–17 days in guinea pig).

It is noted that the effects reported by Jiang et al. (2018) in rats were not reported in guinea pigs (Li et al., 2020). Furthermore, in a repeated dose toxicity study in monkeys dosed 25 mg/kg bw per day up to 24 years (Takayama et al., 1998), no treatment-related histopathological changes were reported in ovaries, testes or seminal vesicles, ureters and urethra or prostate.

Developmental effects (pre- and post-natal)

In a one-generation study in Sprague–Dawley (SD) rats (Schoenig et al., 1985), there was reduced litter size at doses equivalent to ≥ 2700 mg dietary sodium saccharin/kg bw per day (also dose-dependent increased urinary bladder weight and tumours at this dose and increased mineralisation of the kidneys at doses equivalent to 500–900 mg sodium saccharin/kg bw per day in F1 males). In another one-generation dietary study in F344 rats (Cohen, Garland, et al., 1995), mean litter size at birth was reduced (but not statistically significantly) at doses equivalent to 4500 and 6750 mg sodium saccharin/kg bw per day (the only doses tested). The authors reported that, beginning immediately after birth, the high-dose offspring showed growth retardation, and because of clinical deterioration, all either died or became moribund and were killed by 30 days of age. In a one-generation dietary study in SD and F344 rats, focusing on F1 bladder effects (Cohen, Cano, et al., 1995), mean litter size at birth was not affected at a dose equivalent to 2500 mg/kg bw per day (the only dose tested both for saccharin and sodium saccharin). No malformations were reported in pups.

Dietary saccharin in pregnant rats at doses ≥ 2700 mg sodium saccharin/kg bw per day reduced litter size. It is possible that treatment-related reduced body weight in F0 parental animals contributed to reduced litter size. In the absence of data on pre-implantation losses, the Panel considered it not possible to attribute reduced litter size to reproductive toxicity (fertility) or developmental toxicity.

For the HOC 'Reproductive and developmental toxicity', adverse effects were observed in rats at doses ≥ 2700 mg/kg bw per day (increase in prenatal mortality, growth retardation, reduction in F1 litter size).

Additionally, there is moderate confidence in the available evidence that sodium saccharin affects progesterone regulation and oestrus cyclicity in female rats at a dose equal to 140 mg saccharin/kg bw per day. Effects on oestrus cyclicity were not seen in a study in guinea pigs treated with sodium saccharin at doses equal to 95 and 662 mg/kg bw per day for 28 days. This could be due to the shorter duration of exposure in the guinea pig, which has a much longer oestrus cycle than rat. No adverse effects were observed in other measured endpoints. The interaction of saccharins with ovarian taste receptors might be a potential mode of action for the effects on progesterone levels but given the limited data and lack of species consistency, no firm conclusions could be drawn by the Panel (see Appendix B).

Considering the final rating of confidence in the body of evidence for the HOC 'reproductive and developmental toxicity' as 'moderate' and identification of adverse effects (see Tables 12 and 13), the Panel considered that there is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is associated with adverse effects on development and reproduction.

Glucose/insulin homeostasis

Six studies of different duration and design measuring endpoints relevant for the assessment of effects on glucose/insulin homeostasis were included in the assessment (see Table 10). The species tested were rat and mouse. Blood glucose levels (in non-fasting and fasting animals) and blood insulin and insulin sensitivity were assessed. No consistent effects in these endpoints were observed. The Panel noted that in several studies only one dose was tested.

Blood glucose levels in non-fasting animals

Only one study (Azeez et al., 2019), in which rats were treated for 120 days with 2.5, 5 or 10 mg/kg bw per day of sodium saccharin, assessed the blood glucose levels in non-fasting animals. In this study, a non-dose-related increase in blood glucose levels in non-fasting animals was observed.

Fasting blood glucose

Of the five studies included, four reported no effect on fasting blood glucose levels following the exposure to saccharins. In particular, no effects were reported for equivalent daily doses of 1900 mg sodium saccharin/kg bw per day (mice, after

28 days Glendinning et al., 2019), 360 mg sodium saccharin/kg bw per day (rats, after 7 weeks, Boakes et al., 2016), 270 mg sodium saccharin/kg bw per day (rats, 14 weeks, Carraro Foletto et al., 2016) and 15 mg saccharin/kg bw per day (mice, 11 weeks, Shi et al., 2021).

One study reported increased fasting blood glucose in mice treated for 11 weeks with a dose equivalent to 144 mg/kg bw per day (Shi et al., 2019).

Blood insulin and insulin sensitivity

No effect of saccharins on blood insulin or insulin sensitivity was reported in studies in mice or rat (Glendinning et al., 2019; Boakes et al., 2016; Foletto et al., 2016). One study (Shi et al., 2019, mice treated for 11 weeks with a dose equivalent to 144 mg/kg bw day) reported increased fasting plasma insulin (about 150% of control) and decreased insulin sensitivity (< 10%).

Considering the final rating of confidence in the body of evidence for the HOC 'glucose/insulin homeostasis' as 'moderate' and the absence of adverse effects (see Tables 12 and 13), the Panel considered that there is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with an impairment of glucose/insulin homeostasis.

Table 13 provides an overview of the translation of confidence ratings into level of evidence for conclusions of adverse effects or no adverse effect for the included animal studies for each of the HOCs considered in the assessment.

TABLE 13 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 HOC considered in the assessment.

	Final rating of confidence	Level of evidence
General toxicity	High	High There is high confidence in the body of evidence that the exposure to saccharins (E 954) is associated with general toxicity effects i.e. decrease in body weight and increases in clinical signs of toxicity.
Additional clinical chemistry	Moderate	Moderate There is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with adverse changes in clinical chemistry parameters.
Haematotoxicity	High	High There is high confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with adverse haematological effects.
Liver toxicity	Moderate	Moderate There is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with adverse effects on liver.
Nephrotoxicity	Moderate	Moderate There is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with nephrotoxicity.
Other organ toxicity	Moderate	Moderate There is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with other organ toxicity.
Reproductive and developmental toxicity	Moderate	Moderate There is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is associated with adverse effects on development and reproduction.
Glucose/insulin homeostasis	Moderate	Moderate There is moderate confidence in the body of evidence that the exposure to saccharins (E 954) is not associated with an impairment of glucose/insulin homeostasis.

3.5.4.2 | *Human studies*

The studies included in the assessment encompassed 19 human studies (Table 14). Among these studies, four studies were allocated to tier 1 and 15 to tier 2 following risk of bias evaluation. All the included human studies were retrieved from the literature. A detailed description of the included studies is provided in Annex B.

Annex E2 reports all the human studies evaluated, clustered by endpoint within the different health outcome categories (HOCs), for which a weight of evidence (WoE) analysis was performed.

The endpoints considered and evaluated in the WoE for the included human data are shown in Table 15.

TABLE 14 Human studies included in the assessment.

Authors, year (RefID[a])	Type of HCT	Dose (g/person or g/kg bw)[b]	Intervention/ exposure[40]	Number of subjects	Population (mean age in years)	RoB tier
Higgins and Mattes (2019)	Parallel arm single blind control trial	0.73 g/day	Saccharin Continuous, daily, 12 weeks	154, 29 (58.6% F) exposed to saccharin	Adults, 25.8 ± 6.9	1
Gallus et al. (2007)	Network of case–control studies	NA	NA, Food frequency questionnaire	8976 cases (4115M and 4861F) 7028 controls (3301M and 3727F)	Adults, cases median age 58; controls median age 57	1
Bosetti et al. (2009)	Case control study (Ca-Co)	NA	NA, Food frequency questionnaire	3117 (1010 cases, 317M and 693F; 2017 controls, 634M and 1473F)	Adults, cases median age 63, range 22–80; controls median age 63, range 22–80	1
Palomar-Cros et al. (2023)	Case–control study (Ca-Co)	NA	NA, Food frequency questionnaire	4823 cases, 3629 controls	Adults, 63	1
Serrano et al. (2021)	Parallel arm, double blind, placebo-controlled interventional study	0.4 g/day	Sodium saccharin, Continuous, twice daily, 2 weeks	13 (4M and 9F)	Adults, 28.91 ± 2.60	2
Suez et al. (2022)	Open-label, multi-arm randomised control trial	0.18 g/day	Saccharin Continuous, 2 weeks	120 (65% F), 20 exposed to saccharin	Adults, 29.95	2
Bayındır Gümüş, Keser, Tunçer, Altuntaş Yıldız, and Kepenekci (2022)	Randomised crossover trial	240 mg (0.24 g)	Saccharin Single dose[f]	9 (M)	Adults, 23.6 ± 3.17	2
Orku et al. (2023)	Single-blinded control trial	140 mg/day (0.14 g/day)	Saccharin Continuous, daily, 4 weeks	48, 11 exposed to saccharin (F)	Adults, 21.18 ± 1.40	2
Momas et al. (1994)	Case control study (Ca-Co)	NA (estimated lifelong saccharins intake (tablets) ranged 1834–2845)	NA, Food frequency questionnaire	1013 (219 cases and 794 controls) (M)	Adults, 67.8 (cases) 64.6 and 65.5 (controls)	2
Yu et al. (1997)	Case control study (Ca-Co)	NA (use of saccharins times/year: none, 1–18, ≥ 19)	NA, Food frequency questionnaire	381 (127 bladder cancer patient cases and 254 controls) (306M and 75F)	Adults, controls 55.3M and 53.3F; Cases 55.4M and 53.8F	2
Parker et al. (1997)	Prospective cohort study	NA (tertiles: 0, 0.1–28.2, > 28.2 g/day)	NA, Food frequency questionnaire	465 (176M and 289F)	Adults, 46.6 ± 13.5	2
Jensen et al. (2020)	Cohort study	NA	NA, Food-frequency questionnaire	1142	Adults, 42	2
Fulgoni and Drewnowski (2022)	Cohort study	NA	NA, 24-h recall	19,215 (NHANES III 1988–1994), 76.324 (All 1988–2018)	Adults, 44.52 to 47.01	2
Steffen et al. (2023)	Cohort study	NA (tertiles: 0, 0–17.1, > 17.1 mg/day)	NA, Diet history questionnaire	3088	Young adults, 18–30	2
Fernandes et al. (2013)	Cross-sectional study	NA	NA, Food frequency questionnaire	261[c] (85M and 176F)	Adults, 19.3 + 1.4	2

(Continues)

[40]In the case of the non-interventional human studies, the generic term 'saccharins' was used since it is assumed that all the four additives could be consumed and that therefore the information collected in the questionnaires could refer to saccharin and/or to its salts.

TABLE 14 (Continued)

Authors, year (RefID[a])	Type of HCT	Dose (g/person or g/kg bw)[b]	Intervention/ exposure[40]	Number of subjects	Population (mean age in years)	RoB tier
Duran Agüero et al. (2014)	Cross-sectional study	NA	NA, Food frequency questionnaire	571 (281M and 290F)	Adolescents, 13.2 ± 6.3	2
Kuk and Brown (2016)	Cross-sectional (CrSe)	NA	24-h dietary recall	2856[d]	Adults, 51.3–56.2	2
Hess et al. (2018)	Cross-sectional study (CrSe)	NA	NA, 24-h dietary recall	125 (54M and 71F), 6 exposed to saccharins	Adults, 36.7	2
Tapanee et al. (2021)	Cross-sectional study (Cr-Se)	NA (total median saccharins intake was reported to be 0.0254 g/day)	NA, Food frequency questionnaire (semiquantitative)	524 (91M 433F)[e]	Adults, 20.1 ± 1.9	2

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

[a]Numerical identifier generated by the DistillerSR tool.

[b]As reported by the authors.

[c]Number pf participants after drop out.

[d]Saccharins consumer: 125 males and 203 females; non-saccharins consumer: 1238 males and 1290 females.

[e]After removal of outliers or missing data.

[f]Participants were randomly provided with preloads as (i) 300 mL of water or 300 mL of water sweetened with (ii) 75 g of sucrose, (iii) 240 mg of saccharin (adjusted to the sweetness of 75 g of sucrose) 1 h before a standard breakfast. Blood glucose and serum insulin were measured at different intervals.

TABLE 15 Health outcome categories and related endpoints of the appraised human studies subjected to WoE evaluation.

Health outcome categories (HOCs)	Endpoints
Cancer	Bladder, gastric, pancreatic, oral cavity and pharynx, oesophagus, colon, rectum, colorectum, stomach, larynx, breast, endometrial ovary, prostate, kidney cancer, chronic lymphocytic leukaemia, cancer mortality
Cardiovascular risk factors	Low high-density lipoprotein cholesterol, metabolic syndrome, waist circumference, triglycerides, body weight, BMI, body composition/ visceral (VAT), intermuscular (IMAT) and subcutaneous adipose tissue (SAT) volumes, AT volumes, anthropometric measures, 25-year change in anthropometry
Glucose/insulin homeostasis	Fasting blood glucose, insulin, GTT, GLP-1, glycated haemoglobin A1c (HbA1c), diabetes incidence

Based on the included human data, the Panel considered the confidence in the body of evidence for all health outcome categories, see Table 16. The 'final confidence rating' was based on the 'initial confidence rating' followed by downgrading and upgrading considering elements that decrease or increase, respectively, the confidence across studies of the same HOC (see Appendix A and Annex E2). In particular, rating the confidence in the evidence for each identified relevant outcome begins with consideration on 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, imprecision) and four to possibly upgrade the confidence in the body of evidence (residual confounding, magnitude of effects, dose–response, consistency across study population/study design). The final confidence rating was reached by considering these elements across all studies within each HOC The final confidence in the body of evidence was then translated into a level of evidence, as outlined in the revised protocol and in Section 2.2.

TABLE 16 Rating of the confidence in the body of evidence for each health outcome category investigated: Human studies (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	Residual confounding	Magnitude of effect	Dose–response	Consistency across study population/ study design	Upgrading	Final rating of confidence
Cancer	Bladder	Moderate (2)	Serious	Serious	Not serious	Not serious	Yes	No	Not large	Yes	No	No	Very low
	Gastric, pancreatic, oral cavity pharynx, oesophagus, colon cancer, rectum r, colorectum r, stomach	Moderate (3)	Serious	Not serious	Not serious	Not serious	No	No	Not large	N.A.	Yes	No	Low
	Larynx	Moderate (1)	Serious	N.A.	Not serious	Not serious	Yes	No	Not large	N.A.	N.A.	No	Low
	Breast	Moderate (2)	Not serious	Not serious	Not serious	Not serious	Yes	No	Not large	N.A.	Yes	No	Low
	Prostate	Moderate (2)	Serious	Not serious	Not serious	Not serious	Yes	No	Not large	N.A.	Yes	No	Low
	Endometrial	Moderate (1)	Serious	N.A.	Not serious	Not serious	Yes	No	Not large	N.A.	N.A.	No	Low
	Ovary	Moderate (1)	Serious	N.A.	Not serious	Not serious	Yes	No	Large	N.A.	N.A.	No	Low
	Kidney	Moderate (1)	Serious	N.A.	Not serious	Not serious	Yes	No	Not large	N.A.	N.A.	No	Low
	Chronic lymphocytic leukaemia	Moderate (1)	Serious	N.A.	Not serious	Not serious	Yes	No	Not large	N.A.	N.A.	No	Low
	Cancer mortality	Moderate (1)	Serious	N.A.	Not serious	Not serious	Yes	No	Not large	N.A.	N.A.	No	Low
Cardiovascular risk factors		Moderate (9)	Not serious	Not serious	Serious	Not serious	Yes	No	Not large	Yes	Yes	No	Low
Glucose/insulin homeostasis		High (8)	Serious	Not serious	Serious	Not serious	Yes	No	Not large	N.A.	No	No	Low

In this section, the confidence and level of evidence for each HOC are discussed. Further consideration on the overall conclusion will be presented in Sections 3.5.4.3 and 3.6.

Cancer

The included studies encompassed five case–control studies conducted in France, China, Italy and Spain (Bosetti et al., 2009; Gallus et al., 2007; Momas et al., 1994; Palomar-Cros et al., 2023; Yu et al., 1997) and one cohort study conducted in USA (Fulgoni and Drewnowski, 2022).

Case–control studies

Two case–control studies examined the association between saccharins intake and bladder cancer with contradictory results. Momas et al. (1994) conducted a population case–control study (219 cases and 794 controls) in France to investigate risk factors for bladder cancer among males in a 'high risk' area for bladder cancer (Hérault region) and found no association between saccharins use (daily vs. non-daily, OR:1.5, 95% CI: 0.8–3.0) and bladder cancer. Yu et al. (1997), in a hospital-based case–control study conducted in China (127 cases and 254 controls) to explore risk factors for bladder cancer, showed an increased risk for use of saccharins (≥ 19 times/year versus none, OR:3.9, 95% CI: 1.8–8.7, $p_{trend} = 0.0007$). Three case–control studies conducted in Italy and Spain (Bosetti et al., 2009; Gallus et al., 2007, Palomar-Cros et al., 2023) investigated the association between saccharins intake and other types of cancers (oral cavity and pharynx, oesophageal, colorectal, ovarian, breast, prostate, stomach, lymphocytic leukaemia, pancreatic and endometrial cancers) and found no association except for a decreased risk for pancreatic cancer and ovarian cancer associated with saccharins intake. The association between saccharins intake and stomach (Bosetti et al., 2009; Palomar-Cros et al., 2023), breast (Gallus et al., 2007; Palomar-Cros et al., 2023) and prostate cancers (Gallus et al., 2007; Palomar-Cros et al., 2023), was examined in two different case–control studies while for the other cancer types, only one case–control study was available.

Cohort study

In a cohort-study in USA ($N = 15.948$), saccharins intake was not associated with cancer mortality (T3 vs. T0, HR: 0.91, 95% CI 0.50–1.65) (Fulgoni and Drewnowski, 2022).

All studies on cancer (see Table 14) assessed saccharins use through food frequency questionnaires, except for the study of Fulgoni and Drewnowski (2022), who used a 24-h recall method. Assessment of saccharins exposure in the case–control studies, except for the study of Palomar-Cros et al. (2023), was estimated from table-top sweeteners and not from other food items containing saccharins as an additive. The study of Fulgoni and Drewnowski (2022) estimated saccharins intake from diet beverages, table-top sweeteners and sugar free foods. The main issues in the studies were the assessment of the exposure (information bias) and issues related to confounding and the lack of appropriate adjustments in the statistical analysis. The lack of detailed smoking and occupational history data, family history for the specific cancer sites as well as genetic cancer susceptibly data add further uncertainty in the results.

For the HOC 'Bladder cancer' no clear evidence of association between saccharins intake and this type of cancer was identified in the human studies included in the current assessment. For those studies, the Panel considered the confidence in the body of evidence to be very low in particular due to the low quality of the studies (e.g. misclassification of smoking) and the conflicting results (see Annex E2). Therefore, the Panel considered that there is inadequate evidence available in the two human studies included in the current re-evaluation to assess whether the exposure to saccharins (E 954) is associated with bladder cancer. Considering the previous assessments JECFA (1993), SCF (1995) and IARC (1999), and the current knowledge on the mechanism behind bladder cancer in animals, the Panel considered it unlikely that there is an association between exposure to saccharins (E 954) and bladder cancer in humans.

For the other types of cancer, no effects were identified in the human studies included in the current assessment. For those studies, the Panel considered the confidence in the body of evidence to be low (see Table 15) for all cancer types. Therefore, the Panel considered that there is inadequate evidence available in these limited studies included in the current re-evaluation to assess whether the exposure to saccharins is associated with cancer.

In summary, the newly available human data are in line with the previous conclusions by JECFA (1993), SCF (1995) and IARC (1999) that saccharins (E 954) exposure is not associated with cancer.

Cardiovascular risk factors

In this section, epidemiological studies conducted in USA, Chile and Thailand that investigated the role of saccharins on cardiovascular risk factors were reviewed. Five cross-sectional studies (Duran Agüero et al., 2014; Fernandes et al., 2013; Hess et al., 2018; Kuk and Brown, 2016; Tapanee et al., 2021), two cohort studies (Parker et al., 1997; Steffen et al., 2023) and two randomised control trials (Higgins et al., 2019; Orku et al., 2023) were included in the assessment.

Cross-sectional studies

Fernandes et al. (2013) ($N = 294$), in a cross-sectional study showed an increased risk of low levels of high-density lipoprotein cholesterol (< 40 mg/dL in men and < 50 mg/dL in women; odds ratio (OR), 1.047; 95% CI, 1.015–1.080). Hess et al. (2018) in a cross-sectional study ($N = 125$), found that saccharins use was not associated with metabolic syndrome risk factors, but the use of saccharins was associated with high waist circumference ($p = 0.003$).

Three cross-sectional studies, Duran Agüero et al., (2014) ($N = 571$), Kuk and Brown (2016) ($N = 2856$) and Tapanee et al., (2021) ($N = 710$), found no association between saccharin intake and 'overfat/obesity'. In a cross-sectional analysis conducted by Steffen et al. (2023), intake of saccharins at baseline was associated with increased BMI (p-trend < 0.001), increased weight (p-trend < 0.001) and increased waist circumference (p-trend < 0.001).

Cohort studies

Two cohort studies (Parker et al., 1997; Steffen et al., 2023) investigated the association between saccharins intake and weight gain. After 4 years of follow-up, Parker et al. (1997; $N = 465$), observed that subjects in the highest tertile of saccharins intake (> 28.2 g/day[41]) gained more weight (1.4 kg, SE = 0.4, $p = 0.02$) than subjects in the lowest tertile (0 g/day). However, in the multivariate analysis, after controlling for age, BMI, smoking, physical activity and energy intake, the association between saccharins and weight change disappeared. Steffen et al., 2023 ($N = 3088$), in a 25-year cohort study, found that high intake of saccharins (averaged intake T3 = 65.6 mg/day) was associated with an increased risk of obesity (BMI ≥ 30) (HR T3 (> 17.1 mg/day) versus T1: 1.19, 95% CI: 1.13–1.26) and with greater adipose tissue volumes in visceral (p-trend = 0.001), subcutaneous (p-trend = 0.001) and intermuscular compartments (p-trend < 0.001). After 25-year follow-up, increases of weight (p-trend = 0.03) and waist circumference (p-trend = 0.008), but not BMI, were observed with high saccharins intake.

Randomised trials

Two randomised control trials (Higgins et al., 2019; Orku et al., 2023) evaluated the effect of saccharin on body weight. Higgins et al. (2019), in a randomised control trial ($N = 154$) compared the effects of daily consumption of beverages containing sucrose ($N = 39$) and four different sweeteners (0.58 g/day aspartame, 0.73 g/day saccharin, 0.66 g/day rebaudioside A and 0.16 g/day sucralose). After the 12-week trial period, an increase in body weight was observed for participants in the saccharin group (+1.18 ± 0.36 kg, $p = 0.02$), but not for the other sweeteners groups. Orku et al., 2023, in a 4-week randomised single-blinded, controlled trial ($N = 48$) evaluated the effect of saccharin (140 mg/day) or sucralose (66 mg/day) and a mixture of aspartame and acesulfame-K (8 mg aspartame and 88 mg acesulfame-K per day) on body weight, body composition and waist circumference, and found no effect.

The use of non-apical outcomes (e.g. HDL, metabolic syndrome risk factors) and non-representative study populations were the main issues in the cross-sectional studies. Assessment of saccharins intake in the observational studies was mainly estimated from food items containing saccharins as an additive. Food frequency and 24-h food recall were the methods used in the studies, except for the study by Steffen et al. (2023), who used a diet history questionnaire. Main shortcomings in the trials were no double blinding and the lack of intention to treat analysis, while in the cohort studies, selection bias was the main issue.

For the HOC 'Cardiovascular risk factors', effects on weight gain were reported in the included human studies. In particular, results from a prospective cohort study ($n = 3088$, Steffen et al., 2023) and a randomised control trial ($n = 154$; Higgins et al., 2019) suggest that intake of saccharins at 0.73 g/day (Higgins et al., 2019) and at > 17.1 mg/day (averaged intake T3 = 65.62 mg daily; Steffen et al., 2023) are linked to increase in body weight. The Panel considered the confidence in the body of evidence to be low (see Table 15). Therefore, the Panel considered that there is low confidence in the body of evidence for an association between exposure to saccharins (E 954) and weight gain. Studies assessing other cardiovascular disease risk factors were few and inconclusive; the confidence in the body of evidence was low. Therefore, the Panel considered the level of evidence for no effect as inadequate.

Glucose/insulin homeostasis

Eight studies evaluated the association between saccharins intake and endpoints related to glucose homeostasis (Table 14). Five studies represented controlled trials (sample size, range 9–120), one cohort study was identified (Jensen et al., 2020), while two studies were of cross-sectional design with a sample size of 125 and 2856 participants, respectively. Five studies were conducted in the USA, two in Turkey, and one in Israel; the populations under study consisted of adults with weight in the normal range with the exception of one interventional study where the included participants were overweight or obese (BMI between 25 and 40; Higgins et al., 2019).

All five controlled trials were randomised and with generally small sample sizes (range, 9–120). The saccharins interventions under study were of generally short duration and were reported as follows: 0.24 g single dose ($n = 1$), 0.18 g or 0.40 g daily for 2 weeks ($n = 2$), 0.14 g daily for 4 weeks ($n = 1$) and 0.75 g daily for 12 weeks ($n = 1$). The control interventions included

[41]Dose as reported in the publication. The Panel note that this dose seems to be unrealistic considering the daily doses of saccharin reported in similar studies.

aspartame, sucralose, rebA, sucrose, lactisole or water. Compliance was not described in detail. In addition, the source of the intervention was not reported. The following endpoints were assessed usually via the implementation of oral glucose tolerance tests (OGTT): fasting plasma glucose ($n = 6$), glucose through a continuous glucose monitoring system ($n = 1$), OGTT plasma glucose ($n = 4$), fasting insulin ($n = 3$), postprandial insulin ($n = 1$), OGTT insulin ($n = 4$), HOMA-IR (Homeostasis model assessment: insulin resistance; $n = 1$), Matsuda index ($n = 1$), HbA1c ($n = 1$), fasting GLP-1 ($n = 1$) and OGTT GLP-1 ($n = 1$), OGTT C-peptide ($n = 1$) and OGTT glucagon ($n = 1$).

In the largest study, Suez et al. (2022) reported on a randomised-controlled trial in Israel ($n = 120$) where participants were administered a combination of glucose (as bulking agent) and saccharin or glucose (as bulking agent) and other sweeteners for 2 weeks in doses lower than the ADI (20% of the FDA ADI) and were compared to sachet-contained vehicle glucose or no supplement. After the 2-week administration of the interventions, participants were followed up for one more week. The resulting daily dose of saccharin was 0.18 g/day. Saccharin significantly elevated glycaemic response (elevated glucose levels following an OGTT) during exposure compared with the glucose control and with the no-supplement control. When the glycaemic response estimates were normalised with the average baseline glycaemic response, the normalised glycaemic response was also significantly higher in the saccharin group compared with the glucose vehicle group during the 1st ($p = 0.023$) and 2nd week ($p = 0.047$) of exposure. When the glycaemic response was compared to the baseline response within each group, saccharin significantly elevated glycaemic response, starting from the 1st week of exposure ($p = 0.0073$, iAUC mean difference 783.5, 95% confidence interval [CI] 204.3–1363) and persisting to the 2nd week of exposure ($p = 0.0094$, mean 811.2, CI 190.6–1432). At the end of follow-up, no statistically significant differences were observed. Moreover, the daily coefficient of variance (CoV) in glucose (closed glucose monitoring system) did not show higher variability in the saccharin compared to the glucose control group, and no differences were observed for insulin. The remaining four small randomised controlled trials did not report any statistically significant association between saccharins administration and fasting plasma glucose ($n = 3$), OGTT plasma glucose ($n = 3$), glucose through a continuous glucose monitoring system ($n = 1$), fasting insulin ($n = 2$), post-prandial insulin ($n = 1$) and OGTT insulin ($n = 2$), HOMA-IR ($n = 1$), Matsuda index ($n = 1$), HbA1c ($n = 1$), fasting GLP-1 ($n = 1$) and OGTT GLP-1 ($n = 1$), OGTT C-peptide ($n = 1$) and OGTT glucagon ($n = 1$) (Orku et al., 2023; Higgins et al., 2019; Serrano et al., 2021; Bayındır Gümüş, Keser, Tunçer, Altuntaş Yıldız, & Kepenekci, 2022).

Jensen et al. (2020) conducted a cohort study (8-years follow-up) among American Indians ($N = 1142$) to investigate the association between diet soda and non-caloric sweeteners, including saccharins, and incidence of diabetes. The Block food-frequency questionnaire and an additional questionnaire were used to assess diet and diet soda and non-caloric sweeteners consumption, respectively. After adjusting for age, sex, study site, BMI, education, steps per day, smoking, quality of life, total calories, saturated fat, intake of fruit and vegetable, processed meat, fibre and sweetened sugar beverages, no association was found between saccharins use (yes/no) and incidence of diabetes (HR:1.38; 95%CI:0.8–2.37). At baseline, saccharins use was associated with a fasting insulin (GMR: 1.11; 95%CI 1.02–1.21). No association was found for fasting glucose at baseline. Limitations of the study were the high loss of follow-up and missing values.

In the two cross-sectional studies (both evaluated as of moderate risk of bias), saccharins intake was assessed either from the total nutrient intake file (CDC The Total Nutrient Intake File (CDC, NCHS 1996; consumers or non-consumers of saccharins) (Kuk and Brown, 2016; $n = 2856$) or through calculations of the specific amounts of saccharins consumed in milligrams using the Nutrition Data System for Research (NDS-R) and participant data from repeated 24-h recalls (Hess et al., $n = 125$). In the adjusted analyses, neither of these studies reported a statistically significant association between saccharins intake and fasting plasma glucose ($n = 2$), glucose tolerance ($n = 1$) or HOMA-IR ($n = 1$). The cumulative observational evidence was mainly limited by the small number of studies ($n = 2$) and the lack of prospective data.

For the HOC 'Glucose/insulin homeostasis', no adverse effects were identified in the included human studies. For those studies, the Panel considered the confidence in the body of evidence to be 'Low' (see Table 15). The level of evidence for the lack of observed adverse effects was rated as 'Inadequate' (small number of small studies of low or moderate risk of bias). Therefore, the Panel considered that there is inadequate evidence available to assess whether the exposure to saccharins (E 954) is associated with impaired glucose homeostasis.

Table 17 provides an overview of the translation of confidence ratings into level of evidence for conclusions of adverse effects on health or no adverse effect on health for the included human studies for each of the HOC considered in the assessment.

TABLE 17 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 HOC considered in the assessment.

	Final rating of confidence	Level of evidence
Bladder cancer	Very low	The Panel considered that there is inadequate evidence available in the two human studies included in the current re-evaluation to assess whether the exposure to saccharins (E 954) is associated with bladder cancer. Considering the previous assessments JECFA (1993), SCF (1995) and IARC (1999), and the current knowledge on the non-human-relevant mechanism behind bladder cancer in animals, the Panel considered it unlikely that there is an association between exposure to saccharins (E 954) and bladder cancer in humans.

TABLE 17 (Continued)

	Final rating of confidence	Level of evidence
Other types of cancer	Low	The Panel considered that there is inadequate evidence available in these limited studies included in the current re-evaluation to assess whether the exposure to saccharins (E 954) is associated with cancer. In summary, the newly available human data are in line with the previous conclusions by JECFA (1993), SCF (1995) and IARC (1999) that saccharins (E 954) exposure is not associated with cancer.
Cardiovascular risk factors	Low	Effects on weight gain were reported in the included human studies. The Panel considered that there is low confidence in the body of evidence for an association between exposure to saccharins (E 954) and weight gain. Studies assessing other cardiovascular disease risk factors were few and inconclusive; the confidence in the body of evidence was low. Therefore, the Panel considered the level of evidence for no effect as inadequate.
Glucose/insulin homeostasis	Low	The Panel considered that there is inadequate evidence available to assess whether the exposure to saccharins (E 954) is associated with impaired glucose homeostasis.

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 the definitions given in the protocol (EFSA FAF Panel, 2023) and taking into consideration the assessment in 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 saccharins (E 954) and an adverse effect on human health. The integration 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 (SCF, 1995; JECFA, 1993; IARC, 1999) to assess whether the new data support them. In the case of the HOCs with data available only from animal studies (namely 'reproductive toxicity', 'haematotoxicity', 'liver toxicity', 'nephrotoxicity'), the integration of evidence was done considering the option 'missing data' from Figure A.2, Appendix A, see Table 18.

TABLE 18 Overview of the HOCs for which human and animal evidence streams were integrated. Similar HOCs are presented on the same row.

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

The Panel considered it appropriate to integrate aspects of HOCs **'general toxicity'** and **'additional clinical chemistry'** from animal studies with **'cardiovascular risk factors'** addressed in human studies (see Tables 11 and 14). The following was concluded:

- It is noted that, at low doses of saccharins (2.5–730 mg/kg bw per day; Boakes et al., 2016; Azeez et al., 2019; Bian et al., 2017; Jiang et al., 2018; Shi et al., 2019; Shi et al., 2021; Li, Geng, et al., 2019, Li, Ren, et al., 2019), most animal studies reported modest increases (less than 10%) in mean final body weight compared to control. The highest increase was between 10% and 25%., in two studies (Azeez et al., 2019; Li, Geng, et al., 2019; Li, Ren, et al., 2019) The Panel noted that weight gain at low doses might be related to increased feed intake (data insufficient). In the absence of effects on toxicological endpoints in this dose range, the Panel considered this effect as not adverse. The two included human studies (one observational and one interventional), at normally consumed doses or at doses twice the ADI of 5 mg/kg bw per day (SCF, 1995), provided only limited support for these findings (low level of evidence for the observed effect). In the 12-week intervention human study, the effect size was modest (mean 1 kg gain). In consideration of this, the Panel concluded that it is as likely as not that saccharins (E 954) exposure in humans is associated with a small increase in body weight at doses up to twice the ADI of 5 mg/kg bw per day (SCF, 1995).
- It is likely that saccharins (E 954) exposure is associated with a decrease in body weight in animals at high doses, i.e. at or above 4500 mg/kg bw per day. Equivalent high doses were not examined in human studies.
- From the integration of animal and human data, it is unlikely that saccharins (E 954) exposure is associated with effects on body fat mass and percentage in humans.
- Saccharins exposure was not associated with adverse changes in clinical chemistry parameters in animals (moderate level of evidence) and human (low level of evidence). The Panel noted that cholesterol, HDL, LDL and triglycerides were measured in animals while in humans only HDL and triglycerides were measured in a single study. The Panel concluded that it is unlikely that saccharins (E 954) exposure is associated with adverse effects on clinical chemistry parameters in humans.

The HOC **'cancer'** from human studies was integrated with **'other organ toxicity'** in animal studies.

Regarding **bladder cancer**, as already reported in Section 3.5.4.1, the Panel noted that sodium saccharin produces urothelial bladder tumours in rats by a sex-specific mechanism which is not relevant to humans because of interspecies differences in urine composition. As reported in Section 3.5.4.2.1, the Panel considered that there is inadequate evidence available in the two human studies included in the current re-evaluation to assess whether the exposure to saccharins (E 954) is associated with bladder cancer in humans. However, considering the previous assessments JECFA (1993), SCF (1995) and IARC (1999) and the current knowledge on the non-human-relevant mechanism behind bladder cancer in male rats, the Panel concluded that it is unlikely that exposure to saccharins (E 954) causes bladder cancer in humans.

Regarding other types of **cancers**, taking into account the included animal studies, the Panel considered that there is a moderate level of evidence that the exposure to saccharins (E 954) is not associated with cancer. The Panel considered the evidence in human data to be inadequate to assess whether the exposure to saccharins (E 954) is associated with cancer. Based on the integrated level of evidence, the Panel concluded, considering also mechanistic data, that it is unlikely that exposure to saccharins (E 954) is associated with cancer in humans.

Regarding the HOC **'glucose/insulin homeostasis'** in the included animal studies, the level of evidence was rated as 'moderate' while for human studies the level of evidence was rated as 'inadequate'. Therefore, it is as likely as not that saccharins (E 954) is not associated with disturbances of glucose/insulin homeostasis in humans. Based on biological considerations, the Panel concluded that the human and animal studies reviewed provided consistent yet limited evidence for no short-term/acute effect of saccharins (E 954) on glucose homeostasis. Suitable studies addressing long-term/chronic effects were not identified.

Regarding the HOC '**nephrotoxicity'** in the included animal studies, the level of evidence for the lack of effects observed is 'moderate'. No studies in humans were included in the WoE.[42] Therefore, the Panel concluded that it is as likely as not that saccharins (E 954) exposure is not associated with nephrotoxicity in humans.

Regarding the HOC '**reproductive toxicity**', only animal data were available; the level of evidence for effects is 'moderate'. Therefore, the Panel concluded that it is as likely as not that saccharins (E 954) exposure is associated with reproductive toxicity in humans.

Regarding the HOC **'haematotoxicity',** no direct adverse haematological effects were identified in the included animal studies (except for secondary anaemia due to glandular stomach haemorrhage at high dose; see HOC 'other organs toxicity'). For those studies, the Panel considered the level of evidence to be 'high'. Human data were not available. Therefore, the Panel concluded that it is unlikely that saccharins (E 954) exposure is associated with haematological effects in humans.

For the HOC **'liver toxicity',** no adverse effects were observed on liver (weight, histopathology or enzymes) in the included animal studies. For those studies, the Panel considered the level of evidence to be moderate. Human data were not available. Therefore, the Panel concluded that it is as likely as not that saccharins (E 954) exposure is not associated with liver toxicity in humans.

3.5.5 | Hazard characterisation and identification of a reference point

Table 19 provides an overview of the conclusions on the level of likelihood for the association or absence of association between the intake of saccharins (E 954) and an adverse effect on health for each group of integrated HOCs (see Section 3.5.4.3).

TABLE 19 Overview of the conclusions on the level of likelihood of an association or absence of association between the intake of saccharins (E 954) and an adverse effect on human health for each HOCs.

Evidence stream	HOC	Integration of the evidence (likelihood)
Human Animal	Cardiovascular risk factors General toxicity/body weight	It is **likely** that saccharins (E 954) exposure is associated with a decrease in body weight in animals at high doses, i.e. at or above 4500 mg/kg bw per day
Human Animal	Cardiovascular risk factors General toxicity/body weight	It is as **likely as not** that saccharins (E 954) exposure is associated with a small increase in body weight at doses up to twice the ADI (SCF, 1995), adversity is unclear
Human Animal	Glucose/insulin homeostasis	It is **as likely as not** that saccharins (E 954) exposure is not associated with disturbances of glucose/insulin homeostasis. Based on biological considerations, the Panel concluded that the human and animal studies reviewed provided consistent yet limited evidence for no short-term/acute effect of saccharins (E 954) on glucose homeostasis. Suitable studies addressing long-term/chronic effects were not identified
Human Animal	No data available Nephrotoxicity	It is **as likely as not** that saccharins (E 954) exposure is not associated with nephrotoxicity
Human Animal	No data available Reproductive and developmental toxicity	It is **as likely as not** that saccharins (E 954) exposure is associated with reproductive toxicity

[42] A study addressing the incidence of chronic kidney disease based on a metabolomic analysis, with 'saccharin' level measured in serum but no intake data on saccharins reported, was retrieved in the literature search and is summarised narratively, see Appendix C (Su et al., 2023).

TABLE 19 (Continued)

Evidence stream	HOC	Integration of the evidence (likelihood)
Human Animal	No data available Liver toxicity	It is **as likely as not** that saccharins (E 954) exposure is not associated with liver toxicity
Human Animal	Cardiovascular risk factors General toxicity	It is **unlikely** that saccharins (E 954) exposure is associated with effects on body fat mass and percentage
Human Animal	Cardiovascular risk factors Additional clinical parameters	It is **unlikely** that saccharins (E 954) exposure is associated with adverse effects on clinical chemistry parameters
Human Animal	Cancer Organ toxicity	It is **unlikely** that saccharins (E 954) exposure is associated with cancer
Human Animal	No data available Haematotoxicity	It is **unlikely** that saccharins (E 954) exposure is associated with haematological effects

Abbreviations: GLP-1, Glucagon Like peptide-1; GTT, glucose tolerance test; HOC, health outcome category.

For the selection of a reference point, considering the outcome of the assessment as above, the Panel selected the reference point based on HOCs for which adverse effects was considered 'likely'.

The Panel considered it likely that the exposure to saccharins (E 954) is associated with a decrease in body weight and, therefore, considered this endpoint for the identification of a reference point. The Panel considered a decrease in body weight equal or higher to 10% to be adverse. Among the included toxicological studies (see Figure 4), the 8-week study in rats by Uwagawa et al. (1994), in which a decrease in the final body weight of 15% at 4500 mg sodium saccharin/kg bw per day was observed, was selected for the identification of a reference point. A LOAEL of 4500 mg sodium saccharin/kg bw per day was derived. The Panel noted that the exposure estimates reported in Section 3.4.1 are expressed as saccharin free imide. Taking into account the conversion factor of 0.76 reported in Section 3.1.1 based on differences between molecular weights, the Panel converted the LOAEL to 3420 mg saccharin free imide /kg bw per day.

In addition to the default uncertainty **factor of 100**, the Panel applied the following extra factors:

- A factor of 2 for the extrapolation from the LOAEL to the NOAEL; according to EFSA (EFSA Scientific Committee, 2012), in cases where the BMD approach cannot be applied, the LOAEL approach might be used and an additional factor will be needed, the size of which should be determined on a case-by-case basis. Considering the available data (see Figure 4) and the publication from Batke et al. (2013), the Panel considered this factor as appropriate.
- A factor of 2 for the extrapolation to chronic exposure according to EFSA (EFSA Scientific Committee, 2012)

Considering the above, an ADI of 8.55 mg/kg bw per day rounded to 9 mg/kg bw per day was derived expressed as saccharin free imide, for saccharin and its sodium, potassium and calcium salts (E 954).

3.6 | Environmental considerations

The environmental safety of sodium saccharin was previously assessed by the EFSA FEEDAP Panel in the context of its evaluation as a sweetener in feed and water for drinking for piglets, pigs for fattening and veal calve (EFSA FEEDAP Panel, 2018a). The FEEDAP Panel concluded that the use of sodium saccharin at the level considered safe for target species was unlikely to have detrimental effects on the terrestrial and freshwater compartments. The FEEDAP Panel additionally concluded that the high mobility and relative persistence of sodium saccharin and the high persistence of its transformation product 4-hydroxysaccharin indicated that groundwater contamination above the trigger value for groundwater of 0.1 μg/L is likely to occur. In 2023, the FEEDAP Panel assessed newly available data from the applicant and concluded that 1.13 mg sodium saccharin/kg feed could not be considered safe for the environment. The FEEDAP Panel estimated that the use level that would result in a concentration in groundwater below the threshold of 0.1 μg/L is 0.022 mg sodium saccharin/kg feed. The available data did not allow to conclude on the potential effect of the transformation product 4-hydroxysaccharin in groundwater (EFSA FEEDAP Panel, 2023a).

A systematic review collating published data on saccharins (E 954), was performed to identify evidence of potential adverse effects on the environment (Agriculture and Environment Research Unit, University of Hertfordshire, 2021) resulting from the use of saccharins (E 954) as food additive. This review was updated and 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 saccharin is not metabolised in humans. Saccharin has a half-life of ~4 h and is primarily excreted into the urine. Therefore, saccharin when consumed as food additive, has the potential to reach the environment via wastewater. It is expected that the main receiving compartment will be the aquatic environment. Saccharins (E 954), used as food additive, may also reach the terrestrial environment (e.g. via fertilisation with contaminated sewage sludge or flood events), these routes of exposure are expected to be less relevant than the direct emission from waste-water facilities into surface water. The Panel noted that no additional data on the degradation

product of sodium saccharin i.e. 4-hydroxysaccharin (EFSA FEEDAP Panel, 2018a, 2023a) were retrieved in the literature; however, this degradation product is reported as a major metabolite in soil (EFSA FEEDAP Panel, 2018a, 2023a).

The amount of saccharin[43] that may reach the aquatic environment is dependent on how efficiently waste-water treatment plants can remove it from their influent. According to the above-mentioned review (Agriculture and Environment Research Unit, University of Hertfordshire, 2021), there is debate and disagreement regarding the efficiency of treatment plants in removing saccharin from wastewater. In the updated literature search following-up on this review, additional studies dealing with the removal of saccharin from wastewater and with the development of new removal techniques were identified. These papers reported different removal efficiencies depending on the applied removal technique and reconfirmed the previous conclusions (Alipour et al., 2022; Alves et al., 2021; Bernardo et al., 2005, 2006; Brice et al., 2022; Davididou et al., 2017, 2018, 2019; Deng et al., 2019; Diniz & Rath, 2023; Diniz, Crick, & Rath, 2023 Diniz, Gasparini Fernandes Cunha, & Rath, 2023B; Hermes et al., 2019; Jamil et al., 2019; Jamil et al., 2021; Kulandaivelu et al., 2020; Li et al., 2019a; Gaudet-Hull et al., 1994b; Liu, Blowes, & Ptacek, 2019; Liu, Blowes, Ptacek, & Groza, 2019; Ma et al., 2020; Ma, Tang, et al., 2021; Mailler et al., 2016; Moško et al., 2021; Olmez-Hanci et al., 2020; Presumido et al., 2022; Qu et al., 2019; Scheurer et al., 2010; Seller et al., 2021; Semblante et al., 2017; Seo et al., 2016; Song et al., 2018; Toth et al., 2012; Tran et al., 2014; Vymazal & Březinová, 2016; Xu et al., 2022; Yang et al., 2022; Ye et al., 2022; Zelinski et al., 2018).

In the previous review (Agriculture and Environment Research Unit, University of Hertfordshire, 2021), saccharin was reported as not readily biodegradable using the close bottle test (OECD 301D) but was considered readily biodegradable under a manometric respirometry test (OECD 301F; 88% degradation; Bergheim et al., 2015). Additional references were retrieved following the update of the literature search on the biodegradability of saccharin. In Gatidou et al. (2020), a ready biodegradability test (manometric respirometry test according to OECD 301F) reported saccharin met the definition for ready biodegradability with a degradation percentage of about 76%. Seller et al. (2020); Desiante et al. (2021); and Seller et al. (2021) reported that the biodegradation of saccharin depends on the specific microbiological conditions. The study from Chen et al. (2013) (reviewed in Agriculture and Environment Research Unit, University of Hertfordshire, 2021) reported that saccharin is highly persistent in the aquatic environment with a half-life of several years.

From the available literature, there is evidence for the presence of saccharin in several environmental compartments (see Annex F). In the studies where saccharin was measured both in the waste water influent and effluent (Baena-Nogueras et al., 2018; Gan, Sun, Wang, & Feng, 2013; Kokotou & Thomaidis, 2013; Ordóñez et al., 2012; Subedi & Kannan, 2014; Watanabe et al., 2016; Buerge et al., 2009; Arbeláez et al., 2015; Hermes et al., 2018; Tran et al., 2019; Guo et al., 2021; Yue et al., 2023; Kerberová et al., 2022; Ijaz et al., 2023; Alves et al., 2021), the concentration of saccharin was generally lower in the effluent demonstrating to some extent the removal (or conversion to degradation products) of this substance from wastewater.

The highest concentration of saccharin measured in surface water from the available literature studies was 19.7 µg/L (range 0.027–19.7 µg/L). In some studies, saccharin was reported as not detected (Edwards et al., 2017; Tran et al., 2015; Albergamo et al., 2018; Montes et al., 2017; Montes et al., 2019; Gvozdić et al., 2020; Gvozdić et al., 2023; Buerge et al., 2009; Fu et al., 2020; Alves et al., 2021, see also Annex G). The concentration of saccharin in sediment was measured in three of the available literature studies (Gvozdić et al., 2020; Fu et al., 2020; Gvozdić et al., 2023) and saccharin was detected only in one study from China and ranged between 1.63 and 4.17 ng/g dw (Fu et al., 2020). Saccharin was measured and detected in ground water in several studies (0.0027–26.7 µg/L in Berset & Ochsenbein, 2012; Buerge et al., 2009; Edwards et al., 2019; Gan, Sun, Feng, et al., 2013; Gan, Sun, Wang, & Feng, 2013; Han et al., 2022; Khezami et al., 2024; Lee et al., 2019; Ma, Li, & Zhang, 2021; Montes et al., 2017; Moreau et al., 2019; Roy et al., 2014; Tran et al., 2013; Tran et al., 2014; Van Stempvoort, Robertson, & Brown, 2011; Van Stempvoort, Roy, et al., 2011; Watanabe et al., 2016; Yang et al., 2018; Yu, Yu, et al., 2023, and not detected in Sharma et al., 2019; Gvozdić et al., 2020; Datel & Hrabankova, 2020;) while the soil concentration was measured only in one study (Ma, Li, & Zhang, 2021). The international platform of chemical monitoring (IPCHEM)[44] includes data on the concentration of saccharin from different EU and non-EU countries and in different environmental compartments from several EU and no-EU countries (from EMPODAT[45]). The reported values range from 0.001 to 1.7 µg/L for surface water. According to the same database saccharin was not detected in sediment and detected only in few instances in ground water.

Ecotoxicological endpoints for saccharin[46] in aquatic organisms were available from the evaluation of sodium saccharin for its use as feed additive (EFSA FEEDAP Panel, 2018a), and from the EFSA conclusions on several pesticide active substances; saccharin is a metabolite of some pesticides including sulfonylureas (EFSA, 2014, 2015c, 2016, 2017a, 2017b). The updated literature search provided limited information on the toxicity of saccharin to aquatic organisms (Kerberová et al., 2022; Zelinski et al., 2018), see Annex F.

According to the review from Agriculture and Environment Research Unit, University of Hertfordshire (2021) saccharin '*does not appear to be highly toxic to aquatic organisms at current environmental concentrations*'. This conclusion was based on the few available ecotoxicological studies at the time of the review which included data on various taxonomic groups. Of note, the review reported a 96-h LC50 (lethal concentration, median) for the fathead minnow (*Pimephales promelas*) of

[43]It is expected that the salts of saccharin rapidly dissociated into the environment to saccharin as free imide therefore the general term 'saccharin' is used in this section unless further specified.

[44]https://ipchem.jrc.ec.europa.eu/#databaseConsole/EMPODAT/saccharin/81-07-2/none/Water%20(Surface%20Water).

[45]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.

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

150 µg/L as cited in an interlaboratory validation study (Gaudet-Hullet al., 1994).[47] The review also flagged possible concerns for the long-term toxicity to aquatic organisms following the exposure to saccharin at 1 and 10 mg/L, referring specifically to a developmental toxicity study of saccharin in medaka (*Oryzias latipes*) reporting increased heart rate, behavioural and developmental effects (Lee & Wang, 2015). The Panel noted that standard chronic toxicity studies in aquatic invertebrates or fish were not identified in the literature and were not available from previous EFSA assessments.

The concentrations measured in the aquatic compartment are in general substantially lower than most of the available ecotoxicological endpoints.[48] However, this is not the case when the lowest endpoints available (LC50 for *Pimephales promelas* and NOEC for *Lemna gibba*) are considered. The Panel noted that the available data on the environmental concentrations of saccharin 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 levels of saccharin and may not have captured the worst-case exposure for aquatic organisms. Additionally, the measured saccharin concentrations in such studies may result from different sources (e.g. use as feed additive, use in personal care products) and the contribution of the use of saccharins (E 954) as food additive is unknown. This is particularly important for the measured concentrations in soil and groundwater where the use as food additive is expected to be a less relevant source of contamination with respect to other potential sources. The available data on environmental concentrations are therefore to be considered as supportive information only and more evidence might be needed to exclude a concern for the environment. Such evidence could include the modelling of the exposure under realistic worst case scenarios representative of the European situation.

Overall, in the absence of a guidance on the environmental risk assessment of food additives, a full environmental risk assessment for saccharins (E 954) could not be performed.

3.7 | Discussion

The present opinion deals with the re-evaluation of saccharin and its sodium, potassium and calcium salts (E 954), authorised as food additives in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives.

Saccharins (E 954) can be manufactured by the Remsen-Fahlberg process or the Maumee process. No description of the manufacturing processes of E 954 is included in Commission Regulation (EU) No 231/2012. Since only IBOs manufacturing saccharins using the Remsen-Fahlberg process expressed an interest following the EFSA call for technical data,[49] and no analytical data on potential impurities were provided for products manufactured with the Maumee process, the Panel could only evaluate saccharins (E 954) manufactured with the Remsen-Fahlberg process. Thus, the Panel proposed to add a definition in the EU specifications of these food additives, restricted to the manufacturing with the Remsen-Fahlberg process.

The purity of saccharin (E 954 (i)), sodium saccharin (E 954 (ii)) and potassium saccharin (E 954 (iv)) is reported as 'Not less than 99 % on the anhydrous basis' in the EU specifications. The Panel noted that the purity of calcium saccharin (E 954 (iii)) in the EU specifications is 'Not less than 95 % of $C_{14}H_8CaN_2O_6S_2$ on the anhydrous basis', while in the JECFA specifications is 'Not less than 99 % after drying'. Considering the limits of the impurities in the specifications of calcium saccharin (E 954 (iii)), there is no indication for this lower purity of the salt as such. The Panel recommended that, even though no analytical data were provided by the IBOs on the purity of calcium saccharin (E 954 (iii)), the assay could be modified to 'Not less than 99 % of $C_{14}H_8CaN_2O_6S_2$ on the anhydrous basis' in the EU specifications.

In the EU specifications saccharin (E 954 (i)) is defined (according to the chemical name) as an anhydrous substance (molecular weight 183.18), whereas the sodium salt (E 954 (ii)) is defined as a dihydrate (molecular weight 241.19), the potassium salt (E 954 (iv)) as a monohydrate (molecular weight 239.77) and the calcium salt (E 954 (iii)) as a hydrate with two saccharin units and 3.5-waters of crystallisation (molecular weight 467.48). However, the Panel noted that the EINECS numbers 204–886-1 for sodium saccharin (E 954 (ii)) and 229-349-9 for calcium saccharin (E 954 (iii)) reported in the EU specifications refer to the anhydrous substances. The Panel also noted that the CAS numbers for saccharin (E 954 (i)), sodium saccharin (E 954 (ii)) and potassium saccharin (E 954 (iv)) are not included in the EU specifications. The CAS numbers indicated by JECFA for sodium saccharin (128-44-9) and potassium saccharin (10332-51-1) refer to the anhydrous substances. The CAS number indicated by JECFA for saccharin (E 954 (i)) is 81-07-2 (JECFA, 2006).

The Panel noted that the current EU specifications for E 954 only include impurities derived from the Remsen-Fahlberg process. The Panel considered the TTC approach to conduct a risk assessment to organic impurities associated with the Remsen-Fahlberg process. Regarding those impurities included in the EU specifications, the Panel noted that the potential exposure to *o*-toluene sulfonamide, *p*-toluene sulfonamide and benzoic acid *p*-sulfonamide is below the Cramer Class III value of 1.5 µg/kg bw per day, and therefore does not raise a safety concern. The Panel noted that benzoic acid, another impurity of E 954, is an authorised food additive (E 210), with an ADI of 5 mg/kg bw per day (expressed as benzoic acid).

[47] As reported in Agriculture and Environment Research Unit, University of Hertfordshire, 2021. The original study was not available to EFSA.

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

[49] https://www.efsa.europa.eu/en/call/call-technical-data-saccharin-and-its-sodium-potassium-and-calcium-salts-e-954.

Considering that the purity of saccharin and its sodium, potassium and calcium salts is not less than 99% on the anhydrous basis, the maximum amount of salicylic acid, considering that other impurities are not present (worst-case scenario), would be 1%, resulting in a potential exposure to salicylic acid from the use of E 954 up to 77 μg/kg bw per day. When comparing with the lowest NOAEL for salicylic acid of 75 mg/kg bw per day, the Panel noted that the MOE would be at least 1000, and no safety concern was raised. Even if the purity of calcium saccharin was not less than 95% on the anhydrous basis, as indicated currently in the EU specifications, the exposure to this impurity would not be of concern. In addition, the Panel is aware that an Endocrine Disruptor (ED) assessment is ongoing for this substance under the Biocide regulatory framework. The Panel noted that if a HBGV will be established as an outcome of the ongoing assessment in the other regulatory frameworks, a numerical limit of salicylic acid for the EU specifications of E 954 could be considered.

The Panel noted that the specification for 'readily carbonisable substances' in the EU regulation is expressed as 'Absent'. The JECFA specifications (JECFA, 2006) for this parameter are based on formation of colour using a prescribed test method. The data were provided as 'passes test' by the IBOs in five batches of saccharin and five batches of sodium saccharin, without indicating the sensitivity of the method. The Panel noted that the parameter 'readily carbonisable substances' is unspecific, and therefore not needed in the EU specifications of E 954.

Regarding toxic elements, the Panel performed the risk assessment that would result if arsenic, and lead were present in E 954 at the current maximum limits in the EU specifications and at the lowest reported LOD or reporting limit by the IBOs.

Considering the results of the exposure to the toxic element Pb, the Panel noted that its presence in E 954 at the current specification limit value would not give rise to concern. In the case of As, the Panel noted that its presence in E 954 at the current specification limit value would lead to an MOE around 3, which is considered insufficient. The Panel noted that the analytical data provided for Pb and As were reporting limits or below the LODs. The IBOs did not indicate the lowest technologically achievable levels for these toxic elements. The Panel considered that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. The Panel is of the view that the current EU specification limits for Pb and As should be lowered.

A current maximum limit for selenium of 'not more than 30 mg/kg' is set in the EU specifications of E 954. The Panel noted that E 954 may contribute to the total European dietary Se exposure. The daily intakes of Se from the use of E 954, if Se was present at the level of 30 mg/kg (specification limit), would be 4.4 and 16.1 μg/day, which are circa 2% and 6%, respectively, of the UL of Se of 255 μg Se/day for adult men and women (including pregnant and lactating women). Considering the above calculated intakes of Se resulting from the use of E 954, the presence of Se in E 954 at the current specification limit would not be of concern.

In the absence of analytical data on the potential impurities associated with the Maumee process in the food additives, the exposure to the impurities attributed to the Maumee process could not be calculated and a risk assessment was, therefore, not performed.

Considering the microbiological data submitted by the IBOs, the Panel considered that a microbiological contamination is unlikely and, therefore, it is not necessary to recommend inclusion of microbiological criteria in the EU specifications for E 954.

The Panel noted that according to the literature data and the information provided, the solubility of sodium and calcium salt of saccharin ((E 954 (ii) and E 954 (iii), respectively) in water (see Section 3.1.4) is higher than the threshold value of 33.3 g/L as a decision criterion for demonstrating that the material does not require specific assessment at the nanoscale (EFSA Scientific Committee, 2021). Regarding saccharin (E 954 (i)), the reported solubility values (3.2 g/L or 2 g/L (at 20°C)), are lower than the threshold value of 33.3 g/L. The Panel noted that the maximum use levels of saccharin (E 954 (i)) reported by the food industry for various food categories (see Annex A) do not exceed 608 mg/kg and the highest MPL does not exceed 3000 mg/kg. For table-top sweeteners, the food additive is allowed *quantum satis*; however, these are not intended to be consumed as such and will be largely diluted in beverages and, accordingly, particles would be expected to dissolve. Taking into account the maximum reported use levels, the MPLs, the reported solubility values and the volume of gastric secretion (ranging from 215 mL within a single meal to 2000 mL daily; ICRP, 2002; Mudie et al., 2014), the Panel considered that full dissolution of E 954 (i) is to be expected in foods and/or in the gastrointestinal tract and that ingested particles (if any) would not persist. Considering the above, the Panel concluded that there is no concern with regard to the potential presence of small particles, including nanoparticles, in saccharin (E 954 (i)) and its sodium (E 954 (ii)) and calcium (E 954 (iii)) salts at the reported uses and use levels and considered that these food additives can be assessed following the conventional risk assessment, i.e. EFSA Guidance for submission for food additive evaluations (EFSA ANS Panel, 2012).

The Panel noted that, based on the submitted information on the stability of saccharins, E 954 is expected to be stable in food under the normal conditions of use, in accordance with the authorised uses of E 954.

The biological and toxicological data set available to the Panel for the re-evaluation of saccharins (E 954) 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 were 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, the Panel considered that the data on urinary excretion demonstrate that most, if not all, saccharin is absorbed when doses between 2 and 69 mg saccharin per person were administered orally. The Panel considered that saccharin is not metabolised, has a half-life of approximately 4 h and is primarily excreted into the urine. The Panel noted that, after administration, all the salts of saccharin will dissociate in biological fluids to saccharin (as free imide). Saccharin passes into breast milk and is capable of passing the placenta, as indicated by detection in amniotic fluid and cord blood samples, and entering the fetal circulation.

The Panel concluded that the newly available studies do not raise a concern for genotoxicity of saccharins (E 954), which concurs with the conclusion of the previous SCF opinion based on the database available at that time. Based on the available experimental and in silico data, the Panel concluded that saccharins impurities associated with the Remsen-Fahlberg process do not raise concern for genotoxicity. For the potential impurities associated with the Maumee process, a concern for genotoxicity was identified for benzamide, while the genotoxic potential of 2-chlorobenzamide could not be fully assessed.

An evaluation of the risk of bias (RoB) was performed (Annex E1 and E2) and a weight of evidence (WoE) approach for the reliable studies was applied for each health outcome for both human and animal studies (Appendix A, Annexes E1 and E2). Based on the outcome of WoE, the Panel considered it likely that the exposure to saccharins (E 954) at high doses is associated in animals with a decrease in body weight. The body weight decreases observed in animals were higher than 10% at doses equal to or higher than 4500 mg/kg bw per day. Generally, changes in body weight in laboratory rodents of this magnitude are considered adverse (WHO, 2015; van Berlo et al., 2022). These body weight changes did not appear to be clearly associated with a decrease in feed consumption. When the studies assessing doses equal or higher than 4500 mg/kg bw per day are considered it is noted that in the study from Schoenig et al. (Schoenig et al., 1985, one generation) F0 feed intake was increased in all groups. In Cohen, Garland, et al. (1995) (one generation study) and in -Cohen et al. (1996) (10-week study), no effect on feed intake at 6750 mg/kg bw per day was observed. In Uwagawa et al. (1994), feed intake was measured but no data are reported. The Panel noted that reduced body weight was not observed in the included human studies; however, the exposure to equivalent high doses was not examined.

The Panel also noted that at low doses of saccharins (2.5–730 mg/kg bw/day), most animal studies reported modest increases (less than 10%) in mean final body weight compared to the control. The highest increase was between 10% and 25%, in two studies (Azeez et al., 2019; Li, Geng, et al., 2019; Li, Ren, et al., 2019). The Panel noted that this weight gain at low doses might be related to increased feed intake (data insufficient) and may reflect body weight changes which are well known from the use of sodium saccharin as fattening agent in farmed animals (EFSA FEEDAP Panel, 2018a, 2018b). Furthermore, in the absence of toxicological effects in this dose range, the Panel considered this effect not adverse. The two included human studies (one observational and one interventional), at normally consumed doses or at doses twice the ADI previously set by the SCF and JECFA (SCF, 1995; JECFA, 1993), provided only limited support for these findings (i.e. low level of evidence for the observed effect). Overall, the Panel considered that the association between exposure to saccharins (E 954) and increase in body weight has not been convincingly demonstrated by the available studies (WoE analysis in accordance with the protocol: it is 'as likely as not' that saccharins (E 954) exposure in humans is associated with a small increase in body weight at doses up to twice the ADI of 5 mg/kg bw per day set by the SCF and JECFA).

Because of the possible health implications of increases in body weight, the Panel considered that further studies and research would be needed to understand any potential role of saccharins (E 954) in promoting this effect. The Panel is aware of existing evaluations from other bodies on the association between exposure to non-nutritive sweeteners and body weight gain (BfR, 2023; Rios-Leyvraz & Montez, 2022).

The Panel noted that the ADI of 5 mg /kg bw per day (expressed as sodium saccharin, corresponding to 3.8 mg /kg bw per day saccharin as free imide) established by the SCF in 1995 was derived from the NOEL of 500 mg sodium saccharin/kg bw for bladder tumours in male rat (Schoenig et al., 1985) and by applying an uncertainty factor of 100. Based on the studies available at that time, the SCF noted that the mechanistic studies and the epidemiological studies strongly indicated that saccharin is not related to bladder cancer in humans but since it has not been possible to unequivocally demonstrate this, the Committee 'as a matter of prudence' decided to take these lesions into account in setting the ADI. The Panel noted that, according to the current knowledge, the bladder tumours observed in male rats are not considered relevant to humans. The Panel also noted that the ADI of 5 mg/kg bw per day for sodium saccharin established by JECFA in 1993 was set considering the same pivotal study (Schoenig et al., 1985) as in SCF (1995) but identified a NOEL of 500 mg/kg bw per day based on a 'marked disturbance of the homeostasis' described as 'persistent dose-related decreases in body weight gain in the presence of increased food consumption probably related to the inhibitory effects of saccharin on carbohydrate and protein digestion'. The study from Schoenig et al. (1985) has been evaluated in the WoE analysis together with the new evidence.

The Panel considered the decrease in body weight in animal studies as the relevant endpoint for the derivation of a reference point and, following the 2014 ANS Panel conceptual framework approach for the re-evaluation of food additives (EFSA ANS Panel, 2014), considered it appropriate to set a numerical ADI. In the absence of an appropriate NOAEL and suitable data for a BMD modelling, a reference point was identified by the Panel as the LOAEL of 4500 mg sodium saccharin/kg bw per day (corresponding to a LOAEL of 3420 mg saccharin as free imide/kg bw per day) based on the observed body weight decrease (–15%) from the 8-week study in rats by Uwagawa et al. (1994). In derivation of an ADI, the Panel considered that, in addition to the default uncertainty factor of 100, an extra factor of 2 for the extrapolation from the LOAEL to the NOAEL and another factor of 2 for the extrapolation to chronic exposure should be applied (EFSA Scientific Committee, 2012). Consequently, an ADI of 9 mg/kg bw per day, expressed as free imide, was derived for saccharin and its sodium, potassium and calcium salts (E 954).

One of the included studies (Stampe et al., 2022, see Section 3.5.1) reported information on the concentration of saccharin in breast milk following the intake of 20 mg saccharins by the nursing mothers (81.5 ng/mL). Using the maximum concentration measured in breast milk in this study, the Panel estimated the concentration of saccharin as free imide by linear extrapolation in breast milk for an intake of the mother corresponding to the ADI (9 mg/kg bw per day) and assuming a body weight of the mothers of 70 kg. On the basis of this estimation, the intake of saccharin for breast feeding infants was

calculated using the default consumption values from the EFSA Scientific Committee Guidance (daily milk consumption of 200 mL/kg bw per day; EFSA Scientific Committee, 2017) and resulted to be 510 μg/kg bw per day. If compared to the reference point of 3420 mg/kg bw per day, the MOE is more than 1000 for the infant exposure by nursing which the Panel considered as indicative for no health concern.

Dietary exposure to saccharins (E 954), expressed as free imide, was estimated according to different exposure scenarios based on consumers only as described in Section 3.4. Currently, saccharins (E 954) are authorised food additives in the EU in 34 food categories, while IBOs provided EFSA with use levels for seven food categories and analytical data were available for 30 food categories.

The highest mean and P95 chronic exposure to saccharins (E 954), expressed as free imide, among consumers of one or more food categories containing saccharins (E 954) were estimated for the elderly at 2.1 and 7.8 mg/kg bw per day, respectively.

The Panel considered that the exposure to saccharins (E 954), expressed as free imide, from their use as food additives according to Annex II was overestimated in the *regulatory maximum level exposure assessment scenario* as well as in two *refined exposure assessment scenarios* (i.e. *maximum* and *brand-loyal*). This is mostly due to the fact that the exposure calculations were based on MPLs/maximum use levels/highest reliable percentiles of analytical data and these concentrations were considered applicable to all foods within each food category, while the percentage of foods in a subcategory labelled to contain saccharins (E 954) was maximally 20% in Mintel (see Section 3.2.2).

The Panel considered the *refined brand-loyal exposure assessment scenario* the most appropriate exposure scenario for the risk assessment of saccharins (E 954). The Panel noted that the P95 exposure estimates for chronic exposure to saccharins (E 954), expressed as free imide, were below the ADI of 9 mg/kg bw per day in all populations.

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 dietary exposure to saccharins (E 954), expressed as free imide, from their use as food additives, to be overestimated by the regulatory maximum and the two refined exposure assessment scenarios (i.e. maximum and brand-loyal).

Concerning the animal and human studies, the following remaining uncertainties were identified:

- Although some effects on glucose homeostasis were observed in one animal study (Shi et al., 2019) and in one human intervention study (Suez et al., 2022), several other studies showed no effect. The main uncertainty regarding studies on glucose homeostasis is the lack of long-term human intervention that could properly address absence or presence of any long-term effect of chronic exposure.
- Uncertainty exists around the adversity of findings on increased body weight at low doses observed in animal studies and in a human intervention study showing small increases (1 kg) in body weight for saccharins (but not other sweeteners).

Overall, these uncertainties were not considered to influence the conclusions on the safety of saccharins (E 954).

5 | CONCLUSIONS

Based on the included toxicological studies, the Panel derived an ADI of 9 mg/kg bw per day expressed as saccharin free imide, for saccharin and its sodium, potassium and calcium salts (E 954). Accordingly, this ADI replaces the ADI of 5 mg /kg bw per day (expressed as sodium saccharin, corresponding to 3.8 mg /kg bw per day saccharin as free imide) established by SCF (1995).

The 95th percentile exposure estimates for chronic exposure to saccharins (E 954), expressed as free imide, were below this ADI in all population groups, indicating that there is no safety concern.

6 | RECOMMENDATION

The Panel recommends the European Commission to consider:

- including a definition for saccharins (E 954) in the EU specifications, restricted to the manufacturing with the Remsen-Fahlberg process;
- including the CAS numbers 81-07-2 for saccharin (E 954 (i)), 128-44-9 for sodium saccharin (E 954 (ii)) and 10332-51-1 for potassium saccharin (E 954 (iv)) in the EU specifications, indicating that the CAS numbers for sodium saccharin and potassium saccharin refer to the anhydrous substances;
- modifying the purity of calcium saccharin (E 954 (iii)) to 'Not less than 99% of $C_{14}H_8CaN_2O_6S_2$ on the anhydrous basis' in the EU specifications;
- removing the parameter 'readily carbonisable substances' from the EU specifications of saccharins (E 954);
- lowering the limit of lead and arsenic in the EU specifications of saccharins (E 954).

7 | DOCUMENTATION AS PROVIDED TO EFSA

1. Calorie Control Council (CCC), 2018. 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). Submitted on 02 July 2018.
2. International Sweeteners Association (ISA), 2018. 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). Submitted on 21 June 2018.
3. International Sweeteners Association (ISA), 2020. Submission of data in response to the call for technical data on sweeteners authorised as food additives in the EU (EFSA-Q-2019-00318). Submitted on 20 March 2020.
4. JMC Corporation, 2019. Submission of data in response to the call for technical data on sweeteners authorised as food additives in the EU (EFSA-Q-2019-00318). Submitted on 19 June 2019.
5. JMC Corporation, 2021. Spontaneous submission of data in response to the call for technical data on sweeteners authorised as food additives in the EU (EFSA-Q-2019-00318). Submitted on 17 September 2021.
6. JMC Corporation, 2021. Spontaneous submission of data in response to the call for technical data on sweeteners authorised as food additives in the EU (EFSA-Q-2019-00318). Submitted on 22 December 2021.
7. Productos Aditivos S.A., 2022. Submission of data in response to the call for technical data on saccharin and its sodium, potassium and calcium salts (E 954) (EFSA-Q-2011-00736, EFSA-Q-2011-00737, EFSA-Q-2011-00738, EFSA-Q-2011-00739). Submitted on 22 February 2022.
8. Calorie Control Council (CCC), 2022. Submission of data in response to the call for technical data on saccharin and its sodium, potassium and calcium salts (E 954) (EFSA-Q-2011-00736, EFSA-Q-2011-00737, EFSA-Q-2011-00738, EFSA-Q-2011-00739). Submitted on 04 April 2022.
9. Calorie Control Council (CCC), 2022. Submission of data in response to the call for technical data on saccharin and its sodium, potassium and calcium salts (E 954) (EFSA-Q-2011-00736, EFSA-Q-2011-00737, EFSA-Q-2011-00738, EFSA-Q-2011-00739). Submitted on 14 June 2022 and 11 August 2022.[50]
10. International Sweeteners Association (ISA), 2023. Clarification on the data submitted in response to the call for technical and toxicological data on sweeteners authorised as food additives in the EU (EFSA-Q-2017-00500) and the call for technical data on sweeteners authorised as food additives in the EU (EFSA-Q-2019-00318). Submitted on 24 July 2023.
11. Productos Aditivos S.A., 2023. Clarification on the data submitted in response to the call for technical data on saccharin and its sodium, potassium and calcium salts (E 954) (EFSA-Q-2011-00736, EFSA-Q-2011-00737, EFSA-Q-2011-00738, EFSA-Q-2011-00739). Submitted on 17 July 2023.
12. Calorie Control Council (CCC), 2023. Clarification on the data submitted in response to the call for technical and toxicological data on sweeteners authorised as food additives in the EU (EFSA-Q-2017-00500) and the call for technical data on saccharin and its sodium, potassium and calcium salts (E 954) (EFSA-Q-2011-00736, EFSA-Q-2011-00737, EFSA-Q-2011-00738, EFSA-Q-2011-00739). Submitted on 18 September 2023.
13. International Sweeteners Association (ISA), 2024. Spontaneous 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). Submitted on 21 August 2024.
14. Calorie Control Council (CCC), 2023. Submission of the full study report of the pivotal study. Submitted in batches on 22 May 2023, 03 August 2023 and 18 September 2023.
15. European Dairy Association (EDA). Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 28 September 2018.
16. Food Drink Europe (FDE). Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 26 September 2018.
17. European Fruit Juice Association (AIJN) Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 1 October 2018.
18. Food Supplement Europe (FSE). Submission of data in response to call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Data submitted on 29 September 2018.

ABBREVIATIONS

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
As	arsenic
BMDL	benchmark dose lower bound
bw	bodyweight
Cd	cadmium
CFU	colony-forming units
ELISA	enzyme-linked immunosorbent assay
FAF	EFSA Panel on Food Additives and Flavourings
FC	food category
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed

[50]The same documents were submitted on 14 June 2022 and on 11 August 2022 by the IBO Calorie Control Council.

GD	gestational days
GNPD	Global New Products Database
Hg	mercury
HPLC	high performance liquid chromatography
HPLC-UV	high-performance liquid chromatography with ultraviolet detection
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	Limit of detection
LOQ	Limit of quantification
MOE	Margin of exposure
MOS	Margin of Safety
MPL(s)	maximum permitted level(s)
NOAEL	no observed adverse effect level
NTP	US National Toxicology Program
Pb	lead
P95	95th percentile
QS	quantum satis
RoB	Risk of bias
SCF	Scientific Committee on Food
SEM	scanning electron microscopy
TWI	tolerable weekly intake
USDA	United States Department of Agriculture
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 assessment of hazard identification and characterisation applied for saccharins (E 954)

Please refer to steps **1.11–1.15** of the revised protocol for hazard identification and characterisation of sweeteners (EFSA FAF Panel, 2023).

Extensive literature search

Methodology

For step 1.11, open-ended literature searches were conducted in the three selected databases with the search strings and criteria applied as follow:

Web of Science (core collection):

Saccharin and sodium saccharin

TOPIC: (saccharin OR "sodium saccharin" OR E954i OR E954ii OR "E 954i" OR "E 954ii" OR "81–07-2" OR "82,385-42-0" OR "128–44-9") AND LANGUAGE: (English)Timespan: 1994–2024.[51] Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC.

Calcium saccharin

TOPIC: ("calcium saccharin" OR E954iii OR "E 954iii" OR "6485-34-3") AND LANGUAGE: (English) Timespan: 1994–2024.[52] Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC.

Potassium saccharin

TOPIC: ("potassium saccharin" OR E954iv OR "E 954iv" OR "10,332–51-1") AND LANGUAGE: (English) Timespan: 1994–2024.[53] Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC.

PubMed:

Saccharin and sodium saccharin

Quoted phrases not found: "E 954i", "E 954ii", "82,385–42-0", "128–44-9"The following terms were not found in PubMed: E954i, E954ii.
((saccharin OR "sodium saccharin" OR "81–07-2") AND (("1994"[Date - Publication]: "2024"[Date - Publication]))) AND ("english"[Language])

Calcium saccharin

Quoted phrases not found: "E 954iii", "6485-34-3".
The following term was not found in PubMed: E954iii.
(("calcium saccharin") AND (("1994"[Date - Publication]: "2024"[Date - Publication]))) AND ("english"[Language])

Potassium saccharin

Quoted phrases not found: "E 954iv", "10,332–51-1"The following term was not found in PubMed: E954iv.
(("potassium saccharin") AND (("1994"[Date - Publication]: "2024"[Date - Publication]))) AND ("english"[Language])

SciFinder:

Searches to be conducted using the search strings proposed by EFSA and based on the substance CAS number and refined based on the publication date (from 1994 to 2024[54]), language and document type (selected: Clinical Trial, Journal, Preprint, Report, Review; not selected: Biography, Book, Commentary, Conference, Dissertation, Editorial, Historical, Letter, Patent).

[51] 01/01/1994–02/02/2024.
[52] 01/01/1994–02/02/2024.
[53] 01/01/1994–02/02/2024.
[54] 01/01/1994–02/02/2024.

Saccharin

Substance Identifier "81–07-2 "> substances (x) > get references (xxxx) > refine "2021–2024" (xxx) > refine "English" (xxx) > refine "Clinical Trial Journal Preprint Report Review" (xxx).

Saccharin Na salt hydrate

Substance Identifier "82,385–42-0 ">substances (1) > get references (xxx) > refine "2021–2024" (xx) > refine "English" (xx) > refine "Clinical Trial Journal Preprin..." (xx).

Saccharin Na salt

Substance Identifier "128–44-9 ">substances (x) > get references (xxx) > refine "2021–2024" (xxx) > refine "English" (xxxx) > refine "Clinical Trial Journal Preprin..." (xxx).

Saccharin Ca salt

Substance Identifier "6485-34-3 ">substances (x1) > get references (xxx) > refine "2021–2024" (xxx) > refine "English" (xxx) > refine "Clinical Trial Journal Preprin..." (xx)Saccharin K salt.

Substance Identifier "10332–51-1 ">substances (x1) > get references (xx) > refine "2021–2024" (xx) > refine "English" (xx) > refine "Clinical Trial Journal Preprin..." (xx).

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 8699.

For step 1.11.1 (Screening of the studies for relevance), the general principles reported in the protocol applied. 1192 papers were included at the level of title and abstract screening, whereas 7457 papers were excluded. From the 1192 papers included for the full-text screening, 334 were considered as possibly relevant, whereas 858 were either 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 (see Figure A1).

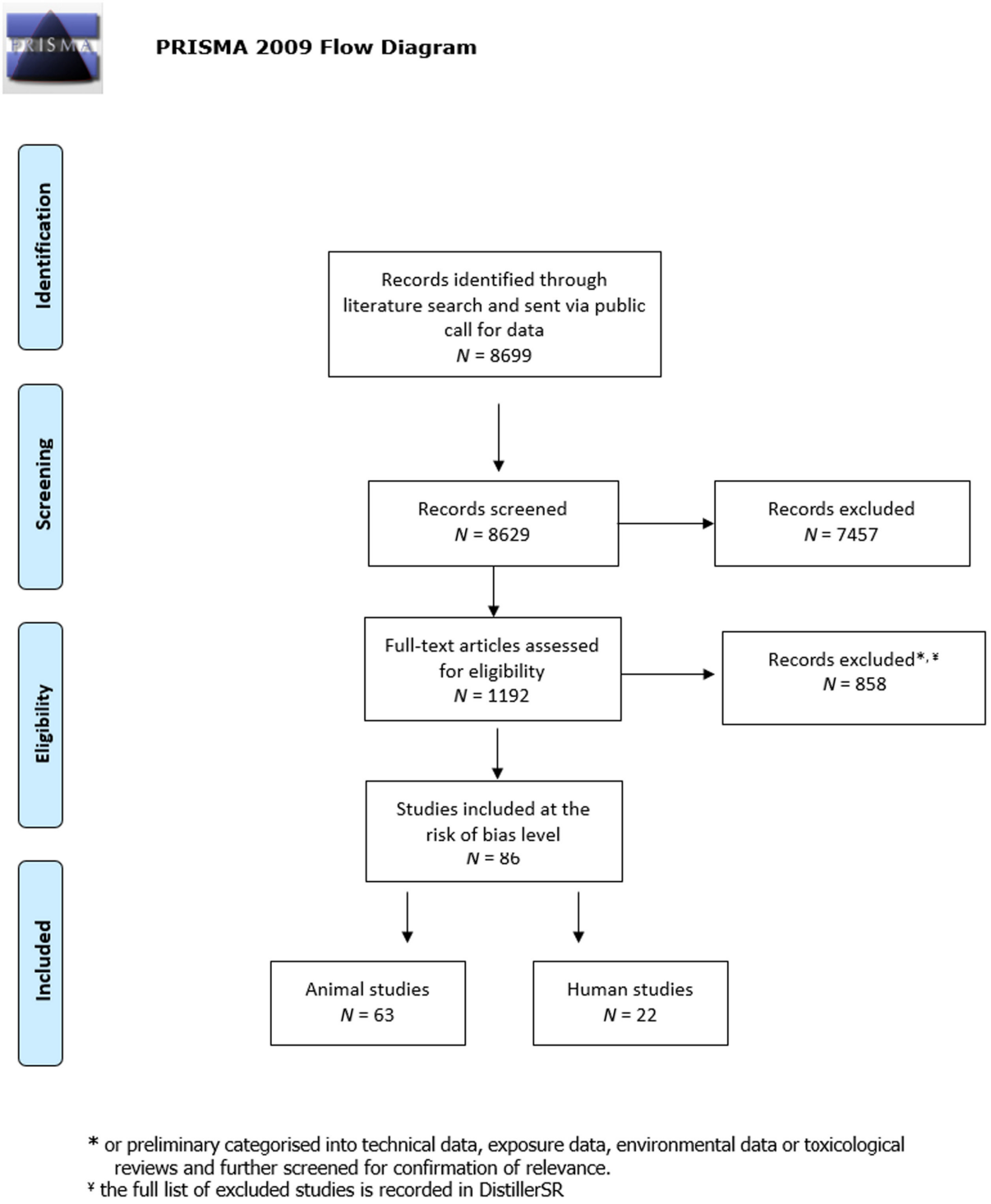


FIGURE A1 PRISMA flow chart (adapted from Moher et al., 2009).

Evaluation of the risk of bias (RoB)

Methodology

For step 1.12, the criteria outlined in the revised protocol for the risk of bias 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 clear justification should have been 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 risk of bias, 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.

18 animal studies and 19 human studies were evaluated as having moderate or low risk of bias and therefore considered further in the assessment, while those having a high RoB were not included.

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 saccharin 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 below.

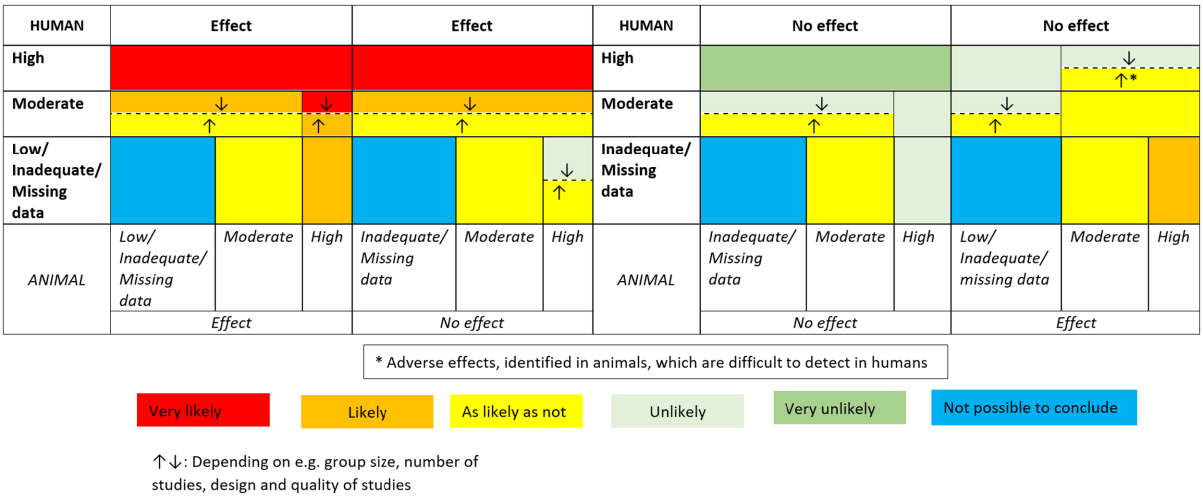


FIGURE A.2 Matrix for integration of animal and human body of evidence based on identification of effect and level of evidence.

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 (SCF, 1995; JECFA, 1993; IARC, 1999) to assess whether the new data support them. In the case of the HOCs with evidence available only from animal (namely 'reproductive toxicity', 'haematotoxicity', 'liver toxicity', 'nephrotoxicity') 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.

APPENDIX B

Other studies

In this appendix, short summaries of studies retrieved in the literature which do not directly contribute to the safety assessment of saccharin as a food additive are provided.

Studies addressing the impact of sweeteners on antimicrobial resistance (AMR)

Three papers (Yu et al., 2022; Yu & Guo, 2022; Yu, et al., 2023) deal with four artificial sweeteners and are showing about the same effect of the four sweeteners analysed (Acesulfame potassium, saccharin, sucralose, aspartame).

Yu et al. (2022) demonstrated a significant increase in transformation efficiency of the model organism *Acinetobacter baylyi* in the presence of sweeteners. They also show in an in vitro experiment (fitness cost experiment based on serial culturing) that the plasmids would persist more stably in the bacterial cells in the presence of the sweetener in the medium. Only doubling in transformation frequency compared to control was confirmed which is, although statistically relevant, a low effect and it is doubtful if it is of any biologically relevance. The increased persistence of a plasmid is shown in an in vitro system which would need further investigation and confirmation in a biological setup closer to practical conditions.

Yu and Guo (2022) demonstrated a significant antimicrobial effect of the four sweeteners tested against both Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) and positive (*Bacillus subtilis*) strains. This effect was, although statistically relevant, quite limited and for most of the results lower than sub-MIC concentrations of antibiotics; not a one log reduction which would be expected to be reached for considering it biologically relevant.

Yu, Henderson, and Guo (2023) demonstrated that the four sweeteners may promote plasmid-mediated conjugative transfer to the gut microbiome. This effect was, although statistically significant, quite limited and it is questionable if this is of biological relevance.

Overall, the Panel noted that these 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 their relevance for the development of AMR is unclear.

Studies on palatability

In Petrov et al. (2004), near expected term (E21) Sprague–Dawley rats delivered by caesarean section were restrained and dosed in six 10-min sessions at 1.5-h intervals beginning 3 h after caesarean delivery via a surrogate nipple with 0.1% saccharin in water; other groups were provided water, milk, 10% sucrose, pedialyte or an empty nipple ($n = 4$/sex/treatment). Overall, neonates ingested significantly more milk than all other fluids available from the surrogate nipple; total ingestion of sucrose, saccharin, pedialyte and water was similar. Neonates gained body weight in the milk, sucrose and pedialyte groups, but lost weight in the water, saccharin and empty nipple groups. Plasma glucose concentrations measured 12 h after delivery were higher in the milk group than all other groups, which did not differ between themselves. A significant reduction in the responsiveness to saccharin was observed starting with the fourth exposure; the authors suggested that this was due to the lack of positive nutritive feedback.

When rats experience an unsignalled decline in the quantity or palatability of a familiar food source, consummatory behaviour falls to a level below that of control rats which have only experienced the lesser reward. The study from **Mitchell and Flaherty (2005)** examined whether this response occurs by varying the orosensory properties of a highly palatable reward. Rats (Sprague–Dawley, 6 per group, no further details) food-deprived to 82% of their ad lib feeding weight displayed successive negative contrast consummatory behaviour decreases (number of licks, number of consummatory bursts initiated and number of licks per consummatory burst) to a level below controls experiencing a lesser reward effect, when given 5 min daily access to a 2% glucose/0.15% saccharin mixture (control rats that received 2% glucose alone). In a second experiment, animals that were shifted from the same glucose/saccharin mixture to 0.15% saccharin alone failed to show a successive negative contrast response. Intake fell to the level of control animals which only received 0.15% saccharin. The authors concluded that reductions in taste hedonics are sufficient to produce SNC; that post-ingestive factors are important in recovery from SNC; that the relationship between absolute and relative properties of glucose and saccharin solutions is not straightforward and that glucose and saccharin do not contribute equally to the enhanced palatability of glucose–saccharin mixtures.

The purpose of the study by **Tordoff et al. (2008)** was to provide information about the taste preferences of rats. Two-bottle choice tests were used to assess the taste preferences of eight male and eight female rats (54–66 days old) from 3 outbred strains (SD, LE, WI) and 11 inbred strains (BN, BUF, COP, DA, Dahl-S, F344, FHH, LEW, Noble, PVG, SHR). Each rat received a series of 109 48-h tests with a choice between water and a 'taste solution'. Saccharin was tested at 0.1, 0.316, 1, 3.16, 10, 31.6 and 100 mM concentrations. The authors concluded that rats show tremendous diversity in taste preferences. However, the Panel noted that all strains had a preference for nearly all concentrations of saccharin with the threshold at which preferences were significantly above indifference between 0.316 and 3.16 mM. There were also very high preferences for moderate concentrations of saccharin, but some strains had less high preferences for the highest concentrations, and 7 strains avoided 100 mM saccharin. Despite the restricted range, there were several significant correlations between

strain mean preferences for the three compounds (0.316 mM saccharin vs. 17.8, 31.6, 56.2 and 100 mM sucrose, 1 mM saccharin vs. 17.8 mM sucrose and 3.16 mM saccharin vs. 0.5%). The FHH strain was an outlying avid consumer of saccharin and the only strain that preferred 100 mM saccharin to water.

The aim of the study of **Johnson and Collier (2001)** was to assess whether taste contributes to the feed choices of foraging rats with the hypothesis that the addition of saccharin to plain chow would enhance its palatability. The rats ($n = 9$, male, 50 days old) were put to the choice which feeder of two feeders to use which were containing chow-based food pellets that were plain (control) or flavoured with saccharin (0.125% or 0.25%) or citric acid (2% or 4%) whereby the position of the feeder containing chow pellets or flavoured with either saccharin or citric acid were changed randomly. The study duration was 4 weeks. When the choice was eating from the feeder with chow or the feeder containing citric acid flavoured food, less pellets were consumed from the feeder with citric acid. When the choice was eating from the feeder with chow or the feeder containing saccharin flavoured food no influence of saccharin on the feed intake, acceptance and preference, both negatively influenced by citric acid, was observed. The hypothesis that saccharin would enhance the palatability is not supported by the data.

The aim of the study of **Warwick et al. (2003)** was to investigate independently the effects of diet palatability and the macronutrient composition on intake. The experiment was performed in male Long Evans rats. The testing procedure was such that intragastric infusion of fluids consisting of either high fat or high carbohydrate diet took place when using an apparatus for drinking a test solution which consisted either of saccharin or of a more palatable mixture of saccharin plus glucose. The test solution was not related to intake. In this respect, saccharin had no effect on daily intake.

Neurobehavioural studies, including studies on feeding behaviour

Furudono et al. (2005) measured hypothalamic neuropeptide mRNA levels (orexin-A and -B, melanin-concentrating hormone MCH and neuropeptide Y (NPY) in male Wistar rats at different times (not specified) after drinking 10 mL of a 5 mM saccharin solution or water. Saccharin increased orexin mRNA compared to water. Orexin levels showed a peak at 60 min, and then decreased gradually to levels obtained by water drinking within 210 min. NPY mRNA levels increased after 30 min compared with the 0 min control point and remained stable at high levels until 210 min. MCH mRNA levels did not differ between saccharin and water intakes, and remained stable throughout the time examined. Orexin-A and MCH directly administered into the lateral ventricle increased both water and saccharin intake, but NPY increased only saccharin intake. The authors concluded that orexin-A, MCH and NPY are related to the facilitation of drinking. Intracerebroventricular injection of orexin-A, MCH and NPY increased saccharin consumption; prior intraperitoneal n'loxone blo'ked this 'orexigenic' effect of orexin-A and NPY, but not MCH. Authors suggested that orexin-A and NPY may exert their palatability-induced ingestion influence via the opioid system. Authors noted that saccharin increased orexin levels, and that orexin-A facilitated draining of the gastric contents; they reported that addition of 0.1 M saccharin increased the draining of mash from the stomach compared with control mash.

Park et al. (2010) measured long-term potentiation (LTP) formation in the hippocampal Schaffer collateral pathway and somatosensory cortex layer IV – II/III pathways in 6-week-old male and female Sprague Dawley rats after ad libitum access to a 0.1% saccharin solution or control drinking water for 2 hrs per day for 3 weeks from weaning on postnatal day 22 ($n > 5$/sex/treatment). Feed consumption and body weight gain were not affected by saccharin ingestion in either sex. Saccharin consumption had no significant effect on LTP formation in either hippocampus (n per treatment > 4 in males, > 5 in females) or somatosensory cortex (n per sex and treatment > 3).

Fukushima et al. (2014) examined the expression of pCREB as a marker of neural activity in hypothalamic orexin and melanin concentrating hormone (MCH) neurons of adult male and female Wistar rats. Comparing animals between, during and after meals ($n = 4$–7/sex/condition), only MCH not orexin neurons expressed pCREB in association with feeding. In 48 h-fasted rats given 10 min drinking access to water (control), 3% glucose or 0.25% saccharin solution ($n = 4$–5/sex/treatment), glucose and saccharin both reduced pCREB expression in the orexin neurons of female rats. In H neurons, glucose attenuated the expression of pCREB, but saccharin had no effect in either sex. The authors concluded that MCH neurons seem to respond to nutritional substance, but orexin neurons appear to respond to a psychological state, suggesting that MCH and orexin peptides play physiologically distinct roles in feeding behaviour.

Oishi et al. (2016) evaluated circadian rhythms of sleep and locomotor activity in young adult male C57BL/6J mice after consumption of drinking water containing 0 or 0.1% saccharin for 2 weeks ($n = 7$/dose). Electroencephalographic (EEG) electrodes were implanted into the skull, steel wires were implanted into the neck muscles to collect electromyographic (EMG) signals; a telemetry recording device was then subcutaneously implanted into the backs of the mice. After 2 weeks of recovery, polygraphic EEG, EMG and spontaneous locomotor activity was continuously recorded for 72 h before and 72 h after 2 weeks of consumption of water with 0 (control) or 0.1% (w/v) saccharin. Food intake was automatically recorded every 10 min using an electronic weighting scale system (FDM-300). Measurement method for locomotor activity was not reported. Total daily food intake was comparable before and after saccharin exposure. Compared to controls, saccharin significantly reduced spontaneous locomotor activity and wakefulness and increased non-rapid eye movement sleep during the first half of the active (dark) phase, and increased wakefulness at the start of the sleep (light) phase. The authors suggested that saccharin-induced orexinergic activation and also changes in gut microbiota might affect the central clock that regulates circadian sleep–wake cycles.

Awad et al. (2020) examined the role of mu opioid receptors (MOP) in binge eating by examining sweet solution intake in mice with genetic deletion of the MOP. Twelve male and 12 female mice lacking MOP receptors and their wild-type controls

(wild-type female $n = 30$; male $n = 24$) were randomly assigned to three groups: limited access groups had 4 h access to food in the home cage combined with limited (4 h) access to sucrose (17.1% w/v) or saccharin (0.09% w/v) whereas continuous group had access to sucrose for 24 h. The animals were treated and examined over a period of 14 days. Knockout mice were generated by homologous recombination (Matthes et al., 1996) on a genetic background of 50% C57/BL6J: 50% 129svPas (weight: 23–46.7 g wild type: 17.7–32.8 weeks; MOP knockout: 18.5–58.8 weeks). Only limited access groups exhibited binge intake, measured as increased solution consumption during the first hour. Limited access groups consumed more food and gained more weight than the continuous access groups, and the effect was magnified in saccharin-consuming mice. Indeed, the increased food consumption in animals given limited access to saccharin was so excessive that caloric intake of this group was significantly higher than either of the sucrose groups (limited or continuous access). Within this group, females consumed more food per bodyweight than males, highlighting important sex differences in feeding behaviours under restricted access schedules.

Dess et al. (2020) 45 male rats with bred for either high (HiS) or low (LoS) saccharin preference were pair-housed with a rat from either their own line (same-line condition (HiS/HiS), (LoS/LoS)) or the other line (other-line condition (HiS/LoS)); weight gain, 0.1% saccharin intake, acoustic startle and open field behaviour were measured. Results after 42 days show for the first time that the lines express different behavioural strategies in a novel open field. In addition, weight gain and open field measures indicate that other-line housing (HiS/LoS) was stressful. Saccharin intake, however, was unaffected by housing condition. A previous finding that the lines possess different gut microbiota was replicated, measuring after 25–27 days. Although microbial transfer occurred between social partners, no clear evidence was obtained that housing-condition effects on weight gain or behaviour were mediated by microbial transfer. Overall, these findings add to the characterisation of non-gustatory correlates of a taste phenotype and suggest that rats differing strikingly on the taste phenotype and/or its correlates may be socially incompatible.

The studies of **Swithers and Davidson (2008)** were designed to test the hypothesis that experiences that reduce the validity of sweet taste as a predictor of the caloric or nutritive consequences of eating may contribute to deficits in the regulation of energy by reducing the ability of sweet-tasting foods that contain calories to evoke physiological responses that underlie tight regulation. Adult male Sprague–Dawley rats (375–425 g) received yogurt for 6 days per week in addition to lab chow and water ad libitum. On the 7th day, only lab chow and water were provided. Rats were assigned to one of three yogurt diet conditions for 5 weeks: yogurt sweetened with 20% glucose (wt/wt; ~ 1.2 kcal/g) for the other 3 days a week (sweet predictive group; 8 rats); yogurt sweetened with 0.3% saccharin for 3 days a week (sweet non-predictive group; 9 rats) or yogurt sweetened with 20% glucose (wt/wt; ~ 1.2 kcal/g) all week (10 rats). At the end of 5 weeks, body composition was determined using dual-energy x-ray absorptiometry to estimate of fat and lean mass. During Weeks 2, 3 and 5, rats in the sweet non-predictive (i.e. saccharin-treated) group gained significantly more weight than did rats in either the sweet predictive or sweet predictive control groups. Analysis of rats indicated that body fat composition was statistically significantly higher in sweet non-predictive (i.e saccharin-treated) rats compared to sweet predictive and sweet predictive control rats. In a second experiment, rats were randomly assigned to a sweet predictive group ($n = 11$) receiving yogurt (0.6 kcal/g) for 7 days and the yogurt sweetened with 20% glucose (wt/wt; 1.2 kcal/g) for 7 days. Rats in the sweet non-predictive group (n = 9) received yogurt for 7 days and yogurt sweetened with 0.3% saccharin for 7 days. After 14 days, rats were given 1 day of chow and water alone, then half of the rats in each group offered a pre-meal of 5 g of a novel sweet diet approximate the viscosity of yogurt (1.4 kcal/g). The remaining rats were given no pre-meal. Chow was then returned to all rats, and chow intake was measured after 1, 2, 4 and 24 hr. Rats then received 3 days of chow and water alone; the chow was then removed overnight, and the rats were tested with the pre-meal conditions reversed. There were significant differences in the quantity of unsweetened and sweetened diets consumed. Rats in the sweet predictive group consumed significantly more sweetened yogurt than unsweetened yogurt yet there was no significant difference in yogurt intake between the predictive and non-predictive groups (187 ±4 g sweetened and 181 ±8 g unsweetened yogurt in the non-predictive (saccharin-treated) group). Total caloric intake at the end of training (chow plus yogurt) was significantly affected, with sweet non-predictive (i.e. saccharin-treated) rats consuming significantly more calories over the course of training than did sweet predictive rats. During training, sweet non-predictive (i.e. saccharin-treated) rats gained significantly more weight than did sweet predictive rats. The authors concluded that the sweet predictive diet exposed rats showed caloric compensation for novel sweet-tasting calories by decreasing subsequent chow intake, whereas sweet non-predictive exposed rats did not. In a third experiment, the effect of predictive and non-predictive exposure on core body temperature changes after a meal were examined. Core body temperatures were determined in rats with intraperitoneal implanted. At the start of training exposures, rats were matched on baseline core body temperature and assigned to sweet predictive ($n = 8$) or sweet non-predictive groups ($n = 6$) as in Experiment 2. Both temperature and activity data were collected at 1-min intervals and then were averaged over 5-min time intervals 30 min prior to and during presentations of taste stimuli. Sweet predictive rats showed significantly greater increases in core body temperature than did non-predictive rats. The authors conclude that consumption of products containing artificial sweeteners may lead to increased body weight and obesity by interfering with fundamental homeostatic, physiological processes.

The aim of the study of **Ishii et al., 2003**, was to compare the postprandial satiety sequence profile of rats with feed which was taste-adulterated with quinine or saccharin. The bitter-tasting quinine increases aversive affective responses to food and reduces intake whereas saccharin is generally regarded as an artificial sweetener with enhanced palatability and is expected to increase consumption. Ten adult male Lister hooded rats (outbred) (221–238 g) received in a random order in a cross over fashion mashed feed, without addition of a substance (control) or containing quinine (0.015% and 0.04%, equivalent to 0.0004 and 0.001 M) or containing saccharin (0.2% and 0.3%; 0.01 and 0.015 M). Thus, five trials were done in

every rat. The test period was 1 h in which in a camera observation food intake and behaviour were recorded and scores were by highly trained observers given. In a further analysis the eating, grooming and resting behaviour was noted in 5-min interval of the 1 h test period. The results show that quinine (0.04%)-adulterated feed resulted in an anorectic response which has a specific behavioural pattern concerning inhibition of the peak feeding response, an elimination of resting and intermittent feed sampling/digging throughout the test session. Saccharin is traditionally considered a highly palatable sweetener that induces hyperphagia. In this study, addition of saccharin to the tested feed did not show any influence on feed intake or behaviour recorded during the 1-h test period.

The aim of the study of **Aoyama and Nagano (2020)** was to evaluate in rats whether consumption of saccharin over some time would increase the consumption of normal food, would increase the activity to receive pellets containing sucrose (sweet tasting food) and to reduce conditioned satiety response to sweet-tal food. Fourteen naive male albino Wistar rats, 80 days, 282–313 g, receiving nutritionally balanced rodent chow and water, were split into placebo group (PLAC, $n = 7$) and saccharin group (SACCH, $n = 7$). The rats were trained and tested in conditioning chambers (operant chambers) fitted with a food opening and two retractable levers. The chamber wall also contained a white cue light above the levers and a tone generator. A ventilating fan masked noises from outside the enclosures. Rats were trained for five consecutive days to press the lever in the operant chamber to get a 45 mg sucrose pellet for 1 h or 0.5 h. A lever response was also accompanied by a compound cue consisting of the white cue light and tone. Chow was provided by another food opening. During this phase the rat received only rodent chow and water. On day 6 a test was performed to measure the response on the clue signal, pressing the lever to receive pellets. Sweetened with sucrose. During the next 3-weeks rats received yogurt daily for 6 days per week in addition to ad libitum rodent chow and water, for the SACCH group the yogurt was sweetened with 0.3% saccharin on 3 of the 6 days. On the day following the last day of the yogurt phase, a second test was conducted identical to the test performed before the yogurt period. The next day, a consumption test was conducted. In which each lever-press was accompanied by a sucrose pellet and the compound cue. Body weight and chow consumption were comparable at the beginning of the yogurt phase. Yogurt consumption did not differ between groups. Concerning body weight gain, significant difference between the SAC and PLA groups were seen in weeks 1 and 2 but not in week 3. Chow consumption was increased to a small, but statistically significant extent within this time period. Rats in the SAC group showed more operant responses in obtaining sugar pellets than those in the PLA group. Whereas the rate of responses decreased by the consumption of each sugar pellet in both groups, the decrease was less in the SAC group compared to the PLA, which the authors interpret as indicating an impairment of the satiety. The authors concluded that consumption of artificial sweeteners reduces the conditioned satiety response to sweet-tasting food. The authors remarked that extrapolating the findings from rats in their highly controlled settings to humans in their complicated environments should carefully be considered.

The aim of the **Hamelin et al. (2022)** study was to evaluate the influence of saccharin on extent and biological mechanisms with brain functions. 297 C57Bl/6 J adult male mice were used for behavioural tests, c-Fos immunohistochemistry analyses, metabolism investigations and measurement of brain monoamines. Mice 6 weeks of age were acclimatised for 4 weeks to the animal facility, and to be manipulated (e.g. by weighting). The animals were food deprived (maintenance at 85% of the free feeding weight) for 3–4 weeks for studying food reward (Skinner chambers and Mouse Gambling Task) with free access to water or sweet solutions. The saccharin dose was 0.012% and 0.1% in drinking water for 5–6 weeks; the author give a dose of 0.48 and 4 mg/day of saccharin, respectively. The Panel calculated a dose of 22.9 and 191 mg/kg bw per day, respectively using the default value of EFSA (REF). Dopamine (DA), and its metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA), as well as 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NA) and 5-HT were quantified in hippocampus, medial PFC and striatum. DA and DA turnover, calculated by DA/(DOAC +3-MT + HVA), were reduced in the prefrontal cortex (PF), however not dose dependent. In the striatum DA and DA turnover was higher than in the control however only DA changes were dose-dependent and not DA turnover. Serotonin concentrations were not different between saccharin and control in any brain region. The saccharin 0.1% group showed an increased noradrenaline concentration in the striatum. In the same brain regions mRNA of dopaminergic receptor and dopamine transporter expression was quantified. Consumption of 0.1% saccharin decreased DRD2mRNA level in PFC whereas of 0.012% saccharin decreased DRD2mRNA level in the PFC and increased DRD2mRNA level in the striatum. Neither effect was dose-dependent. The density of cells with c-FOS positive cell nuclei per millimetre was measured in prelimbic (PrL), infralimbic (IL), orbitofrontal lateral, median, dorsolateral and ventral cortex (OFC) nucleus accumbens (NAcc), caudate putamen (CPU), basolateral amygdala (BLA), hippocampus (Hpc), motor cortex (Mot), cingular cortex (Cing), anterior agranular and granular insular cortex, dorsal and ventral (Ins) and lateral hypothalamus (LH), areas known to be involved in decision-making, novelty exploration, reward, motivation, emotion and nutrition. The number of c-Fos-positive cells was different between control and saccharin treated animals in several brain regions (8 out of 12), however never dose –dependent. An intraperitoneal Glucose Tolerance Test (GTT) was performed with a dose of 2 mg/kg; and blood sampling from the tail at 15, 30, 60 and 120 min after i.p. injection. An intraperitoneal Insulin Tolerance Test (ITT) was performed with a dose of 0.75 U/kg and blood sampling from the tail at 15, 30, 60 and 120 min after i.p. injection. Saccharin had no effect on glucose tolerance, insulin responsiveness, body weight or food intake and insulin plasma levels, nor the expression of genes implicated in the control of energy homeostasis, in the hypothalamus and the hippocampus. The mouse gambling task lasted 5 days with two sessions of 10 trials per day, 4 h apart. The used maze had four different arms: two of them gave access to long-term advantageous choices and the two others to long-term disadvantageous choices. In advantageous arms a small 'reward' (i.e. 1 food pellet) is located near the entry, followed by an award (i.e. 3–4 food pellets) located further in the arm in 18 out of 20 trails. In 2 of the 20 trails the further pellets contain quinine. In disadvantageous arms a 'reward' (i.e. of 2 food pellets), followed by 4 to 5 quinidine containing pellets in 19 out of 20 trials and in 1 out of 20 trails 5 food

pellet. Localization of advantageous and disadvantageous arms was randomised between each mouse. Scoring included arm chosen, food consumption, number of quinine pellets obtained but not eaten, and time to make a choice from the start box. A rigidity score was calculated based on the number of choosing the same arm during the task. The saccharin group (0.1%) made more advantageous choices, had a shorter latency, had a higher pellet gain score and both saccharin groups (0.012% and 0.1%) showed a higher rigidity score. The authors offer the interpretation that low dose saccharin alters decision-making performance and strategies, leading to a more risky and more rigid behaviour. They concluded that saccharin had no effect on metabolic parameters.

Kendig et al. (2018) assessed the effect of switching from sugar-sweetened 'diet' beverages to low-calorie sweeteners using 10 female Sprague–Dawley rats bred in-house aged 6–7 weeks per group. Experiment 1 exposed rats for 4 weeks to each stage, experiment to had prolonged exposure times of 8 weeks stage 1 and 7 weeks stage 2. At stage 1 rats had unrestricted access to 10% sucrose solution in addition to chow, at stage 2 they were either exposed to 0.1% saccharin (Suc-Sacch), water (Suc-Water) or remained on 10% sucrose (Suc-Suc). In both experiments energy intake and weight gain in Stage 2 was reduced for Suc-Sacch and Suc-Water groups relative to the Suc-Suc groups and at cull the Suc-Suc groups showed poorer insulin sensitivity and greater g/kg fat than Suc-Water and Suc-Sacch groups. In Experiment 2 short-term place recognition memory was impaired at the end of Stage 1 but recovered to a similar extent in the Suc-Water and Suc-Sacch groups; when the latter groups were compared with the Water–Water group, recovery was found to be essentially complete. Overall, the authors concluded that switching from sucrose to either water or saccharin produces equivalent improvements on both metabolic and cognitive measures, i.e. saccharin had no negative effects.

In **Tonosaki et al. (2007)**, the authors examined whether taste stimuli placed on the tongue could induce cephalic phase insulin release (CPIR). After a 12-h fast, blood samples from 5 male Wistar rats (weighing 120–220 g) were obtained from a cardiac catheter at −5, −1, 1, 3, 5, 7, 9, 11 and 15 min after taste stimulation (1.0 mL of 10mM saccharin dissolved in distilled water, given for 45 s into the oral cavity via an oral catheter) performed without anaesthesia and non-restraint using 'customary methods'. Administration of saccharin resulted in a transient increase (at 1 and 3 min post stimulation) in plasm insulin but no change in plasma glucose levels. No separate controls (e.g. distilled water) were included in this study. The authors conclude that that sweetness information conducted by this taste stimuli provides essential information for eliciting CPIR.

Studies on pain response

Suri et al. (2010) investigated the effects in male Wistar rats of 10% sucrose or 0.1% saccharin (presumably in drinking water, not reported) on pain responses to various noxious stimuli; latency of heat-induced tail flick and paw lick and threshold for electrical stimulation-induced tail flick in 5 sessions (0, 0.25, 1, 3 and 5 h; n = 6/treatment) and pain-related behavioural response to forepaw subcutaneous formalin test in 3 sessions (1, 3 and 5 h; *n* = 9/treatment). The authors reported initial tail flick and paw lick analgesia in saccharin dosed rats which extended until session III (3 h), followed by eualgesia and hyperalgesia in sessions IV and V (5 h); in the forepaw formalin test, saccharin induced only analgesia; the magnitude and duration of the saccharin-induced effects was greater than the sucrose-induced effects. The authors concluded that this is the first study to report biphasic nociceptive responses to noxious pain stimuli in the same adult rat after sucrose and saccharin ingestion.

Studies on animals on a high fat diet

In **Becker et al. (2020)**, C57BL/6 J mice (*n* = 40, 20 males, 20 females) were divided into four groups, each consisting of 5 males and 5 females. One group remained on normal diet whereas 3 groups consumed a high fat diet. From the groups receiving a high fat diet, 2 groups received sweeteners in their drinking water, i.e. saccharin and stevia, resulting in the following groups (i) low fat (low fat food and drinking water), (ii) high fat (high fat food and drinking water), (iii) saccharin (high fat food and drinking a saccharin solution) and (iv) stevia (high fat food and drinking a stevia solution). Dosage of the sweeteners was calculated to reflect the United States Federal Drug Administration's (FDA) allowable daily intake (5 mg/kg) by multiplying the allowable by the average mouse weight of the saccharin or stevia group and dividing by the average daily liquid intake of the group. The treatment duration was 10 weeks. Caloric consumption was recorded. Glucose tolerance tests were conducted before and after treatments. Ten animals were randomly selected to establish a baseline area under the curve (AUC) prior to the start of the high fat diet and all animals were tested post-treatment intraperitoneally with 1 mg glucose per kg body weight. Blood glucose was measured at 0, 15, 30, 60, 90 and 120 min in the initial phase (before treatment) and at 0, 20, 40-, 60-, 90- and 120-min post-treatment. Furthermore, gut microbiota samples were collected, and PCR sequencing followed by sequence clean-up was performed. The consumption of saccharin did not lead to a change in caloric consumption. Concerning the glucose tolerance test there was a significant difference between the low-fat group and the groups on the high fat diet compared to the AUC. No differences were observed between the high fat group and the saccharin group on high fat diet. The authors reported differences in species richness and relative abundances of several phyla in low fat groups compared to high fat, and saccharin group on high fat diet. In this study, an effect of high fat diet could be observed, however, no effect of the addition of saccharin to high fat diet was noted.

Respiratory quotient, energy expenditure and thermogenic effect

The aim of the study of **McGregor and Lee (1995)** was to test several feed types (froot loops, casein, lard, lab chow and saccharin) concerning their respiratory quotient, energy expenditure and thermogenic. Six experimentally naive male Wistar

rats, 235–320 g, at the start of testing were included. Testing occurred during the light phase. During the testing phase, the rats were food-deprived, but water was available at all times. An indirect calorimetry apparatus was used whereby the operator was able to open and close food hoppers fitted with a lid. Six feed categories were tested: Froot Loops, lard, casein, chow, saccharin and control (empty food hopper). After several days of acclimatisation, to the experimental settings the rats were tested for 75 min. The food hopper was opened remotely by the experimenter at exactly 25 min into the test session. The 25 min baseline period allowed for the stabilisation of respiratory parameters before food presentation. The lid was closed 5 min later to prevent the rats returning for any leftover food. Another 45 min of postprandial observation followed. For each minute of testing, mean O_2 consumption, mean CO_2 production and total activity counts were recorded. Saccharin had a small effect on respiratory quotient, and no effect on energy expenditure and activity.

Sensitising potential and allergenicity

The aim of the study of **Warbrick et al. (2001)** was to test the hypothesis that a broader association may exist between carcinogenicity in rodents (including non-genotoxic carcinogenesis) and skin sensitising activity. Two substances without a carcinogenic hazard (limonene and saccharin) and three genotoxic rodent carcinogens (vinylidene dichloride, ethyl acrylate and bisphenol A diglycidyl ether) were tested concerning their skin sensitising activity using the local lymph node assay (OECD 429). Saccharin did not induce a positive response in the local lymph node assay. According to this study saccharin has no sensitising potential.

Yamashita et al. (2017) investigated the effects of sodium saccharin on the induction of oral tolerance to ovalbumin (OVA) in female BALB/c mice. The study has some limitations, e.g. age and weight of animals not reported, randomisation not mentioned, methods are poorly described, and a small number of animals ($n = 4$) were used for some experiments.

The working hypothesis is that food additives may prevent acquisition of oral tolerance, which may lead to adverse immune responses to food proteins, including food allergy. To induce tolerance, mice were administered OVA (30 mg/mice) with food additives, including saccharin, or PBS as vehicle control (oral tolerance group) for 5 sequential days. After the last administration, the mice were sensitised intraperitoneally with 1 μg OVA, control mice received PBS, mixed with Imject Alum every other week. Positive control was sensitised by OVA/alum without induction of tolerance.

In an first study, mice ($n = 5$-6/group) were subjected to the tolerising procedure with or without sodium saccharin (10 mg/mouse; approximately equivalent to 10 times the current ADI), followed by the sensitization procedure. After the last administration, blood was collected for OVA-specific IgE on day 24. Ovalbumin-specific IgE was present in the positive control (~ 31 ± 5 ng/mL) and in saccharin treated mice (~ 20 ± 4 ng/mL) but not detectable in the oral tolerance (OT) group.

In a second study, mice (5–7/group) underwent the same procedure to that described in the initial study, except that mice were treated with either 0, 10 or 100 mg saccharin during the tolerising procedure (i.e. approximately equivalent to 0, 10 or 100 times the current ADI). After sensitization, blood was collected and then the mice were then additionally repeatedly orally administered 30 mg OVA/mouse to induce anaphylaxis (8 treatments over 18 days), followed by a final blood collection.

Ovalbumin-specific IgE (~ 54 ± 10 ng/mL) was present in the FA (food allergy positive control) control and in mice treated with saccharin during tolerisation (~ 10 ± 4 and 40 ± 8 ng/mL for 10 and 100 mg saccharin treated groups, respectively) but not detectable in the OT control.

Ovalbumin-specific IgG1 was present in the FA (food allergy positive control?) control (~ 180 ± 30 ng/mL) and in saccharin treated mice (~ 10 ± 2 and 40 ± 4 ng/mL for 10mg and 100mg saccharin treated groups, respectively) but not detectable in the OT control.

After the final administration of OVA, body temperature had significantly fallen in the FA and 100mg saccharin-treated groups. Diarrhoea was also apparent in these groups.

Analyses of antigen-presenting cells in the mesenteric lymph nodes (MLN) revealed that sodium saccharin treatment during tolerisation influenced their migration patterns, facilitating an increase in allergic CD11b + dendritic cells and attenuating tolerogenic CD103+ dendritic cells. MLNs were collected after the last challenge, and expressions of cytokine-related allergy and tolerance were measured: expression of IL-4 in the oral tolerance control group decreased in comparison with the food allergy control group and was up-regulated by sodium saccharin treatment. Expression of IFN-g and TGF-b were down-regulated in the saccharin-treated group compared with the oral tolerance group. Furthermore, saccharin reduced the proportion of CD25hi regulatory T cells among CD4+ T cells in the MLNs.

The authors concluded that oral exposure to sodium saccharin inhibited acquisition of oral tolerance to ovalbumin based on hypothermia and allergic diarrhoea responses accompanied by elevations in blood ovalbumin specific IgE and IgG1 levels. An analysis of antigen-presenting cells in MLNs suggested that, according to the authors, sodium saccharin decreased the proportion of CD25hi regulatory T cells and affected their migration.

The Panel noted that some endpoints indicative of an inhibition of tolerance to ovalbumin (elevations in blood ovalbumin-specific IgE and IgG1 levels) were seen at a dose level of 10 mg/day sodium saccharin (according to the authors approximately equivalent to 10 times the 'ADI from Food Safety Commission in Japan or the Joint FAO/WHO Expert Committee on Food Additives'). At a dose level of 100 mg/day sodium saccharin (according to the authors approximately equivalent to 100 times the current 'ADI from Food Safety Commission in Japan or the Joint FAO/WHO Expert Committee on Food Additives'), there was also an inhibition in acquisition of oral tolerance to ovalbumin based on induction of anaphylaxis endpoints (hypothermia and allergic diarrhoea responses).

Studies in animal disease models

In **Mchunu et al. (2019)** 49 days old Sprague–Dawley rats were made diabetic by treating them for 2 weeks with fructose following by intraperitoneal injection of 40 mg/kg bw streptozotocin. Saccharin was added to drinking water in a concentration equivalent to the sweetness of 10% sucrose. Ad libitum access was allowed for a 12-week experimental period. Catalase, glutathione reductase, superoxide dismutase and glutathione peroxidase activity were increased in the serum and most organs following diabetes induction. Saccharin administration reduced diabetes-induced lipid peroxidation and boosted serum, hepatic, renal, cardiac and pancreatic glutathione (GSH) levels.

Kumar and Chail (2019) assessed the withdrawal effect of sucrose or saccharin in Swiss albino diabetic mice (streptozotocin: 135 mg/kg, 6–8 weeks, 20–25 g, male and female, 6 animals per group) by initial exposure over 28 days followed by 7 days of withdrawal of sucrose and saccharin and 3 days of reintroduction of saccharin). Monoamine oxidase (MAO), corticosterone, thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) were quantified after behavioural tests.

After induction of diabetes, mice were exposed to a two-bottle water–water, 10% sucrose-water or 10% saccharin-water choice paradigm for 28 days. Diabetic mice manifested preference towards 10% sucrose or saccharin over water. Sucrose-overeating by diabetic mice amplified symptoms of depression and anxiety; however, withdrawal further exaggerated these behavioural abnormalities. Substitution of sucrose by 10% saccharin attenuated the depressive and anxiety-like behaviour in comparison to diabetic mice that were exposed separately to water–water or sucrose-water alone, and with respect to normal mice. Although withdrawal from saccharin resurfaced behavioural anomalies in diabetic mice, however, these were significantly low in comparison with withdrawal from sucrose or normal group. Reinstatement of exposure to saccharin mitigated symptoms of depression and anxiety in diabetic mice.

The authors concluded that the preference for sucrose overeating augments while preference for saccharin mitigates depressive and anxiety behaviour during diabetes.

The aim of the study by Fujita et al., 2009 was to determine whether artificial sweeteners, among them saccharin, could induce incretin release from the gut and thereby influence glucose homeostasis in normal and diabetic rats (Male Wistar rats, 290–530 g, male fed ZDF rats 17 weeks) in vivo, to test the hypothesis that release of incretins from enteroendocrine cells is triggered by carbohydrates via a pathway identical to the sensation of 'sweet taste' in the tongue. The rats were given 1 g/kg bw artificial sweeteners, among them saccharin or 1 g/kg glucose by oral gavage, 15 min before an intraperitoneal glucose tolerance test. In contrast to glucose, artificial sweeteners, among them saccharin did not influence maximal glucose concentration or AUC. Plasma glucose-dependent insulinotropic polypeptide (GIP) levels increased when rats were administered an oral glucose load (2 g/kg) to a level 4-fold higher than the basal level whereas no increase in plasma GIP was observed following oral administration of artificial sweeteners, among them saccharin. Likewise, no increase in plasma glucagon-like peptide-1 (GLP-1) concentration was seen following gavage administration of artificial sweeteners, among them saccharin whereas glucose increased the plasma GLP-1 level 2.5-fold. Artificial sweeteners, among them saccharin did also not influence the blood glucose level. The authors concluded that the findings do not support the hypothesis that sweet taste triggers the release of incretins or is a signal influencing the blood glucose level.

Activation of taste receptors in non-gustatory tissues as a possible mode of action

There is some evidence that saccharin induces biological effects via taste receptors in non-gustatory tissues, but given the limited data no firm conclusions can be drawn.

There is limited conflicting evidence that saccharin can affect serum progesterone levels, possibly via ovarian taste receptors (TR).

Jiang et al. (2018) reported that rats dosed via drinking water for 48 days with 1.5 or 7.5 mM sodium saccharin (equal to 0, 140 or 730 mg/kg bw per day) had dose-related increases in abnormal oestrous cycles, serum progesterone and number of ovarian cysts and CL; also increased expression of progesterone synthesis-related StAR and 3b-HSD, and of steroidogenesis-related factors (StAR, CYP11A1, 3b-HSD, CYP17A1). **Li et al. (2020)** reported that postweaning guinea pigs dosed via drinking water with 1.5 or 7.5 mM (equivalent to 95 and 662 mg/kg bodyweight per day) for 28 days had increased body and ovary weight and higher ovarian expression of T1R2 at 7.5 mM, but at 7.5 mM there was reduced ovarian expression of T1R2 and changes in uterus and ovary (reduced number of stromal cells surrounding the narrowed uterine cavity, less secondary follicles). Since saccharin had no effects on day of vaginal opening, oestrous cyclicity, serum progesterone and oestradiol, or number of antral follicle and corpora lutea, these effects are not clearly adverse. The lack of effect on serum progesterone, oestrous cyclicity and ovary are clearly in conflict with 6365-Jiang-2018 (increased serum progesterone, abnormal oestrous cycles, corpora lutea and ovarian cysts). Jiang et al. (2021), investigated the effects and potential mechanisms underlying ovarian taste receptor activation on progesterone production using saccharin sodium as the receptor agonist in a pseudo-pregnant rat model. They reported that taste 1 receptor member 2 (TAS1R2) and taste 2 receptor member 31 (TAS2R31) are abundantly expressed in rat corpora lutea, and that intraperitoneal injection of saccharin sodium (5 mg/kg bw) can activate both of them and initiate their downstream signalling cascades. They concluded that the activation of ovarian taste receptors by saccharin reduced the protein expression of steroidogenesis-related factors, causing the inhibition of ovarian progesterone production. The authors noted the apparent discrepancy with their previously reported saccharin-induced increase in serum progesterone (6365- Jiang et al., 2018). They suggested a number of possible reasons, including the use of prepubertal pseudo-pregnant rat model in their current study versus mature rats

in 6365-Jiang-2018. There is thus inconsistent evidence that saccharin can alter progesterone production via ovarian taste receptors. Human-relevant adversity has not been demonstrated.

There is limited conflicting evidence that saccharin can affect serum testosterone levels and spermatogenesis, possibly via Leydig cell TR.

Gong et al. (2016) reported saccharin effects in male ICR mice dosed in feed and water with 40, 210 or 460 mg/kg body weight per day for 35 days. Saccharin at all doses increased water intake and increased 6h fasting serum glucose (but no effect on triglyceride or total cholesterol). At low- and mid-dose, there was increased body and testis weight, and increased expression of taste receptor T1R3 and its G alpha protein transducer gustducin, and of steroidogenesis enzymes StAR, 3b-HSD and 17b-HSD, and at the mid-dose increased CYP11A1 and CYP17A1. At the high dose, there was reduced serum oestradiol and testosterone, testis histopathology and reduced sperm quality (lower sperm count, sperm viability and sperm motility, higher sperm abnormality rates) and reduced expression of T1R3 and gustducin, and of StAR, CYP11A1, 3b-HSD, CYP17A1 and 17b-HSD. It is unknown whether or how effects on testicular T1R3 are related to other testis effects; Liu et al., 2022, have recently reviewed this topic. The Gong et al. (2016) study has not been considered in the weight of evidence since it was considered having a high risk of bias (Tier 3, due to unexplained attrition).

Wang et al. (2023) reported that sodium saccharin increased testosterone and steroid hormone synthases (StAR, 3ß-HSD, CYP17A1, 17ß-HSD) in ex vivo pig Leydig cells. Leydig cell TAS1R3 expression was also altered by NaS treatment, but effects were bidirectional and not dose-related (NaS 0.1–20 mM, 0.5–72 h; Figure S3). The relationship between T1R3 expression and steroid metabolism is thus not clear.

Concerning other possible hormonal effects, **Serrano et al**. **(2022)** presented evidence suggesting that saccharin may transiently potentiate insulin secretion through the activation of non-gustatory sweet taste receptor pathways.

Other studies

Tsurugizawa and Uneyama (2014) compared the blood oxygenation level-dependent signal intensity in male Sprague–Dawley rats (10 weeks) in awake functional magnetic resonance imaging after intragastric infusion (without stimulation of oral sensation) of 8% glucose (caloric, $N=6$), 0.2% saccharin (noncaloric, $N=6$) and physiological saline (control, $N=6$). The infusion rate was 1 mL/min per kg body weight for 10 min. Glucose induced a positive signal increase in the amygdala and nucleus accumbens, both of which receive dopaminergic input from the ventral tegmental area. In contrast, saccharin administration did not activate these areas. Both glucose and saccharin increased the blood oxygenation level-dependent signal intensity in the insular cortex and the nucleus of the solitary tract compared to control. These results show that there were significant differences between post-ingestive glucose and saccharin-induced increases in the blood oxygenation level-dependent signal in rats.

The aim of **Sato et al. (2013)** was to compare the effects of luminal nutrients on the stimulation of GLP-2 secretion in vivo using lymph samples. The thoracic ducts of male Wistar rats (250–300 g body weight) were cannulated prior to administration of a 3-ml single oral bolus of sucrose (5% solution; 146.1 mM; $n=5$), saccharin sodium (4% solution; 218.4 mM; $n=5$) or saline as control. The administration of sucrose increased lymph (glucagon like peptide-2 (GLP-2) concentrations at 2 h (5.75 ± 1.73 ng/h) compared with control saline (concentration ~1.5 ± 0.5 ng/h), the only time point reported. Saccharin did not cause a statistically significant increase in lymph GLP- 2 (2.52 ± 0.76 ng/h).

The aim of **Cheng et al. (2021)** was to explore the effects of sucrose, xylose and saccharin on the oral microbiota and immunoglobulins in saliva of Sprague Dawley (SD) rats. Six-week-old female SD rats ($n=6$ per group) were given 0.83 mg/mL xylose, 0.83 mg/mL sucrose or 0.83 mg/mL saccharin for 12 h/day, and purified water for the 12 h at night and the control received water for 24 h. After 8 weeks, saliva was collected, and swabs were taken from tongue, palate and upper throat of the rat's oral cavity for evaluating changes in the composition, community structure and function of the oral microbiomes in the treated vs. control rats. In the saliva SIgA, IgE, IgM and IgG levels were measured. IgE was not found in the saliva and no significant differences were observed in SIgA, IgM and IgG concentrations between the saccharin and control group and the abundance of the top 4 bacteria genus was the same for control and saccharin treated animals.

In a study by **de Mercau et al. (1997)**, 4-month-old CH3 male and female mice (5 per sex/treatment group) were fed a diet with or without the addition of 0.1% (w/w) sodium saccharin (Sigma) for 180 days. Control and treated mice were then both fed control diet for the following 180 days prior to sacrifice, removal of the descending colon, fixation and processing for examination by electron microscopy. The authors reported colonic epithelial cells in the large intestine presented 'pleomorphic microvilli and membrane fusion with formation of excrescences' in treated male and female mice, but not in controls. No quantitative data were reported.

In Li et al.'s (2021) study, 12, 4-week-old female Harley-white guinea pigs with a body weight of 240.7 ± 7.7 g were treated, a control group ($n=6$) received normal water and a saccharin group ($n=6$) received water with 5 mM sodium saccharin solution for 28 days. It is noted that there is uncertainty about the dose, as in the abstract the added saccharin concentration is given as 1.5 mM whereas in the methods the concentration of the added saccharin solution is given with 5 mM. TG (triglyceride), ALT (glutamic-pyruvic transaminase), AST (glutamic-oxalacetic transaminase), TC (total cholesterol), CRE (creatinine), ALP (alkaline phosphatase), UA (uric acid), GLU (glucose), CHO (cholesterol) and TP (total Protein) as well as growth hormone-releasing peptide (GHRP), glucagonlike peptide 1 (GLP-1), cholecystokinin (CCK), peptide-YY (PYY) were measured in serum. The averaged villus height and crypt depth were measured using ImagePro Plus software. Lactic acid and SCFA were measured in the ileal digesta. Furthermore, from hypothalamus and from ileum mucosa RNA-Seq and RT-qPCR was performed. The microbiome composition was analysed. Saccharin supplementation had no significant effects

on serum levels of TG, ALT, AST, CRE, ALP, CHO, TP, glucagonlike peptide 1 (GLP-1), cholecystokinin (CCK) and peptide-YY (PYY). The serum level of GHRP was slightly elevated. The villus height and villus height/crypt depth ratio were slightly but significantly increased by SS and the crypt depth decreased compared with the control group. Adding SS to drinking water increased the level of lactic acid, acetic acid, propionic acid and N-valeric acid in the ileal digesta to a significant extent. The gut microbiota in the guinea pigs treated with saccharin showed changes of the composition compared to the controls. Hypothalamic RNA data demonstrated that saccharin had gene expression profiles which showed up and down regulation in comparison with that of controls not followed by a clear interpretation of the data.

Wilinski et al. (2013). The aim of the study was to evaluate the influence of artificial sweeteners (stevia, sodium cyclamate, saccharin), 5 mg/kg daily for 5 days, given by oral injection, on the sulfane concentration in various tissues (brain, heart, liver and kidney). CBA female mice (7 weeks old; ~20 g body weight; 7 animals/group) were gavaged with 5 mg/kg bw saccharin in a water solution for 5 consecutive days. The control group received physiological saline. Two hours after the final administration, animals were sacrificed and the level of sulfane determined in several tissues. There were no statistically-significant changes compared with control in sulfane levels following saccharin administration in brain, heart, liver and kidney.

APPENDIX C

Additional human studies

In this appendix, short summaries of studies retrieved in the literature which do not directly contribute to the safety assessment of saccharin as a food additive are provided.

Andreatta et al. (2008) conducted in Argentina, a case–control study on 197 patients with histologically confirmed urinary tract tumours (UTT), and 397 controls with acute, non-neoplastic and non-urinary tract diseases to study the effect of sweeteners use (saccharin, cyclamate, aspartame, acesulfame-K) and risk of UTT. Out of 146 sweeteners consumers, 111 (76%) consumed saccharin and/or cyclamate. The risk of developing UTT was significantly increased in long-term (≥ 10 years, OR: 2.18; 95% CI: 1.22–3.89) sweeteners users compared with no users after adjustment for age, gender, smoking and BMI.

Hasan et al., (2023) conducted a cross-sectional study ($N = 181$ type II diabetic subjects mellitus; 82 healthy individuals) to study the relationship between consumption of both saccharin and cyclamate (40 mg of cyclamate-sodium and 4 mg of saccharin-sodium) and levels of serum catalase activity, peroxynitrite, ceruloplasmin, malondialdehyde, glycated haemoglobin, fasting glucose, creatinine, alanine transaminase and lipid profile. Subjects were classified into two groups based on saccharin and cyclamate use (yes/no). Then they were grouped according to the amount and duration of saccharin and cyclamate use. Saccharin and cyclamate intake versus no use were associated with high levels of HbA1C ($p < 0.001$), MDA ($p < 0.001$), ceruloplasmin ($p = 0.002$) and lower levels of catalase ($p = 0.001$) whereas in diabetic patients high levels of blood glucose ($p = 0.002$), ceruloplasmin ($p = 0.025$) and MDA ($p = 0.03$) and lower levels of catalase ($p = 0.001$) were observed. Among healthy subjects, BMI was higher among consumers of saccharin and cyclamate versus non consumers ($p = 0.001$). Among diabetic patients, high consumption of saccharin and cyclamate (> 5 tables daily) was associated with high levels of creatinine ($p = 0.008$) and decreased levels of ceruloplasmin ($p = 0.049$) and catalase ($p = 0.05$). Duration of saccharin and cyclamate use (> 10 years) was associated with higher triglyceride levels and (TC) and high TC/HDL ratio.

Bryant et al. (2014) studied, on ten healthy subjects (18–24 years), the effect on glycaemic response and appetite, of a test drink containing aspartame (150 mg + 45 glucose) or saccharin (20 mg + 45 glucose) and acesulfame-K (85 mg + 45 glucose) administrated in four separate days. The mean blood glucose levels peaked 30 min after the consumption of the glucose and saccharin group. However, post hoc analysis revealed no differences between the glucose and saccharin. No effect of saccharin on perceptions of hunger or fullness was observed.

Biagini and Albi (2020) conducted a randomised, single blinded trial on 16 amateur athletes (8 men; 8 women, mean age 39.1, SD = 7.8 years) to study the effect of 4 different carbohydrates glycaemic index meals (banana, dried apricots, energy gel containing glucose, fructose, maltodextrin, trehalose, isomaltulose) versus placebo (water, sodium cyclamate, sodium saccharin and acesulfame K) on blood glucose levels during (30 min) and after physical exercise (40 min run of 70% of their VO_2max.) Three blood samples were taken from the athlete's finger by glucometer. No difference in run performance was found between groups. Plasma glucose levels were not changed after 30 or 40 min run in the dried apricots group and in the placebo group.

Ehlers, Niggemann, Binder, and Zuberbier (1998) conducted at the Pediatrics and Dermatology of the Charite, Berlin, Germany a study to investigate the role of nonallergic hypersensitivity to food (not IgE-mediated allergy) in children ($N = 16$, mean age 12 years, range 3–17 years) suffering from chronic continuo's urticaria (at least 3 months' duration). A special strict elimination diet, until remission of symptoms, was followed by all patients. Pseudoallergen-induced urticaria was diagnosed in 12 cases (75%). Out of 16 patients, 13 (81%) went into remission under a stringently controlled low-pseudoallergen diet. A double-blind, placebo-controlled food challenges (DBPCFC) with encapsulated food additives were performed in six children and showed that food additives (colouring agents, preservatives, monosodium glutamate, saccharin/cyclamate) provoked 83% of nonallergic hypersensitivity reactions.

Bakali et al. (2017) conducted a randomised, single blinded crossover trial on healthy Caucasian ($N = 20$) and Asian females ($N = 20$) aged 18 years or more to compare bladder sensation during a forced diuresis experiment (sweetened water with 5 mg/kg body weight saccharin versus drinking water). Voided volume and time required to achieve maximum sensation were measured at experimental session 1 and 2 (28 days later). Median diuresis with saccharin was 16.7 mL/min (8.6–35) compared to 13.2 mL/min (7.1–25) with water ($p = 0.008$). No differences in maximum voided volume or diuresis rate were seen by ethnicity. In a secondary analysis ($N = 24$), in which 16 women were excluded (> 5 mL/min difference in diuresis rate), time to achieve maximum sensation was lower with saccharin (37.5 min (20–85) than with water (50.0 min; 20–80), $p = 0.002$.

Bayındır Gümüş, Keser, Tunçer, Altuntaş Yıldız, et al. (2022) conducted a randomised, controlled cross-over trial in Turkey ($N = 9$ males, aged 20–29) to evaluate if 300 mL water containing 75 g sucrose and 240 mg saccharin in comparison with 300 mL water would change levels of serum ghrelin and appetite measured by Visual Analog Scale. No statistically significant difference in serum ghrelin levels at the beginning and 30th min after consuming test drinks ($p > 0.05$). However, at 60th and 120th min, mean ghrelin level was higher in the test drink containing saccharin compared to drink containing sucrose ($p = 0.001$, $p = 0.003$, respectively). Mean food consumption and desire to eat score at 120th min after drink consumption was higher in saccharin test drink than sucrose test drink ($p < 0.05$).

Su et al. (2023) using the Atherosclerosis Risk in Communities (ARIC) cohort study in the US ($n = 3751$; mean age = 54 years), evaluated the association between 359 serum metabolites and ultra-processed food consumption cross-sectionally and the association between ultra-processed food–associated metabolites and incident chronic kidney disease. Dietary intake was

assessed using a semiquantitative 66-item FFQ, ultra-processed foods were classified using the NOVA classification system, and an untargeted metabolomics quantification protocol was implemented. In the adjusted analyses, saccharin (and other metabolites; homostachydrine, stachydrine, N2, N2-dimethylguanosine, catechol sulfate, caffeine, 3-methyl-2-oxovalerate, theobromine, docosahexaenoate, glucose, mannose and bradykinin) was statistically significantly associated with ultra-processed food consumption, however not with incident chronic kidney disease (HR 1.02; 95% CI: 0.99–1.04).

Wolraich et al. (1994) evaluated the effects of sucrose and aspartame on hyperactivity and other behavioural problems in preschool and school children and used saccharin as control. They conducted a three-week three-arm double-blind controlled trial in US children ($n = 48$) identified as sugar sensitive by their parents. The following interventions were tested: (a) diet high in sucrose with no artificial sweeteners, (b) diet low in sucrose with aspartame as a sweetener, (c) diet low in sucrose with saccharin (placebo) as a sweetener. Among the multiple cognitive variables assessed, no association was observed in the school children. In preschool children, a few statistically significant associations were observed that did not point to a consistent effect pattern.

Reid et al. (1995) assessed the effects of a sucrose drink (160 kcals/40 grams of cane sugar) on mood state (Profile of Mood States, POMS) in Glasgow, UK, using a tree-arm single-blind before-after intervention study ($n = 60$; 40 g sucrose vs. 4.34 g saccharin vs. water). Although no association was observed with hunger or eating, a delay in food intake was noted after the ingestion of sucrose. No safety-related observations were reported for the saccharin group.

Allen et al. (1996) evaluated the role of glucose in the performance of a series of memory and non-memory neuropsychological tests ($n = 28$) using saccharin as a control (27.3 mg) in a single-dose cross-over controlled trial setting. Statistically significant findings were reported for the glucose group for the recall of the Rey/Taylor Figure, verbal fluency, figural fluency and divided attention. No safety-related observations were reported for the saccharin group.

Manning et al. (1997) also assessed whether glucose intake enhances performance on explicit declarative verbal memory tasks implementing a single-dose cross-over controlled trial ($n = 47$) using saccharin as control (27.3 mg). Glucose compared to saccharin enhanced explicit declarative verbal memory in healthy elderly people. In the younger study participants, no differences were observed. No safety-related observations were reported for the saccharin group.

Messier et al. (1999) assessed the effect of glucose (50 g) and glucoregulation on memory in adults in Canada ($n = 36$) using saccharin (50 mg) as placebo. No additional control group was used and no safety-related observations were reported for the saccharin group. Among the multiple analyses performed, the 100 mg/kg glucose group performed worse on measures of commission errors, post-commission responses and post-commission response time variability compared to all other groups including saccharin. A similar assessment was done in Messier et al. (1997) in an elderly population.

Parent et al. (1999) evaluated whether a memory-enhancing emotional narrative would further increase blood glucose levels in US adults ($n = 41$) after administration of oral glucose (50 g) using saccharin as control (23.7 mg). Glucose administration produced a larger increase in blood glucose levels than did the emotionally arousing narrative and prevented the memory-enhancing effect of emotional arousal. No safety-related observations were reported for the saccharin group.

Reid and Hammersley (1999) evaluated the effects of sucrose and maize oil on subsequent food intake and mood in UK adults ($n = 80$) using a randomised single-blind intervention study setting with saccharin as placebo (4.34 g). Men ate more after the saccharin preload than after the other preloads but did not vary the time of their next solid food and the saccharin preload decreased rated tiredness at 2 h compared with the sucrose preload. No safety-related observations were reported for the saccharin group.

Metzger (2000) in a randomised single-blind study assessed the acute effect of glucose consumption on a nonverbal, facial recognition task in young US adults ($n = 34$) using saccharin as control (23.7 mg). There were no statistically significant differences on target identification between the saccharin or glucose groups. No safety-related observations were reported for the saccharin group.

Mohanty and Flint Jr (2001) in a randomised single-blind trial evaluated the differential effects of glucose on modulation of emotional and non-emotional spatial memory tasks in US adults ($n = 70$) using saccharin as control (23.7 mg). Among other findings, the authors reported that glucose administration may enhance memory performance, and that the effects of exogenously administered glucose may interact with the emotionality of the task to modulate performance. No safety-related observations were reported for the saccharin group.

Flint Jr and Turek (2003) assessed the glucose effects (10, 100 and 500 mg/kg, or 50 g) on attention in US adults ($n = 67$) using saccharin (23.7 mg) as placebo. No additional control group was used and no safety-related observations were reported for the saccharin group. Among the multiple analyses performed, the 100 mg/kg glucose group performed worse on measures of commission errors, post-commission responses and post-commission response time variability compared to all other groups including saccharin. No safety-related observations were reported for the saccharin group.

Metzger and Flint (2003) in a randomised single-blind trial evaluated the association of glucose enhancement of face recognition with face features in US adults ($n = 18$) using saccharin (23.7 mg) as placebo. No safety-related observations were reported for the saccharin group.

Messier et al. (2003) in a cross-over double-blind intervention study evaluated the effect of age and glucoregulation on cognitive performance in adults in Canada using two arms as follows: (a) 50 g glucose plus 5 mg saccharin and (b) 50.6 mg saccharin. Cognitive performance was assessed via the subtests in the Working Memory and Processing Speed indices of the Wechsler Adult Intelligence Scale-Third Edition and the Wechsler Memory Scale-Third Edition, as well as via the Verbal Free Recall test, an order reconstruction recall test and the Modified Brown–Peterson task. No safety-related observations were reported for the saccharin group.

Morris (2008) in a single-blind trial evaluated the association between elevating blood glucose level and the retention of information from a public safety video in UK adults ($n = 72$) using saccharin as control (2 g). No safety-related observations were reported for the saccharin group.

Yılmaz et al. (2023) in a randomised double-blind trial evaluated the effect of dietary sugars on cognitive performance and reaction time in Turkey ($n = 75$) using five study arms as follows: Glucose (10 g), fructose (10 g), sucrose (10 g), saccharin (0.24 g) and placebo. The saccharin group had a higher reaction and response time score compared to other sweeteners. No safety-related observations were reported for the saccharin group.

Guo et al. (2014) prospectively evaluated the association between the consumption of various types of beverages and self-reported depression in USA ($n = 263{,}923$). At baseline, participants were asked what kind of sweetener they regularly add to coffee or tea. In the adjusted analysis, saccharin use was statistically significantly associated with self-reported depression (OR 1.14; 95% 1.02–1.27). No information was available on the level of consumption.

González-Domínguez et al. (2021) assessed the association between saccharin levels (as part of the food-related and microbiota-derived circulating metabolome) and cognitive decline using two case–control studies as discovery ($n = 418$) and validation ($n = 424$) datasets nested within the 3C study, a population-based cohort on dementia in France. Metabolomics analysis was performed using a large-scale quantitative targeted multi-metabolite platform. In the discovery dataset, a LASSO penalised conditional logistic regression was employed to select the metabolites that were significantly associated with the odds of subsequent cognitive decline. The unpenalised multivariable adjusted odds ratio for saccharin was 1.26. This finding was replicated in the validation dataset with a multivariable adjusted odds ratio for saccharin of 1.34.

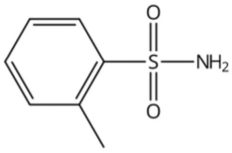
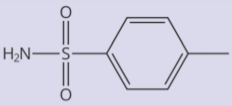
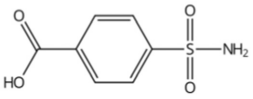
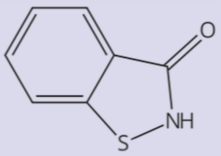
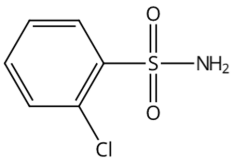
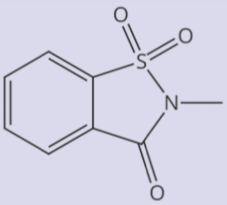
A number of included studies have examined GIT microbiota changes in response to oral exposure to saccharin. Serrano et al. (2021) performed a double-blind, placebo-controlled, parallel arm study exploring the effects of sodium saccharin on gut microbiota (and glucose tolerance, see Section 3.5.4.2) in healthy men and women. The authors concluded that short-term sodium saccharin consumption at maximum acceptable levels is not sufficient to alter gut microbiota. **González-Domínguez et al. (2021)** assessed the association between food and microbiota metabolites with cognitive decline in a 12 year prospective study. **Suez et al. (2022)** assessed the impacts of non-nutritive sweeteners in humans and their microbiomes in a randomised-controlled trial (see also Section 3.5.4.2). The authors concluded that non-nutritive sweeteners consumption may induce person-specific, microbiome-dependent glycaemic alterations, necessitating future assessment of clinical implications. Ramne et al. (2021) examined if the intake of added sugar, sugar-sweetened beverages or artificially sweetened beverages is associate with changes in the gut microbiota composition. The authors concluded that the cross-sectional associations between added sugar and sweet beverage intake and the gut microbiota are modest, but the results suggest that sugar sweetened beverage intake is associated negatively with some changes in the gut microbiota. In addition to the above studies, studies addressing changes in the microbiota in vitro were also retrieved in the literature (**Vamanu et al., 2019**; **Markus et al., 2021**; Shil and Chichger, **2021**; de Souza Lopes et al., 2023).

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.[55]

[55] https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2024.EN-8597.

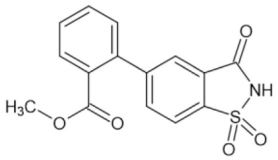
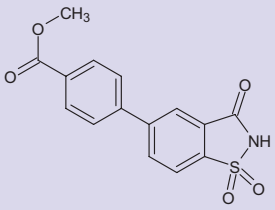
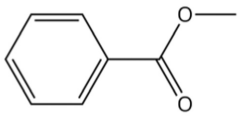
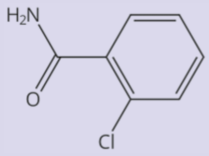
APPENDIX D

QSAR analysis of impurities of saccharin and its sodium, potassium and calcium salts (E 954)

Chemical name	CAS no.	Structure	QSAR ToolBox
o-Toluene sulfonamide	88-19-7		DNA binding OASIS: none DNA binding by OECD: none Protein binding OASIS: none Protein binding by OECD: none Carcinogenicity (genotox & non genotox): none DNA alerts for Ames, CA and MNT by OASIS: none In vitro mutagenicity (Ames) by ISS: none In vivo mutagenicity (Micronucleus) by ISS: none Protein binding alerts for CA by OASIS: none
p-Toluene sulfonamide	70-55-3		DNA binding OASIS: none DNA binding by OECD: none Protein binding OASIS: none Protein binding by OECD: none Carcinogenicity (genotox & non-genotox): none DNA alerts for Ames, CA and MNT by OASIS: none In vitro mutagenicity (Ames) by ISS: none In vivo mutagenicity (Micronucleus) by ISS: none Protein binding alerts for CA by OASIS: none
Benzoic acid p-sulfonamide	138–41-0		DNA binding OASIS: none DNA binding by OECD: none Protein binding OASIS: none Protein binding by OECD: none Carcinogenicity (genotox & non-genotox): none DNA alerts for Ames, CA and MNT by OASIS: none In vitro mutagenicity (Ames) by ISS: none In vivo mutagenicity (Micronucleus) by ISS: none Protein binding alerts for CA by OASIS: none
1,2-benzisotiazolin-3-one (BIT)	2634-33-5		DNA binding OASIS: none DNA binding by OECD: none Protein binding OASIS: no alert Protein binding by OECD: none Carcinogenicity (genotox & non-genotox): none DNA alerts for Ames, CA and MNT by OASIS: none In vitro mutagenicity (Ames) by ISS: none In vivo mutagenicity (Micronucleus) by ISS: none Protein binding alerts for CA by OASIS: none
2-Chlorobenzene sulfonamide	6961-82-6		DNA binding OASIS: none DNA binding by OECD: none Protein binding OASIS: none Protein binding by OECD: none Carcinogenicity (genotox & non-genotox): Halogenated benzene (non-genotox) DNA alerts for Ames, CA and MNT by OASIS: none In vitro mutagenicity (Ames) by ISS: none In vivo mutagenicity (Micronucleus) by ISS: none Protein binding alerts for CA by OASIS: none
N-methyl saccharin	15448-99-4		DNA binding OASIS: none DNA binding by OECD: none Protein binding OASIS: none Protein binding by OECD: none Carcinogenicity (genotox & non-genotox): none DNA alerts for Ames, CA and MNT by OASIS: none In vitro mutagenicity (Ames) by ISS: none **In vivo mutagenicity (Micronucleus) by ISS: H-acceptor-path3-H-acceptor** **Protein binding alerts for CA by OASIS: Acylation**

(Continues)

(Continued)

Chemical name	CAS no.	Structure	QSAR ToolBox
4- Methyl (saccharin) benzoate	–		DNA binding OASIS: none DNA binding by OECD: none Protein binding OASIS: none Protein binding by OECD: none Carcinogenicity (genotox & non genotox): none DNA alerts for Ames, CA and MNT by OASIS: none In vitro mutagenicity (Ames) by ISS: none In vivo mutagenicity (Micronucleus) by ISS: **H-acceptor-path3-Hacceptor** **Protein binding alerts for CA by OASIS: Acylation**
2- Methyl (saccharin) benzoate	–		DNA binding OASIS: none DNA binding by OECD: none Protein binding OASIS: none Protein binding by OECD: none Carcinogenicity (genotox & non genotox): none DNA alerts for Ames, CA and MNT by OASIS: none In vitro mutagenicity (Ames) by ISS: none In vivo mutagenicity (Micronucleus) by ISS: **H-acceptor-path3-Hacceptor** **Protein binding alerts for CA by OASIS: Acylation**
Methyl benzoate	93-58-3		DNA binding OASIS: none DNA binding by OECD: none Protein binding OASIS: no alert Protein binding by OECD: none Carcinogenicity (genotox & non-genotox): none DNA alerts for Ames, CA and MNT by OASIS: none In vitro mutagenicity (Ames) by ISS: none In vivo mutagenicity (Micronucleus) by ISS: none Protein binding alerts for CA by OASIS: none
2-Chlorobenzamide	609-66-5		DNA binding OASIS: none DNA binding by OECD: none Protein binding OASIS: none Protein binding by OECD: none Carcinogenicity (genotox & non-genotox): Halogenated benzene (non genotox) DNA alerts for Ames, CA and MNT by OASIS: none In vitro mutagenicity (Ames) by ISS: none In vivo mutagenicity (Micronucleus) by ISS: none **Protein binding alerts for CA by OASIS: Acylation**

Abbreviations: CA, chromosomal aberration; MNT, micronucleus test.

APPENDIX E

Exposure calculations to organic impurities, associated with the Remsen-Fahlberg process, from the use of E 954

The Panel considered the TTC approach to conduct a risk assessment for the impurities o-toluene sulfonamide, p-toluene sulfonamide and benzoic acid p-sulfonamide, as suitable based on the absence of compound-specific toxicity data and given their lack of genotoxic potential.

The impurities o-toluene sulfonamide, p-toluene sulfonamide and benzoic acid p-sulfonamide were classified into Cramer Class III (Cramer et al., 1978), for which a TTC of 90 μg/person per day or 1.5 μg/kg bw per day is applicable provided that the substance is not genotoxic (EFSA Scientific Committee, 2019). The specification limits of 'not more than 10 mg/kg expressed on dry weight basis' for o- and p-toluene sulfonamide and 'not more than 25 mg/kg expressed on dry weight basis' for benzoic acid p-sulfonamide were considered for the assessment, being the worst-case scenario (Table E.1). The Panel noted that the potential exposure to these impurities is below the Cramer Class III value of 1.5 μg/kg bw per day, and therefore does not raise a safety concern.

The Panel noted that benzoic acid is an authorised food additive (E 210), with an acceptable daily intake (ADI) of 5 mg/kg bw per day (expressed as benzoic acid) (EFSA ANS Panel, 2016).

Considering that the purity of saccharin and its sodium, potassium and calcium salts is not less than 99% on the anhydrous basis, the maximum amount of salicylic acid, considering that other impurities are not present (worst-case scenario), would be 1%, resulting in a potential exposure to salicylic acid from the use of E 954 up to 77 μg/kg bw per day. When comparing with the lowest NOAEL for salicylic acid of 75 mg/kg bw per day (EFSA CEP Panel, 2020), the Panel noted that the MOE would be at least 1000, and no safety concern was raised. Even if the purity of calcium saccharin was not less than 95% on the anhydrous basis, as indicated currently in the EU specifications, the exposure to this impurity would not be of concern.

TABLE E.1 Potential exposure (μg/kg bw per day) to different impurities.

Exposure to E 954 (mg/kg bw per day)	Exposure to o-toluene sulfonamide at a concentration of 10 mg/kg in the additive (μg/kg bw per day)	Exposure to p-toluene sulfonamide at a concentration of 10 mg/kg in the additive (μg/kg bw per day)	Exposure to benzoic acid p-sulfonamide at a concentration of 25 mg/kg in the additive (μg/kg bw per day)	Exposure to salicylic acid at a concentration of 10,000 mg/kg in the additive (μg/kg bw per day)
2.1[a]	0.02	0.02	0.05	20.8
7.7[b]	0.08	0.08	0.19	76.7

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

[b]Highest exposure level among the different population groups (brand-lo[ya]l scenario – the elderly – 95th percentile (Table 5)).

APPENDIX F

Chemical structures of potential impurities analysed in samples of E 954 by the IBOs

TABLE F.1 Chemical structures of potential impurities analysed in samples of E 954 manufactured with the Remsen-Fahlberg process by the IBOs.

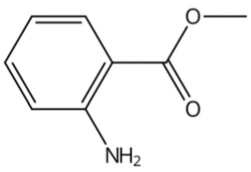
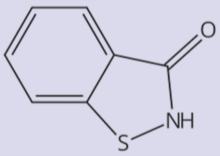
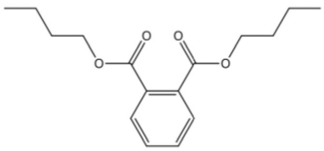
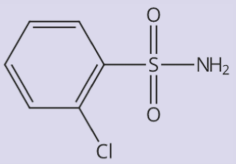
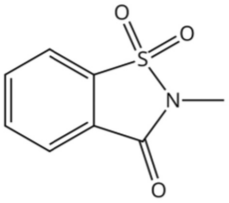
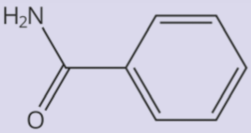
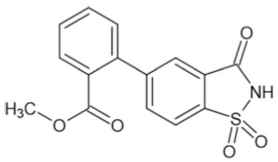
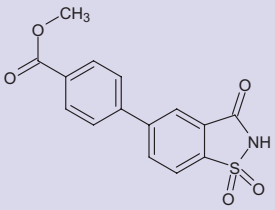
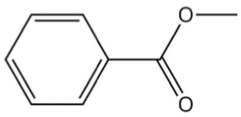
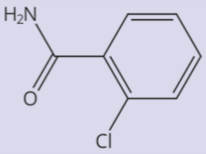
Chemical name	CAS no.	Structure	Analytical results provided by the IBOs	
			Sodium saccharin (E 954 (ii))	Saccharin (E 954 (i))
Methyl anthranilate	134–20-3		5 batches: < 0.1 (LOD) mg/kg in all[a]	5 batches: < 0.1 (LOD) mg/kg in all[a]
1,2-benzisotiazolin-3-one (BIT)	2634-33-5		5 batches: < 0.1 (LOD) mg/kg in all[b] 5 batches: < 0.1 (LOD) mg/kg in all[a]	5 batches: < 0.1 (LOD) mg/kg in all[b] 5 batches: < 0.1 (LOD) mg/kg in all[a]
Dibutyl phthalate (DBP)	84–74-2		5 batches: < 0.1 (LOD) mg/kg in all[a]	5 batches: < 0.1 (LOD) mg/kg in all[a]
2-Chlorobenzene sulfonamide	6961-82-6		5 batches: < 0.1 (LOD) mg/kg in all[a]	5 batches: < 0.1 (LOD) mg/kg in all[a]
N-methyl saccharin	15448–99-4		5 batches: < 0.1 (LOD) mg/kg in all[a]	5 batches: < 0.1 (LOD) mg/kg in all[a]
Benzamide	55–21-0		5 batches: < 0.1 (LOD) mg/kg in all[a]	5 batches: < 0.1 (LOD) mg/kg in all[a]
4- Methyl (saccharin) benzoate	–		1 batch: not detected[c]	–
2- Methyl (saccharin) benzoate	–		1 batch: not detected[c]	–

TABLE F.1 (Continued)

Chemical name	CAS no.	Structure	Analytical results provided by the IBOs	
			Sodium saccharin (E 954 (ii))	Saccharin (E 954 (i))
Methyl benzoate	93–58-3		5 batches: < 0.1 (LOD) mg/kg in all[a]	5 batches: < 0.1 (LOD) mg/kg in all[a]
2-Chlorobenzamide	609–66-5		5 batches: < 0.1 (LOD) mg/kg in all[a]	5 batches: < 0.1 (LOD) mg/kg in all[a]

[a]Documentation provided to EFSA n. 8 and 9.
[b]Documentation provided to EFSA n. 7.
[c]Documentation provided to EFSA n. 4.

APPENDIX G

Exposure calculations to toxic elements and selenium from the use of saccharin and its sodium, potassium and calcium salts (E 954) as a food additive

One IBO provided analytical results on one batch of saccharin (E 954 (i)) for As (< 1 mg/kg), Pb (< 1 mg/kg) and selenium (Se) (< 1 mg/kg) (Documentation provided to EFSA n. 9). The Panel noted that those values are reporting limits. The same IBO provided analytical results on five batches of saccharin (E 954 (i)) for As (< 2 mg/kg in all batches), Pb (< 1 mg/kg in all batches) and Se (< 30 mg/kg in all batches (Documentation provided to EFSA n. 8, 9)). The Panel noted that those values are also reporting limits. The IBO mentioned a LOQ and LOD of 0.1 mg/kg for As, Se and Pb. Another IBO provided analytical results on five batches of saccharin (E 954 (i)) for As (< 1.5 mg/kg in all batches), Pb (< 0.5 mg/kg in all batches), Se (< 15 mg/kg in all batches), copper (Cu) (< 0.5 mg/kg in all batches) and iron (Fe) (< 5 mg/kg in all batches) (Documentation provided to EFSA n. 7). The Panel noted that those values are reporting limits. The analytical method used in all the cases was inductively coupled plasma mass spectrometry (ICP-MS).

In the case of sodium saccharin (E 954 (ii)), one IBO provided analytical results on one batch for As (< 1 mg/kg) and Se (8 mg/kg) (Documentation provided to EFSA n. 3). The Panel noted that the value of As is a reporting limit. Another IBO provided analytical results on one batch of sodium saccharin (E 954 (ii)) for As and cobalt (Co) (below the LOD of 1 mg/kg), Cd, Pb, mercury (Hg) and titanium (Ti) (below the LOD of 0.5 mg/kg), Se, Fe, osmium (Os), palladium (Pd), platinum (Pt), rhodium (Rh), ruthenium (Ru), molybdenum (Mo), gold (Au), Se, silver (Ag), lithium (Li), antimony (Sb), barium (Ba), tin (Sn) (below the LOD of 5 mg/kg) and chromium (Cr), nickel (Ni), Cu (below the LOD of 2 mg/kg) (Documentation provided to EFSA n. 4). In addition, a third IBO provided analytical results on five batches of sodium saccharin (E 954 (ii)) for As (< 1.5 mg/kg in all batches), Pb (< 0.5 mg/kg in all batches) and Se (< 15 mg/kg in all batches), analysed with ICP-MS (Documentation provided to EFSA n. 7). The Panel noted that these values are reporting limits. Another IBO provided analytical results on five batches of sodium saccharin (E 954 (ii)) for As (< 2 mg/kg in all batches), Pb (< 1 mg/kg in all batches) and Se (< 30 mg/kg in all batches) analysed with ICP-MS (Documentation provided to EFSA n. 8, 9). The same IBO provided analytical results on one batch of sodium saccharin (E 954 (ii)) for As (< 1 mg/kg), Pb (< 1 mg/kg) and Se (< 1 mg/kg), using ICP-MS (Documentation provided to EFSA n. 9). The Panel noted that these values are reporting limits.

EFSA requested actual measured concentrations for each element to be indicated, but no respective information was received.

The Panel noted that no information on the lowest technologically achievable levels for the toxic elements in E 954 was provided by the IBOs.

The potential exposure to toxic elements and selenium from the use of saccharin and its sodium, potassium and calcium salts (E 954) can be calculated by assuming that the impurities are present in the food additive up to a limit value, and then by calculation pro-rata to the estimates of exposure to the food additive itself.

With regard to the dietary exposure to E 954, the Panel considered the refined brand-loyal exposure assessment scenario (Table 5). For the current assessment, the highest exposure levels for the mean and 95th percentile among the different population groups were considered, i.e. 2.1 and 7.7 mg/kg bw per day, for the elderly.

For As and Pb, the levels of these toxic elements in E 954 combined with the estimated intakes of E 954 could result in an exposure which can be compared with the following reference points for the toxic elements potentially present in E 954 (Table G.1).

TABLE G.1 Reference points for toxic elements potentially present in E 954.

Element/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 arsenic (iAs)/0.06 µg/kg bw per day (BMDL05)	The reference point is based on a benchmark dose lower confidence limit (BMDL05) of 0.06 µg/kg bw per day identified for skin cancer. The reference point is considered to cover lung cancer, bladder cancer, skin lesions, ischemic heart disease, chronic kidney disease, respiratory disease, spontaneous abortion, stillbirth, infant mortality and neurodevelopmental effects. A MOE of 1 would correspond to the exposure level that is associated with a 5% increase relative to the background incidence for skin cancer, based on the available data'. A MOE of 1 raises a health concern. Because there are no precedents in EFSA for identification of an MOE of low concern, when using a BMDL derived from human cancer data the CONTAM Panel decided not to determine a value for an MOE of low concern. EFSA CONTAM Panel (2024)

Abbreviations: BMDL, lower confidence limit of the benchmark dose; MOE, margin of exposure; RP, Reference point.

The risk assessment of toxic elements helps determine whether there could be a possible health concern if these impurities would be present at the limit values in the food additive. The assessment is performed by calculating the MOE (margin of exposure) by dividing the reference point (e.g. BMDL Table G.1) by the exposure estimate (Table 5).

The IBOs provided analytical data on the levels of lead (Pb) and arsenic (As), in commercial batches of saccharin and sodium saccharin (see Section 3.1.3). No proposal for the lowest achievable levels for those toxic elements in E 954 was provided by the IBOs.

The Panel performed the risk assessment that would result if lead and arsenic, were present in E 954 at the current maximum limits in the EU specification and at the lowest reported LOD or reporting limit by the IBOs. For the assessment it is considered that any arsenic in the samples corresponds to the element in the inorganic form rather than an organic form. Consequently, for the comparison, the RP for inorganic arsenic is used (Table G.1). The outcome of the risk assessment is presented in Table G.2.

Taking into account the fact that E 954 is a product of chemical synthesis using well defined chemical precursors, involving several separation and purification steps, the Panel did not consider it necessary to propose specifications for additional toxic elements.

The Panel emphasised that the choice of the maximum limit values as well as other considerations, such as on multiple sources of exposure to conclude on the maximum limits for toxic elements in the specifications is in the remit of risk management. The numbers used here are merely taken to support the risk assessment of these toxic element as presented below.

TABLE G.2 Risk assessment for toxic elements.

Exposure to E 954 (mg/kg bw per day)	(i) Considering the presence of lead (Pb) and arsenic (As) at the current limits of the EU specifications for E 954 (Commission Regulation (EU) No 231/2012)	
	MOE for Pb at 1 mg/kg	MOE for iAs at 3 mg/kg
2.1[a]	240	10
7.7[b]	65	3
	(i) Considering the presence of lead (Pb) and arsenic (As) at the lowest reported LOD or reporting limit by the IBOs (Documentation provided to EFSA n. 4, 7)	
Exposure to E 954 (mg/kg bw per day)	MOE for Pb at 0.5 mg/kg	MOE for iAs at 0.5 mg/kg
2.1[a]	481	58
7.7[b]	130	16

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

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

Considering the results of the exposure to the toxic element Pb, the Panel noted that its presence in E 954 at the current specification limit value would not give rise to concern. In the case of As, the Panel noted that its presence in E 954 at the current specification limit value would lead to a MOE around 3, which indicates the need to lower the maximum limit for As in the EU specifications.

The Panel noted that the analytical data provided for Pb, and As were reporting limits rather than actual measured values or below the LODs. The IBOs did not indicate the lowest technologically achievable levels for these toxic elements.

The Panel considered that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. Although, no quantitative data were provided, the Panel is of the view that the current EU specification limits for Pb and As should be lowered.

The Panel noted that selenium may be present in the food additive, due to its co-occurrence with sulfur in sulfuric acid, used in the manufacturing of E 954. A current maximum limit for selenium of 'not more than 30 mg/kg' is set in the E 954 EU specifications. The Panel noted that E 954 may contribute to the total European dietary Se exposure. Therefore, the highest mean and 95th percentile of daily dietary exposure of E 954 (2.1 and 7.7 mg/kg bw per day, respectively, for the elderly) were considered to calculate the daily intake of Se from the use of this food additive, if Se was present at the level of 30 mg/kg (specification limit). This exposure was compared with the tolerable upper limit (UL) value of 255 μg Se/day applicable to adult men and women (including pregnant and lactating women) (mean body weight 70 kg) (EFSA NDA Panel, 2023). The daily intakes of Se from the use of E 954 would be 4.4 and 16.1 μg/day, which are circa 2% and 6%, respectively, of the UL of Se for this age group. Considering the above calculated intakes of Se resulting from the use of E 954, the presence of Se in E 954 at the current specification limit would not be of concern.

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