DOI: https://doi.org/10.17590/20230405-084145-0

Do Mixtures of Several Sweeteners Pose Risks for Human Health?

BfR Opinion no. 005/2023, issued 07 February 2023

Sweeteners are food additives. The use of food additives is authorised according to Regulation (EC) No. 1333/2008. One requirement for authorisation is that they do not present any health risk. In the European Union (EU), 20 sweeteners are currently authorised. For each single substance, a dose is derived indicating the quantity of a substance which consumers can ingest every day of their lives without any recognisable health risks. This dose is defined as acceptable daily intake (ADI).

How about using a combination of sweeteners? The German Federal Institute for Risk Assessment (BfR) has investigated whether the available data can provide indications of health risks associated with the combination of sweeteners. Such mixtures are often found in non-alcoholic soft drinks, for example. One reason for this is that some sweeteners at higher concentrations have a bitter metallic aftertaste. In order to avoid this, they are combined with other sweeteners.

The BfR has examined which sweeteners were used most frequently in newly released products on the German market within the period from 2016 to 2020. These are sucralose, acesulfame K, steviol glycosides, cyclamate, saccharin and aspartame. As a basis for the assessment of possible combined effects of these sweeteners, the BfR has considered the data that were taken into account for the authorisation of the single sweetener as food additive in the EU. Other relevant studies were also included.

On the basis of the existing data, it has been examined whether more than one sweetener adversely affect the same organ in the animal model. The sweeteners sucralose, saccharin and aspartame, for example, caused adverse effects on the kidney and the urinary tract in rat studies in which the animals were administered with one of these sweeteners via the diet or drinking water. The observed effects were considered of little relevance for the authorisation of the single substances at that time.

The BfR concludes that effects due to the combination of sweeteners (in the animal model) can in principle be observed in the same organ. A model calculation made by the BfR also states: If the effects are considered toxicologically relevant, and the sweetener dose corresponds to its respective ADI value, the combined intake of the sweeteners could lead to adverse health effects on the kidney and lower urinary tract. At present, it cannot yet be clarified whether several sweeteners in combination compared to the single substances become mutually reinforcing, weakening or do not influence each other. A study addressing this question would be helpful for the assessment of potential effects of combined exposure. Whether the effects observed in the animal model can be transferred to humans cannot be evaluated at present due to the limited data available on the combined effects of sweeteners.

1 Subject of the Assessment

The aim of the National Reduction and Innovation Strategy for Sugar, Fats and Salt in Finished Products (NRI) adopted by the Federal Cabinet in Germany in December 2018 is to significantly reduce the levels of sugar, fats and salt in finished products. In this context, appropriate target agreements have been made with associations of the food industry from the individual sectors,

which are to be implemented step-by-step. For example, the participating companies from the "Wirtschaftsvereinigung Alkoholfreie Getränke e.V. (wafg)", which according to their own statements account for over 90% of the market volume of soft drinks, are aiming for a sugar and calorie reduction of 15% by 2025 within the “soft drinks” category. As part of the strategy the use of sweeteners are given a central role (wafg 2018).

Assessments by the Scientific Committee on Food (SCF) and the European Food Safety Authority (EFSA) form the basis for the authorisation of sweeteners as food additives in the European Union (EU). Within the framework of single substance assessments, an acceptable daily intake (ADI) was derived for each specific sweetener from data from animal experiments. Reliable animal experimental data on potential adverse health effects when combining sweeteners, such as those found in non-alcoholic soft drinks, for example, are not yet available. For this reason, this aspect has not been taken into account in the toxicological assessment by international expert committees.

The German Federal Institute for Risk Assessment (BfR) has examined whether the available data, especially from animal studies, provide indications of health risks associated with the combined use of relevant sweeteners. The considerations focused on the combined use of sweeteners in non-alcoholic soft drinks. For this purpose, three main questions were addressed:

(1) Do the scientific opinions on single substances conducted by the SCF and EFSA, which were carried out as part of the authorisation of sweeteners as food additives in the EU, provide indications of combined effects?

(2) Are the identified effect doses relevant for the assessment of health risks due to the effects upon combined exposure to humans?

(3) Do any identified combined effects pose health concerns?

2 Results

In order to answer the question whether the animal experimental data provide indications of adverse effects when sweeteners are used in combination, a search in the *Mintel Global New Products Database* initially identified sweeteners that were most frequently mentioned in the lists of ingredients for beverages and other foodstuffs, newly launched on the German market between 2016 to 2020. These include sucralose, acesulfame K, steviol glycosides, cyclamate, saccharin and aspartame.

The animal experimental data from the assessments of the single substances conducted by the SCF, EFSA and JECFA (the Joint Expert Committee for Food Additives of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO)) (Joint FAO/WHO Expert Committee on Food Additives) and other animal studies served as the basis for the toxicological evaluation. The animal studies showed effects that were observed in the same target organ/system with more than one of the tested sweeteners sucralose, acesulfame K, steviol glycosides, cyclamate, saccharin and aspartame. In particular, effects on kidney and testicular tissue, on the reproductive system, on the gastrointestinal tract and changes in biochemical parameters in blood, liver and lymphatic organs were considered.

As part of the assessment approach presented here, the kidney and the lower urinary tract were identified as a possible common target organ in rat studies after administration of the sweeteners sucralose, saccharin and aspartame. For hyperplasia and mineralisation of the renal pelvis, the data from the animal studies was considered to be sufficiently reliable for the comparative assessment performed here. With regard to the other effects considered, the data available was

not considered to be sufficiently reliable in order to assess possible combined effects of different sweeteners. Therefore, hyperplasia and mineralisation of the renal pelvis were considered further as examples.

The ADI value of each single sweetener is usually based on the highest dose at which no adverse health effects were observed in animal models (no observed adverse effect level, NOAEL). Uncertainties regarding the transfer of the study results from animals to humans and individual differences are considered by a factor. The quotient of NOAEL and ADI is usually 100.

Supposing that

- the effects considered are cumulative,
- the exposure corresponds to the respective acceptable daily intake (ADI) and
- weighting factors for the different toxicological potencies of the three sweeteners are applied,

the combined intake of the three sweeteners sucralose, saccharin and aspartame would only result in a factor (or "range") of 21.5 between the dose at which mineralisation of the renal pelvis was observed in rat studies and the weighted total exposure (Table 8). In comparison, considering the sweeteners separately, the "range" between the effect dose and the respective ADI would be 60 for sucralose, 100 for saccharin and 50 for aspartame. The "range" is smaller when considering the combined intake compared to the single substances. In regard to the combined intake of the sweeteners sucralose and aspartame, a similar picture emerges when considering the effect of hyperplasia of the renal pelvic epithelium of rats (Table 9).

The examples show that, in principle, combined effects can be observed. If the effects are considered as toxicologically relevant and the assumed exposure corresponds to the respective ADI, the long-term daily intake of sucralose, saccharin and aspartame could possibly no longer be regarded as harmless to health.

The observed effects (mineralisation and hyperplasia of the renal pelvis in rats) were considered as less relevant for the risk assessment of the three sweeteners by the international evaluation panels. However, the BfR points out that changes in the mineral and water balance can cause an imbalance in the renal system and urinary tract. In particular, crystal formation and retention can be key factors in the development of kidney stones in humans. Hence, from the BfR's point of view, potential effects on the kidney and urinary tract due to the combined use of the three sweeteners cannot be finally assessed on the basis of the available data. As already described, the data on the other observed effects (testicular tissue, reproductive system, gastrointestinal tract, changes in biochemical parameters in the blood, in the liver and in the lymphatic organs) were not considered to be sufficiently robust for comparative assessment of combined effects.

An animal study in which several sweeteners are combined, and also administered separately at the same time, would be very helpful to assess potential combined effects.

Current assessments of exposure to sweeteners via non-alcoholic flavoured drinks and other product groups are currently not available for Germany. It is therefore presently unknown to what extent the values for the acceptable daily intake (ADI) of the single sweeteners are exhausted or even exceeded in Germany. It is however expected that increased use of sweeteners as part of the NRI strategy will lead to an increase in exposure to these sweeteners. In this respect, it seems advisable to determine the current exposure in consumers in Germany prior to a possible expansion of use of sweeteners.

3 Rationale

3.1 Database

3.1.1 Selection of considered Sweeteners

According to Annex I of Regulation (EC) No. 1333/2008, the functional class of sweeteners includes substances that are used to impart a sweet taste to food. Excluding the subgroup of sugar alcohols, eleven sweeteners are currently authorised as food additives in the EU. They have low or no nutritive value, are classified as non-cariogenic and are therefore used in numerous product groups.

According to Regulation (EC) No. 1333/2008, sweeteners are authorised for use in a large number of foodstuffs, such as dairy products, breakfast cereals and flavoured drinks, which are energy-reduced, non-cariogenic or with no added sugar. Certain maximum levels apply to the sweeteners considered here.

A search in the *Mintel Global New Products Database*[1] was carried out to identify the sweeteners that were most frequently used in Germany. This database contains, among other things, information about food and its ingredients according to the packaging information. The product number and range do not reflect the entire market, but provide a good overview of the existing products. The search covered a period of 5 years (2016 - 2020). The data was used to determine which sweeteners were most frequently mentioned in the lists of ingredients for beverages and other foodstuffs, newly launched on the German market during this period (Figures 1 and 2). Evaluation of the search in the Mintel database shows that sucralose, acesulfame K, steviol glycosides (including stevia extract), cyclamate (including sodium and calcium cyclamate), saccharin (including sodium, potassium and calcium saccharin) and aspartame were among the non-nutritive sweeteners that were most frequently mentioned in the lists of ingredients for newly launched beverages and other foodstuffs.

By contrast, the authorised sweeteners thaumatin, neohesperidine DC, neotame and salt of aspartame-acesulfame were rarely mentioned in the list of ingredients for newly launched beverages or other foodstuffs within the selected period (<10 mentions). There was no product hit for advantame.

In the assessment of the combined use of sweeteners, the six sweeteners sucralose, acesulfame K, steviol glycosides, cyclamate, saccharin and aspartame are considered accordingly.

[1] Mintel GNPD - Global New Products Database. © 2021 Mintel Group Ltd, 11 Pilgrim Street, London, UK EC4V 6RN, https://www.mintel.com/global-new-products-database

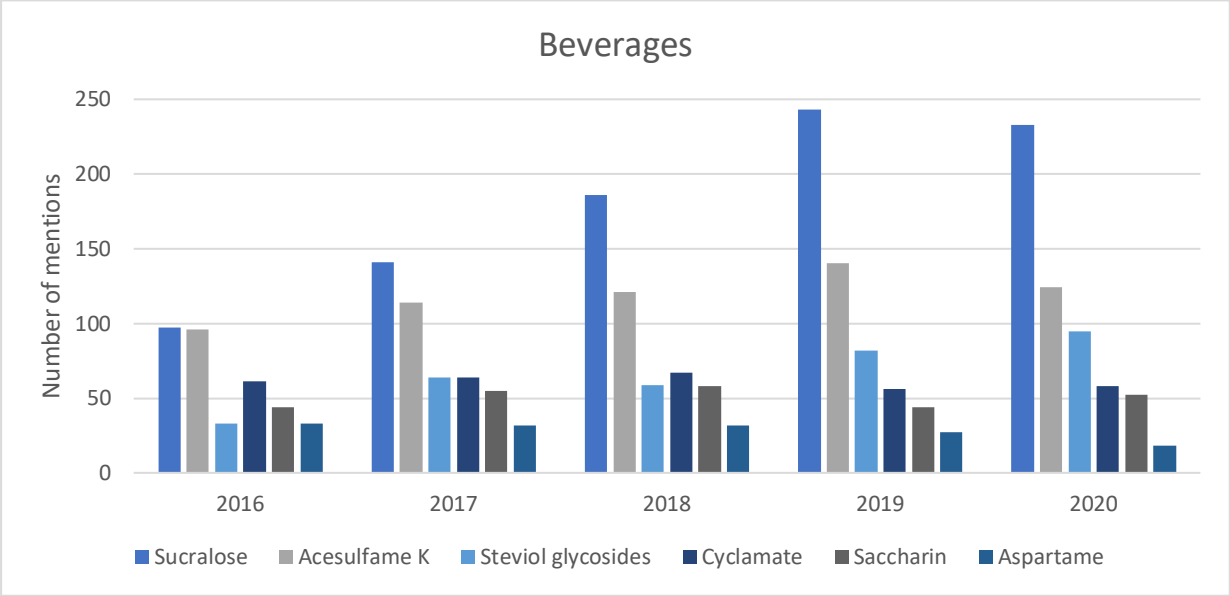

Figure 1: Number of mentions of sweeteners as an ingredient in beverages newly launched on the German market within the period from 2016 to 2020.

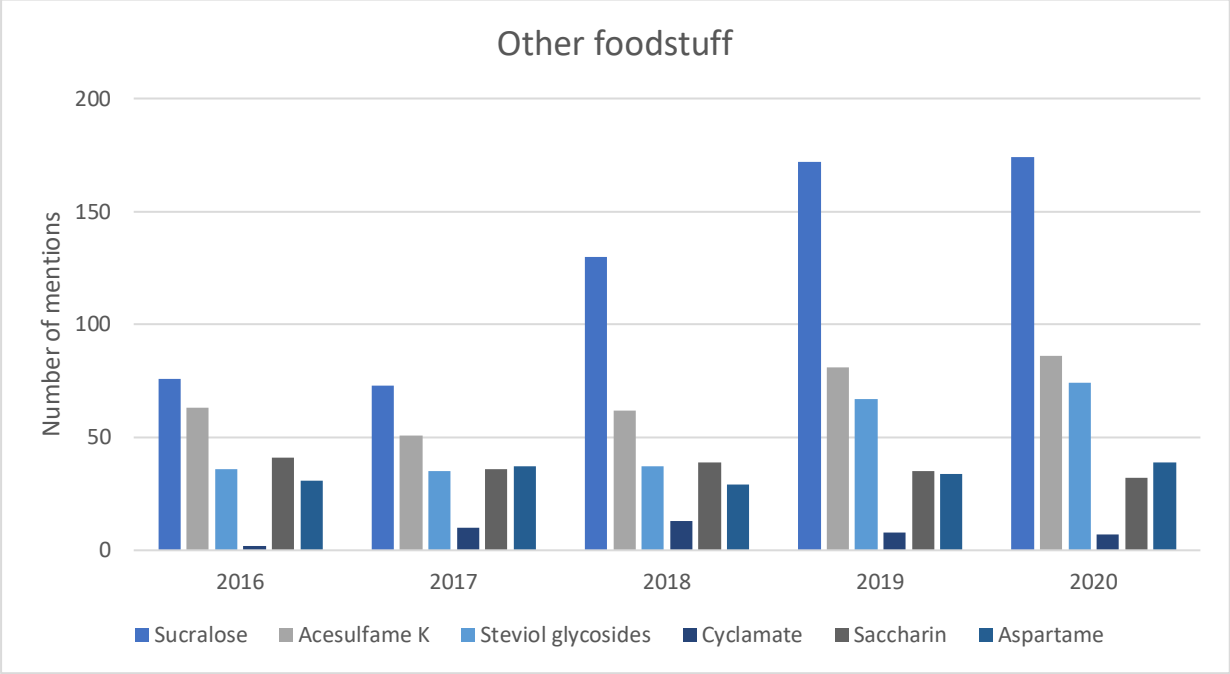

Figure 2: Number of mentions of sweetening agents as an ingredient in other foods newly launched on the German market within the period 2016-2020.

3.1.2 Evaluations of Sweeteners by International Institutions

The basis for authorisation as food additive in the European Union are assessments by the Scientific Committee on Food (SCF) of the EU Commission and the European Food Safety Authority (EFSA). Assessments by the Joint FAO/WHO Expert Committee on Food Additives

(JECFA) are also available. As part of the assessment of a single substance, acceptable daily intake levels (ADI) were derived from data determined from animal experiments.

In the included animal studies, the animals received the relevant food additive in varying concentrations with their diet, primarily on a daily basis and over a long period. All adverse health effects were identified using the lowest dose at which the adverse effect (lowest observed adverse effect level, LOAEL) and the dose at which no adverse effects (no observed adverse effect level, NOAEL) were observed. The NOAEL for the adverse health effect that were observed at the lowest dose (most sensitive effect) was used to derive the ADI value. This NOAEL is generally divided by a factor (usually 100). Thus, uncertainties regarding the transfer of the study results from animals to humans and individual differences are considered. The ADI value is commonly one-hundredth of the NOAEL. The ADI value is expressed in milligrams per kilogram (mg/kg) of body weight (bw) per day. This amount can be consumed daily over a lifetime without expecting any adverse health effects.

However, no reliable animal experimental data on effects when combining sweeteners, such as those found in non-alcoholic soft drinks, are currently available, so this aspect could not be taken into account in the toxicological assessment by the international expert committees.

Sucralose (E 955)

Sucralose is produced by chlorination of sucrose and its flavour is comparable without have an off-taste or after-taste. After oral intake, the sweetener is absorbed to a comparatively small extent and excreted largely unchanged in the faeces and to a lesser extent in the urine (Grice & Goldsmith 2000). Sucralose is used in numerous food groups, but also as an additive in pharmaceutical products. Sucralose is used commercially as a table-top sweetener, e.g. marketed as a mixture of sucralose (1%) and maltodextrin (99%).

Taking into account the available data, the SCF derived an ADI value for sucralose of 15 mg/kg of body weight per day in 2000. This value is based on the dose of 1,500 mg/kg of body weight per day (NOAEL or NOEL according to the SCF report) at which no reduction in body weight gain was observed in rats. The lowest dose administered in the diet at which a lower weight gain was identified after administration of sucralose in the diet (LOAEL or LOEL according to the SCF report) was 3% (corresponding to 1,973 to 2,455 mg/kg of body weight per day) (SCF 2000c).

Acesulfame K (E 950)

Acesulfame K refers to the potassium salt of acesulfame. The sweetener is often used in combination with other sweeteners because high concentrations exert a metallic after-taste. Acesulfame K can be stored for a long time, is very stable and withstands conventional pasteurisation and sterilisation processes without losing its sweetness. Because of its resistance to heat, it can also be used for cooking and baking. Acesulfame K is also found in drinks and toothpastes. The maximum permitted level in non-alcoholic flavoured drinks is 350 milligrams per litre (mg/L). Acesulfame K is mainly absorbed from the gastrointestinal tract (> 90%) and excreted in the urine. Only a small part is excreted in the faeces. The additive was re-evaluated by the SCF in 2000. An ADI of 9 mg/kg of body weight per day was set. The derivation is based on the highest administered dose of 3% in the diet (corresponding to 900 mg/kg of body weight per day in dogs or 1,500 mg/kg of body weight per day in rats), at which no significant toxicological effects were observed (NOAEL). No LOAEL could be identified in the studies used to derive the NOAEL (SCF 2000b).

Steviol glycosides (E 960)

Steviol glycosides are extracted from the leaves of the *Stevia rebaudiana* Bertoni plant, which contain various substances from the glycoside group. The food additive E 960 must contain not less than 95% of the steviol glycosides steviolbioside, rubusoside, dulcoside A, stevioside, rebaudiosides A, B, C, D, E, F and M on the dried basis (Regulation (EU) No. 231/2012), whereby stevioside and rebaudioside A are essentially responsible for the sensory properties of the extracts made from the stevia leaves and thus represent the main components. Steviol glycosides are hydrolysed to the aglycone steviol by intestinal flora and then metabolised to steviol glucuronides and excreted primarily in the urine but also in the faeces.

Because of their bitter taste, reminiscent of liquorice, steviol glycosides are used in combination with other sweeteners or sugar. Steviol glycosides are authorised in the EU according to Regulation (EC) No. 1333/2008 in numerous food categories, whereby non-alcoholic flavoured drinks make up the majority of the estimated total exposure (EFSA 2010).

The EFSA has established an ADI value for steviol glycosides of 4 mg/kg of body weight per day. This value was derived from the lowest dose of stevioside administered in the diet at 2.5% (corresponding to 388 mg steviol equivalents /kg of body weight per day or corresponding to 967 or 1,120 mg stevioside /kg of body weight per day in male and female rats) at which no adverse health effects were observed after two years of administration in the rat. In contrast, the administration of 5% stevioside in the diet (corresponding to 1,997 or 2,387 mg/kg of body weight per day in male or female rats) led to adverse health effects (lower body weight, changes in various organ weights such as kidney, ovaries and brain), and was accordingly identified as LOAEL (EFSA 2010).

Cyclamate (E 952)

The sweetener cyclohexane-sulphamic acid and its sodium and calcium salts are summarised under the name cyclamate. Cyclamate is rapidly absorbed in the intestine and excreted primarily via the urine. The intestinal flora can metabolise cyclamate to cyclohexylamine (CHA). CHA is also absorbed and excreted in the urine (Bopp *et al.* 1986). Cyclamate is heat stable, can be stored for a long time and can also be used for cooking and baking. High concentrations have a bitter metallic after-taste. Cyclamate is often used together with saccharin because the two substances intensify each other's sweetness.

The SCF established the ADI for cyclamate (more precisely for cyclamic acid and its sodium and calcium salts) of 0 – 7 mg/kg of body weight per day. The value is based on a NOAEL of 100 mg/kg of body weight per day for cyclohexylamine (CHA) including a conversion rate to cyclamate of 85% and an uncertainty factor of 32 (SCF 2000a). Higher doses of CHA led to adverse health effects on testicular tissue in rats. Administration of 300 mg/kg of body weight per day or 0.6% in the diet led to changes in the testicular tissue (testicular atrophy) in male rats and was accordingly identified as the LOAEL (Gaunt *et al.* 1976; Brune & Mohr 1978).

Saccharin (E 954)

Saccharin was the first industrially produced sweetener and also includes the sodium, potassium and calcium salts of saccharin. The sweetener is almost entirely (> 90%) absorbed in the gastrointestinal tract and excreted unchanged in the urine. Only a small part is excreted in the faeces (about 3%). To date, there is no evidence that saccharin is metabolised in detectable amounts. Saccharin is mostly used in combination with other sweeteners, because a too high concentration leads to a bitter-metallic after-taste (Renwick 1986). The substance is heat and freeze resistant and is also stable in aqueous and acidic products.

In 1995, the SCF established an ADI value of 0 – 5 mg/kg of body weight per day for saccharin and its sodium, potassium and calcium salts, and 0 – 3.8 mg/kg of body weight per day for the free acid. The derivation is based on a NOAEL of 1% sodium saccharin in the diet (corresponding to 500 mg/kg of body weight per day) of male rats, where no relevant toxicological effects were observed. A dose three times higher (3% in the diet, corresponding to 1,500 mg/kg of body weight per day) in rats led to a general disturbance of homoeostasis with reduced body weight gain and other adverse health effects, including an increased incidence of bladder tumours. The relevance of bladder tumours was discussed by the JECFA and SCF (JECFA 1993; SCF 1995).

Aspartame (E 951)

Aspartame is a methyl ester of the dipeptide of the amino acids L-aspartic acid and L-phenylalanine. Aspartame can be hydrolysed by intestinal esterases and peptidases to aspartic acid and phenylalanine and methanol. The degradation products enter systemic circulation where they are further metabolised (Magnuson *et al.* 2007). Accordingly, the sweetener has a calorie content of 4 kilocalories per gram (kcal/g). Aspartame contains a source of phenylalanine, which must be labelled on products and should be taken into account by those suffering from the metabolic disease phenylketonuria. Aspartame is useful for sweetening a variety of foodstuff (e.g. chewing gum, breakfast cereals, other dry goods and beverages). Aspartame is unstable at sustained high temperatures and is therefore unsuitable for baking and cooking purposes.

The EFSA last published a full risk assessment of aspartame in 2013. The previously established ADI value of 40 mg/kg of body weight per day was confirmed and is based on a NOAEL of 4,000 mg/kg of body weight per day. However, the EFSA emphasised that the possibility of developmental toxicity occurring at doses lower than 4,000 mg/kg in the tested animals could not be excluded: "*[…] The possibility of developmental toxicity occurring at lower doses than 4,000 mg/kg in animals could not be excluded. Based on MoA and weight-of-evidence analysis, the Panel concluded that developmental toxicity in animals was attributable to phenylalanine. Phenylalanine at high plasma levels is known to cause developmental toxicity in humans. […]*". The administration of 8,000 mg aspartame /kg of body weight per day showed numerous adverse health effects in rats, including the kidney (EFSA 2013).

Table 1: Overview of toxicological parameters for the sweeteners tested.

	Sucralose	**Acesulfame K**	**Steviol glycosides**	**Cyclamate**	**Saccharin**	**Aspar-tame**
Description of sweetener	Trichlorogalacto-sucrose (TGS)	Potassium salt of acesulfame	Not less than 95% ste-viol glycosides[a]	Sodium and calcium salts of cyclohex-ane-sulphamic acid	Saccharin and its calcium, potassium and sodium salts	
Evaluation panel Year	SCF 2000	SCF 2000	EFSA 2010	SCF 2000	SCF 1995	EFSA 2013
NOAEL in mg/kg bw per day	1,500	900	388 steviol equivalents[b]	100 CHA-HCL[c]	500 Na saccharin[d]	4,000
LOAEL in mg/kg bw per day	1,973 – 2,455	No LOAEL described	2,500 stevioside	300 CHA-HCL[c]	1,500 Na saccharin[d]	8,000
ADI in mg/kg bw per day	15	9	4	7	5	40

ADI (acceptable daily intake); NOAEL (no observed adverse effect level); LOAEL (low observed adverse effect level); bw (body weight); SCF (Scientific Committee on Food); EFSA (European Food Safety Authority); a Steviolbioside, rubusoside, dulcoside A, stevioside, rebaudiosides A, B, C, D, E, F and M; b Corresponding to 967 or 1,120 mg/kg of body weight per day of stevioside in male or female rats; c Cyclohexylamine hydrochloride; d Sodium salt of saccharin

3.2 Identification of Relevant Target Organs/Tissues

The animal experimental data from the assessments of food additives conducted by SCF, EFSA and Joint FAO/WHO Expert Committee on Food Additives (JECFA) and other animal studies served as basis for the toxicological evaluation. However, potential effects resulting from the combination of sweeteners were not taken into account in the toxicological assessment by the international expert panels, because no reliable animal experimental data on potential effects resulting from the combination of sweeteners were available at the time of the assessment.

To identify potentially identical adverse health effects, all effects observed with the sweeteners sucralose, acesulfame K, steviol glycosides, cyclamate, saccharin and aspartame in the relevant animal studies were considered. Effects were identified that were observed with more than one of the tested sweetener in the same target organ/system. The current SCF and EFSA assessment reports on sucralose (SCF 2000c), acesulfame K (SCF 2000b), steviol glycosides (EFSA 2010), cyclamate (SCF 2000a), saccharin (SCF 1995) and aspartame (EFSA 2013) were used and NO(A)EL and LO(A)EL were identified for the relevant effects. In addition, the monographs of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) were also taken into account. Further study results on the identified and relevant effects, which were published after the risk assessments of the SCF and the EFSA, were also included.

Effects that were not used to establish the NOAEL or ADI as part of the evaluation of the sweeteners, but which could be considered relevant to health, as well as effects that only were observed at doses higher than the LOAEL, were also included in the assessment. The research and selection of the studies makes no claim to completeness.

3.2.1 Effects on Kidney and Urinary Bladder

The evaluation of the data from animal experiments, which were taken into account for the authorisation of the sweeteners tested as food additives in the EU, showed that adverse health effects were observed in the kidney and the urinary bladder after administration of the sweeteners sodium saccharin, sucralose and aspartame in rats (**Table 2**).

Of particular note are the histopathological findings in the urinary bladder and in the renal pelvis of rats. In a two-generation study in male F1 rats treated with 3% **Na saccharin** in the diet (equivalent to 1,500 mg/kg of body weight per day), an increased incidence of primary neoplasia in the urinary bladder was observed compared to the control group, at higher doses (≥ 4% in the diet, corresponding to ≥ 2,000 mg/kg of body weight per day), an increased number of bladder tumours were reported. In the same animal model, the dose of 1% Na saccharin in the diet (corresponding to 500 mg/kg of body weight per day) led to increased mineralisation in the kidney, which were observed within or directly beneath the pelvic epithelium (Schoenig *et al.* 1985). Morphological changes in the bladder epithelium (pleiomorphic microvilli, hyperplasia of the epithelium) were observed in male rats after administration of 1,250 mg/kg of body weight per day for ten weeks (Murasaki & Cohen 1981).

Sucralose also led to effects on the renal pelvis. Dietary sucralose administered to female rats at concentrations of 0; 0.3; 1 and 3% (corresponding to 0, 200, 900 and 2,200 mg/kg of body weight per day) over a period of two years, increased hyperplasia of the renal pelvic epithelium at all doses, while an increase in the mineralisation of the renal pelvis was observed additionally at 900 and 2,200 mg/kg of body weight per day. These effects were not observed in male rats (Mann *et al.* 2000).

Increased mineralisation of the renal pelvis was also observed after two years of administration of **aspartame** at doses of 2,000 and 4,000 mg/kg of body weight per day in the diet of male and female rats. This effect was not observed in the renal pelvis at 1,000 mg/kg of body weight per day (Ishii *et al.* 1981). Deposits of iron-containing haemosiderin in the epithelial cells of the renal tubules and renal pelvis and an increased occurrence of focal hyperplasia of the renal pelvic epithelium and degeneration of the renal tubules were observed in male rats that were administered a dose of 8,000 mg aspartame /kg of body weight per day in the diet for two years. Lower doses did not lead to the effects described (EFSA 2013).

Table 2: Effects on the kidney and urinary bladder following administration of sweeteners with respective study details.

Sweeteners	Effect	NOEL* in mg/kg of bw per day	LOEL* in mg/kg of bw per day	Species	Duration in weeks	Route	Reference
Sucralose (ADI 15mg/kg bw per day)	Hyperplasia of the renal pelvic epithelium		200[b]	Rat, f	104	Diet	Mann *et al.* 2000[#]
	Mineralisation of the renal pelvis	200	900[c]	Rat, f	104	Diet	Mann *et al.* 2000[#]
Na saccharin (ADI 5 mg/kg bw per day)	Mineralisation of the renal pelvic epithelium		500[d]	Rat, m (F1)	Multigeneration	Diet	Schoenig *et al.* 1985[#]
	Primary neoplasms of the urinary bladder, increase in the relative weight of the urinary bladder	500	1,500[d]	Rat, m (F1)	Multigeneration	Diet	Schoenig *et al.* 1985[#]
	Morphological changes in the bladder epithelium (pleiomorphic microvilli, hyperplasia)	500[a]	1,250[e]	Rat, m (n=10)	10	Diet	Murasaki *et al.* 1981
Aspartame (ADI 40 mg/kg bw per day)	Mineralisation of the renal pelvis	1,000	2,000[f]	Rat, m, f	104	Diet	EFSA 2013 (Ishii *et al.* 1981)[#]
	Liver: Pigment deposits, focal tubular degeneration and focal tubular hyperplasia in the kidney	1,000, 2,000, 4,000	8,000	Rat, m (n=40)	104	Diet	EFSA 2013 (E33-34)[#]

* Specified as NOEL and LOEL (and not as NOAEL or LOAEL), because the evaluation panels did not consider the effects observed to be relevant for the establishment of the respective ADI; m Male; f Female; [a] Corresponds to 1% in the diet, further effect doses 0.1%, 0.5% in the diet; [b] Dose-dependent increase in incidence: 0 mg/kg of bw/day (2/50); 200 mg/kg of bw/day (6/50); 900 mg/kg of bw/day (9/50); 2,200 mg/kg of bw/day (10/50); [c] Dose-dependent increase in incidence: 0 mg/kg of bw/day (7/50); 200 mg/kg of bw/day (12/50); 900 mg/kg of bw/day (16/50); 2,200 mg/kg of bw/day (15/50); [d] Corresponds to 1% in the diet, further effect doses: 3%, 4%, 5%, 6.25%, 7.5% in the diet; [e] Further effect dose: 5% in the diet; f Dose-dependent increase in incidence: 0 mg/kg of bw/day (m:1/57; f: 16/59); 1,000 mg/kg of bw/day (m: 5/55, f: 23/59); 2,000 mg/kg of bw/day (m: 10/60, f: 30/59); 4,000 mg/kg of bw/day (m: 15/59; f: 46/60); [#] Conduction primarily according to OECD test guidelines

In conducting the literature review, further effects after the administration of sweeteners in the kidney tissue and changes in renal and urological parameters were also identified. The findings described below and presented in Table 3 represent a selection of the results from the literature review.

Increased calcification of the renal cortex, pelvis and renal papillae was observed in male rats that were administered a dose of 50, 100 or 150 mg **cyclohexylamine (CHA)**/kg of body weight per day in the diet for 2 years. According to the authors, however, the study results must be interpreted with caution because of the lack of a dose-response relationship (Oser *et al.* 1976). In their comprehensive review, Bopp et al. comes to the conclusion that sodium and calcium cyclamate cause renal disorders and nephrocalcinosis only at very high doses of 5% (corresponding to 2,500 mg/kg of body weight per day) or 10% in the diet of rats. However, these observations have not been confirmed in other species (mouse, dog, monkey) treated with cyclamate and CHA, respectively (Friedman *et al.* 1972; Bopp *et al.* 1986).

Four-week administration of 10 or 500 mg/kg of body weight per day of **Na saccharin** via gavage increased creatinine, albumin and urea values in the serum of male rats (Amin *et al.* 2016). Treatment of male rats for nine weeks with 70 mg **aspartame** / kg of body weight per day by gavage increased serum urea and creatinine levels. These changes in the parameters were not observed with 15 or 35 mg aspartame/kg of body weight per day (Adaramoye & Akanni 2016). An increase in the urea and creatinine concentrations in male rats was also observed after administration of 500 mg aspartame/kg of body weight per day in the drinking water for six weeks (Saleh 2014). In contrast, administration of about 7 mg aspartame/kg of body weight per day (60 mg/l) via the drinking water to male rats on a standard and high-fat diet for eight weeks led to a decrease in the serum urea concentration (Palmnäs *et al.* 2014). Gaunt et al. observed lower blood urea levels in male rats that were administered 0.06% **CHA** and higher doses of 0.2% and 0.6% in the diet (corresponding doses in mg/kg of body weight per day see Tab. 3). Administration of 0.06% CHA in the diet did not lead to a change in the albumin concentration in the blood, but an increase in the albumin content was observed at 0.2 and 0.6% (Gaunt *et al.* 1976).

Other parameters of the urinary tract were the increase in bladder weight and changes in pH and mineral content in the urine that were altered by the treatment of sweeteners. 1,500 mg/kg of body weight per day and higher doses of sodium saccharin in the diet led to an increase in bladder weight in rats, whereas a dose of 500 mg/kg of body weight per day had no effect on bladder weight (Schoenig *et al.* 1985). Anderson et al. also observed an increase in the weight of the bladder and a reduction in the pH value and an increase in the phosphate content in the urine after administration of 2,500 mg/kg of body weight per day of Na saccharin for ten weeks (Anderson *et al.* 1988). A pH reduction and an increased calcium concentration in the urine were also determined after administration of 2,000 or 4,000 mg aspartame /kg of body weight per day for two years. Such effects were not observed after administration of 1,000 mg aspartame/kg of body weight per day (Ishii *et al.* 1981).

Table 3: Other renal or urological effects following administration of sweeteners with respective study details.

Sweeteners	Effect	NOEL* in mg/kg bw per day	LOEL* in mg/kg bw per day	Species	Duration in weeks	Route	Reference
Cyclamate (ADI 7 mg/kg bw per day)	Calcification of the kidney		50/100/150 CHA	Rat, m	104	Diet	Oser *et al.*1976[#]
	Decrease in blood urea		~19 CHA**[b]	Rat, m (n=10)	104	Diet	Gaunt *et al.* 1976[#]
	Increase in serum albumin	~19 CHA	~77 CHA**[c]	Rat, m (n=10)	104	Diet	Gaunt *et al.* 1976[#]
Na saccharin (ADI 5 mg/kg bw per day)	Increase in serum creatinine, urea, albumin		10[d]	Rat, m (n=8)	4	Gavage	Amin *et al.* 2016
	Decrease of urine pH; increase in urinary phosphate, increase in rel. bladder weight		2,500	Rat, m (n=5)	10	Diet	Anderson *et al.* 1988
Aspartame (ADI 40 mg/kg bw per day)	Increase in serum creatinine, urea		500	Rat, m (n=6)	6	Drinking water	Saleh *et al.* 2014
	Increase in serum creatinine, urea	15, 35	70	Rat, m (n=5)	9	Gavage	Adaramoye *et al.* 2016
	Decrease in serum urea		7	Rat, m (n=9-12)	8	Drinking water	Palmnäs *et al.* 2014
	Decrease in urine pH, increase of calcium in urine	1,000	2,000[e]	Rat, m, f	104	Diet	EFSA 2013 (Ishii *et al*. 1981)[#]

* Specified as NOEL and LOEL (and not as NOAEL or LOAEL), because the evaluation panels did not consider the effects observed to be relevant for the establishment of the respective ADI; m Male; f Female; [a] No dose dependence with respect to incidence -> 0 mg/kg bw per day: 2/33; 15 mg/kg bw per day: 5/24; 50 mg/kg bw per day: 11/35; 100 mg/kg bw per day: 10/27; 150 mg/kg bw per day: 8/41; [b] Other effect doses: 77 and 257 mg/kg bw per day; [c] Other effect doses: 257 mg/kg bw per day; [d] Other effect doses: 500 mg/kg bw per day; [e] Other effect doses: 4,000 mg/kg bw per day, ** Doses depending on week 1→104: 0.06% in the diet (equivalent to 71→19 mg/kg bw per day); 0.2% in the diet (corresponding to 201→77 mg/kg bw per day); 0.6% in the diet (corresponding to 545→257 mg/kg bw per day); [#] Conduction primarily according to OECD test guidelines

3.2.2 Effects on Testicular Tissue

For the evaluation of cyclamate as a food additive, the pathological change in testicular tissue was identified as the most sensitive effect for establishing the NOAEL (SCF 2000a). In male rats, the administration of 200 mg **cyclohexylamine (CHA)** /kg bw per day of in the diet led to differences in the testicular score after 90 days and provides an indication of reduced spermiogenesis in the tubules (Johnsen 1970) compared to the control group. After administration of 300 mg CHA/kg bw per day, degenerative changes in the testicular tubules and indication of testicular atrophy were observed. The CHA doses administered of 50 or 100 mg/kg bw per day had no effect on the testicular tissue (Brune & Mohr 1978; JECFA 1982). Testicular atrophy was also observed in male rats that were administered 150 mg CHA/kg bw per day for two years (Oser *et al.* 1976). A dose of 0.6% CHA in the diet increased the incidence of testicular atrophy and also resulted in increased calcium deposition in the seminiferous tubules after treatment for two years (Gaunt *et al.* 1976). James et al. observed a reduction in the sperm count with an increased number of abnormal sperm in the testicular tissue both in male rats and in dogs (beagles) after administration of 200 or 250 mg CHA/kg bw per day via gavage for nine weeks (James *et al.* 1981). In mice, on the other hand, the dose of 300 mg/kg bw per day of CHA did not lead to any histopathological changes in the testicular tissue (Hardy *et al.* 1976).

Within the literature review performed, which also included studies that were published after the SCF and EFSA opinions, a study was identified where administration of 460 mg/kg bw per day of **Na saccharin** in mice for eight weeks led to adverse health effects on the testicular tissue. Gong *et al.* observed effects on testicular morphology and on sperm function and quality in male mice after treatment with Na saccharin via the drinking water for five weeks. Damage to the seminiferous tubules from the periphery to the lumen was found in the testicular tissue of mice treated with 460 mg Na saccharin/kg bw per day Na saccharin. The histological examinations showed an increase in exfoliated spermatogenic cells from the epithelium (spermatids, pachytene spermatocytes) and a cluster of discohesive cells. Histopathological changes in the testicular tissue of animals treated with lower Na saccharin (40 and 210 mg/kg bw per day) were not observed. In addition, sperm count, motility and viability were reduced, and structural abnormalities of the sperm head and tail were observed when the high dose of Na saccharin (460 mg/kg bw per day) was administered. These effects were not observed with Na saccharin doses of 40 or 210 mg/kg bw per day. Comparable histopathological changes in testicular tissue and adverse effects on sperm quality (exfoliated cells in the lumen, lower sperm viability, abnormal sperm tail structure) were observed in mice that were treated with sucrose (22.28 and 53.7 g/kg bw per day), which is comparable to the sweetness of the medium and high administered doses of Na saccharin (210 or 460 mg/kg bw per day). The analysis of hormones in the serum relevant to reproduction showed a reduction in the estradiol and testosterone levels at 460 mg/kg bw per day Na saccharin, whereas the effects were not observed at lower Na saccharin doses (40 or 210 mg/kg bw per day) or sucrose doses. After administration of Na saccharin, changes in the luteinizing hormone (LH) (not dose-dependent) and in the expression levels of various proteins relevant to steroid hormone synthesis were also observed in the testicular tissue (Gong *et al.* 2016).

In a chronic toxicity study in male rats that were treated with **aspartame** in the diet for two years, the incidence of vesicle atrophy increased in the animals treated with the highest aspartame doses (4,000 and 8,000 mg/kg bw per day), although no clear dose dependence was observed. No other effects on testicular tissue or sperm function were described in the EFSA report (EFSA 2013) (**Table 4**).

Table 4: Effects on testicular tissue following administration of sweeteners with respective study details.

Sweeteners	Effect	NOEL* in mg/kg bw per day	LOEL* in mg/kg bw per day	Species	Duration in weeks	Route	Reference
Cyclamate (ADI 7 mg/kg bw per day)	Degenerative changes in the tubules, testicular atrophy	50,100,200 CHA	300 CHA	Rat, m	13	Diet	Brune *et al.* 1978[#]
	Changes in spermiogenesis	50.100 CHA	200 CHA				
	Testicular atrophy[a]	15,50,100 CHA	150 CHA	Rat, m	104	Diet	Oser *et al.* 1976[#]
	Testicular atrophy[b], calcification of tubules[c]	~30, 100 CHA**	~300 CHA**	Rat, m	104	Diet	Gaunt *et al.* 1976[#]
	Decrease in the number of "late" spermatids		200 CHA	Rat, m	9	Gavage	James *et al.* 1981
	Decrease in sperm count, increase in abnormal sperm		250 CHA	Dog, m	9	Gavage	James *et al.* 1981
Na saccharin (ADI 5 mg/kg bw per day)	Decrease in sperm count, motility, viability; Histopathological changes in testicular tissue	40, 210	460	Mouse, m (n=8)	5	Drinking water	Gong *et al.* 2016
Aspartame (ADI 40 mg/kg bw per day)	Incidence of seminal vesicle atrophy[d]	1,000, 2,000	4,000, 8,000	Rat, m	104	Diet	EFSA 2013 (E33-34)[#]

*** Except for the CHA effects,** NOEL and LOEL (and not as NOAEL or LOAEL) are presented, because the evaluation panels did not consider the effects described to be relevant for the establishment of the respective ADI; m Male; f Female; ** Corresponds to 0.06 % and 0.2% or 0.6% in the diet, doses depending on week 1→104: 0.06% in the diet (equivalent to 71→19 mg/kg bw per day); 0.2% in the diet (corresponding to 201→77 mg/kg bw per day); 0.6% in the diet (corresponding to 545→257 mg/kg bw per day);[a] Incidence -> 0 mg/kg bw per day 5/19, 150 mg/kg bw per day of CHA: 12/20); [b] Incidence -> 0 mg/kg bw per day: 0/34, 300 mg/kg bw per day of CHA: 18/46; [c] Incidence -> 0 mg/kg bw per day: 1/34, 300 mg/kg bw per day of CHA: 10/46; [d] Incidence -> 0 mg/kg bw per day: 2/23, 4,000 mg/kg bw per day: 4/23, 8,000 mg/kg bw per day: 6/21); [#] Conduction primarily according to OECD test guidelines

3.2.3 Effects on Reproduction and (Postnatal) Development

In a multi-generational study by Kroes *et al.* embryotoxic effects were observed in mice after administration of 0.5% **cyclohexylamine (CHA)** (with a conversion factor of 0.15 corresponding to 750 mg/kg bw per day). The foetuses of the mice had a lower body weight and the administration of CHA led to a lower number of liveborn foetuses and an increase in postnatal mortality. In animals treated with 5% **sodium cyclamate** (equivalent to 7,500 mg/kg bw per day), a lower body weight was also observed in the offspring. Other sweetener dose groups (2% Na cyclamate; 5% Na cyclamate + 0.5% saccharin; 2% Na cyclamate + 0.2% saccharin; 0.5% saccharin; 0.2% saccharin) showed no embryotoxic or teratogenic effects compared to the control group (Kroes *et al.* 1977).

In a two-generation study with female rats, Schoenig *et al.* observed a decrease in the average litter size after treatment with 3% (corresponding to 1,500 mg/kg bw per day) **Na saccharin** in the diet of the dams. Higher Na saccharin doses of 5 or 7.5% in the diet (corresponding to 2,500 or 3,750 mg/kg bw per day) reduced the body weights of the offspring, and higher fluid intake was observed in the F1 animals after 30 months of the study. The offspring survival rate increased in dams that were treated with 5 or 7.5% Na saccharin in the diet, but not in the 6.25% dose group (Schoenig & Anderson 1985). In mice treated with 5% (equivalent to 2,500 mg/kg bw per day) Na saccharin in the diet of the parent animals, a decrease in the number of live pups per litter and a lower body weight of the pups were observed in mice, together with a lower body weight in the dams. The mortality of the parent animals increased in the 5% Na saccharin group. The water intake of the parent animals increased with 1.25 and 2.5% Na saccharin, but decreased after treatment with 5% saccharin (NTP 1997).

Postnatal development disorders were observed in numerous rat studies with aspartame, described in detail in EFSA's risk assessment of aspartame. The offspring whose dams were treated with 4,000 mg/kg bw per day of aspartame in the diet had reduced body weight and size compared to the control animals. In addition, the number of viable pups per litter at birth and at weaning was reduced and the abortion rate increased. Treatment with 4,000 mg/kg bw per day of aspartame decreased feed consumption and body weight of the dams during gestation and lactation. The effects described in the dams and the F1 generation were not observed with 2,000 mg aspartame/kg bw per day. In female rabbits, treatment with 2,000 mg/kg bw per day of aspartame via gavage also led to lower body weights and reduced feed intake in the dams. In addition, the abortion rate increased. The offspring had reduced body weight and skeletal anomalies were observed. Treatment with 500 and 1,000 mg aspartame/kg bw per day did not lead to any of the effects described above. In female rabbits that were treated with 4,000 mg aspartame/kg bw per day in the diet, the fetal weight was reduced compared to the control group, and congenital anomalies were determined (EFSA 2013).

Table 5: Effects relevant to reproduction following administration of sweeteners with respective study details.

Sweeteners	Effect	NOEL* in mg/kg bw per day	LOEL* in mg/kg bw per day	Species	Time of observation	Route	Reference
Cyclamate (ADI 7 mg/kg bw per day)	Embryotoxic (Decrease in fetal body weight and lower number of liveborn foetuses, increase in postnatal mortality)		750 CHA[b]	Mice	F1-F6	(Diet)	Kroes *et al.* 1977[#]
Na saccharin (ADI 5 mg/kg bw per day)	Decrease in average litter size of dams	500	1,500[c]	Rat (F0 w n=418)	Gestation period	Diet	Schoenig *et al.* 1985[#]
	Decrease in bw F1 Increase in water intake F1		3,750		until the end of lactation (30 months)		
	Increase in survival rate F1	500[a]	2,500[d]				
	Decrease in number of live young per litter, decrease in water intake and body weight	1.25%, 2.5% in diet	5 % in diet	Mice, m, f	F0	Drinking water	NTP 1997[#]
Aspartame (ADI 40 mg/kg bw per day)	Decrease in body weight and body size of young, decrease in the number of live pups per litter at birth, decrease in feed intake and body weight of the dams, increase in the abortion rate	2,000	4,000	Rat, f	Gestation and lactation period	Diet	EFSA 2013 (E5, E11, E39, E47, E48)[#]
	Decrease in fetal weight, Congenital anomalies, decrease in feed intake of the dams	1,000, 2,000	4,000	Rabbits, f	Gestation and lactation period	Diet	EFSA 2013 Appendix I E54, E53, E55[#]
	Decrease in body weight and skeletal anomalies in the offspring, decrease in feed intake and body weight of the dams, increase in the abortion rate	0, 500, 1,000	2,000	Rabbits, f	Gestation and lactation period	Gavage	EFSA 2013 Appendix I E90[#]

* Specified as NOEL and LOEL (and not as NOAEL or LOAEL), because the evaluation panels did not consider the effects described to be relevant for the establishment of the respective ADI; m Male; f Female; [a] Corresponds to 1% in the diet, further doses: 3 %, 4 %, 6.25 %; [b] Corresponds to 0.5 % in the diet, conversion factor: 0.15; [c] Corresponds to 3 % in the diet, further effect doses: 4 %, 5 %, 6.25 %, 7.5 %; [d] Corresponds to 5 % in the diet; [e] Further effect dose: 7.5 %; [#] Conduction primarily according to OECD test guidelines

3.2.4 Gastrointestinal Effects

As part of the literature review, studies were identified that observe a link between sweetener intake and gastrointestinal effects.

Based on the current (limited) data, no statement can be made whether the intake of sweeteners has a clinically relevant effect on the intestinal microbiome in humans or model animals.

3.2.5 Effects on Biochemical Parameters

In the course of the literature review and the review of the opinions of the evaluation panels, studies were identified that showed changes in biochemical parameters in the blood and in organs such as the liver or spleen after administration of sweeteners.

Table 6 presents a selection of the identified literature (no claim to completeness) with regard to the observed effects on haematological parameters.

The JECFA monograph (WHO FAS 28) reported a slight increase in the haemoglobin content after 13-week administration of 10% **acesulfame K** in the diet of male rats (JECFA 1991a).

Gaunt *et al.* observed a reduced haemoglobin content and a lower proportion of erythrocytes in the total blood volume (PCV) in female rats treated with 0.6% **cyclohexylamine (CHA)** in the diet for one year. In contrast, male rats showed increased haemoglobin and PCV levels after two years when treated with 0.6% CHA in the diet (Gaunt *et al.* 1976). Changes in haemoglobin levels and in the proportion of erythrocytes in total blood volume were also observed in rats after treatment with 0.6% CHA for 13 weeks(Gaunt *et al.* 1974).

In male and female mice that were treated with 1000 mg/kg bw per day of **saccharin** via the drinking water for one year, the erythrocyte count and volume as well as the haemoglobin content in the blood decreased (Prasad & Rai 1987). A reduction in the haemoglobin and haematocrit values was also seen in the male and female offspring four weeks postpartum when the dams were treated with 3% (corresponding to 1500 mg/kg bw per day) Na saccharin in the diet. Higher doses (7.5%) also altered blood levels of iron and blood iron-binding capacity (Garland *et al.* 1991). Schoenig *et al.* also observed anaemic effects in rats with Na saccharin doses of 5 and 7.5% (Schoenig *et al.* 1985).

Administration of **aspartame** at 70 mg/kg bw per day by gavage increased the proportion of conjugated bilirubin, a water-soluble breakdown product of haemoglobin, in male rats after nine weeks (Adaramoye & Akanni 2016). In the blood of male rats that were treated with 500 mg/kg bw per day of aspartame via the drinking water for six weeks, lower haemoglobin, iron and ferritin levels were also measured, together with increased TIBC and UIBC values, which represent a measure of the iron-binding capacity (Saleh 2014).

Table 6: Effects on haematological parameters following administration of sweeteners with respective study details.

Sweeteners	Effect	NOEL* in mg/kg bw per day	LOEL* in mg/kg bw per day	Species	Duration in weeks	Route	Reference
Acesulfame K (ADI 9 mg/kg bw per day)	Increase in haemoglobin	1 %, 3 % in the diet	10 % in feed	Rat, m (n=10)	13	Diet	JECFA 1991
Cyclamate (ADI 7 mg/kg bw per day)	Increase in haemoglobin, PCV	0.2 % CHA in the diet**	0.6 % CHA in the diet**	Rat, m (n=10)	104	Diet	Gaunt *et al.* 1976[#]
	Decrease in haemoglobin, PCV (no effect after 104 weeks)	0.2 % CHA in the diet**	0.6 % CHA in the diet**	Rat, f (n=10)	52	Diet	
Saccharin (ADI 5 mg/kg bw per day)	Decrease in erythrocyte count (erythropenia), decrease in haemoglobin, decrease in PCV, increase in neutrophils, splenomegaly	500	1,000[a]	Mouse, m, f (n=10)	52	Drinking water	Prasad *et al.* 1987
	Decrease in haemoglobin, haematocrit, decrease in iron at 7.5%, m); Change in iron binding capacity at 7.5% (decrease f, increase m)	500	1,500[b]	Rat, m, f (F1)	4 post partum	Diet	Garland *et al.* 1991[#]
Aspartame (ADI 40 mg/kg bw per day)	Decrease in haemoglobin, iron, ferritin, increase in iron-binding capacity		500	Rat, m (n=6)	6	Drinking water	Saleh *et al.* 2014
	Increase in conjugated bilirubin	15	35[c]	Rat, m (n=5)	9	Gavage	Adaramoye *et al.* 2016

* Specified as NOEL and LOEL (and not as NOAEL or LOAEL), because the evaluation panels did not consider the effects described to be relevant for the establishment of the respective ADI; m Male; f Female; [a] Doses dependent on week 1→104: 0.06% in the diet (equivalent to 71→19 mg/kg bw per day); 0.2% in the diet (corresponding to 201→77 mg/kg bw per day); 0.6% in the diet (corresponding to 545→257 mg/kg bw per day); [a] Further effect dose: 1,500 mg/kg bw per day; [b] Other effect doses: 3,750 mg/kg bw per day; [c] Other effect doses: 70 mg/kg bw per day; [#] Conduction primarily according to OECD test guidelines

Studies were also identified in which the administration of sweeteners led to altered concentrations of various biochemical (including inflammatory) parameters in the blood and in various organs (liver, lymphatic organs). A selection of the identified effects is shown in Table 7 (no claim to completeness).

Changes in blood parameters

5% **sucralose** in the diet decreased serum alanine aminotransferase (ALT) activity in male rats after four weeks and reduced leukocyte and lymphocyte counts (Goldsmith 2000). A reduction of leukocytes in serum was also observed when 0.06% **CHA** was administered in the diet to male rats for two years, whereas the number of lymphocytes increased and that of neutrophils decreased (Gaunt *et al.* 1976).

Administration of 10 or 25 mg/kg bw per day of **Na saccharin** by gavage or drinking water to male rats increased the enzyme activity of ALT, aspartate aminotransferase (AST) and alkaline phosphatase (ASP) in the blood after four and eight weeks, respectively (Amin *et al.* 2016). Furthermore, a decrease in catalase (CAT) activity and in antioxidant capacity, measured by the total amount of antioxidants, in the blood was observed in rats after 8-week administration of 25 mg Na saccharin /kg bw per day via the drinking water (Alkafafy Mel *et al.* 2015).

The enzyme activity of ALT and ALP in male rats was also increased after administration of 250 mg/kg bw per day of **aspartame** for eight weeks (Alkafafy Mel *et al.* 2015). Prokic et al. observed increased concentrations of markers for oxidative stress (superoxide anion (O2•−), hydrogen peroxide (H2O2), peroxynitrite (ONOO), lipid peroxides (LPO)) and increased CAT activity in isolated erythrocytes from the blood of male rats that were treated with 40 mg aspartame /kg bw per day of via the drinking water for eight weeks. Reduced glutathione (GSH) levels decreased in the aspartame group (Prokic *et al.* 2014).

Changes in hepatic parameters

Administration of 500 mg/kg bw per day of **Na saccharin** by gavage in male rats for four weeks reduced enzyme activities of superoxide dismutase (SOD) and CAT as well as the levels of GSH. However, the hepatic level of malondialdehyde (MDA), which is a marker for lipid peroxidation, increased (Amin *et al.* 2016). Bian *et al.* observed increased mRNA levels of inducible nitric oxide synthase (iNOS) and tumour necrosis factor-α (*TNF*-α) in the livers of male mice after administration of 15 mg/kg bw per day of Na saccharin via the drinking water for 24 weeks (Bian *et al.* 2017b). Histological examinations showed changes in the liver parenchyma with indication of increased infiltration of immunocompetent cells, increased periportal fibrosis and congestion of the central vein and liver sinusoids after administration of 25 mg/kg bw per day of Na saccharin for eight weeks (Alkafafy Mel *et al.* 2015).

Effects on hepatic enzymes were also observed in male rats after nine-week treatment with 35 mg aspartame /kg bw per day by gavage. Adamamoye *et al.* observed lower GSH concentrations and enzyme activities of glutathione transferase (GST), glutathione peroxidase (GPx), SOD and CAT as well as increased levels products of lipid peroxidation (TBARS) in the aspartame group. A decrease in the catalytic activity of GST and SOD as well as reduced GSH and LPO levels after aspartame treatment was also observed in the kidney. Histological examination of the liver indicated necrotic tissue, periportal infiltration and impaired venous drainage. Micrographs of kidney sections also showed indication of congestion of vessel in the medulla and cortex and hyperplasia of the cuboidal cells (Adaramoye & Akanni 2016).

Finamor *et al.* studied the effect of aspartame on hepatic parameters of the methionine transmethylation and reverse transsulfuration pathway in male rats. The 13-week administration of 80 mg aspartame /kg bw per day by gavage reduced the concentration of the sulphur-containing metabolites methionine adenosyl transferase (MAT1A/2A), S-adenosylmethionine (SAM), homocysteine, cysteine, glutamate-cysteine ligase (GCLc), gamma-glutamylcysteine (γ-GC)

and oxidised and reduced glutathione (GSSG, GSH) in the liver of male rats. In addition, administration of 80 mg aspartame /kg bw per day led to increased hepatic leukocyte infiltration (Finamor *et al.* 2017). Alkafafy *et al.* observed an infiltration of immunocompetent cells and histopathological findings in the liver (periportal fibrosis, congestion of the central vein and liver sinusoids) even after eight weeks when 250 mg aspartame /kg bw per day were administered via the drinking water (Alkafafy Mel *et al.* 2015).

Indication of liver injury were found by Dhurandhar *et al.* in the livers of male rats treated with **sucralose** by gavage at 3000 mg/kg bw per day for four weeks. Histopathologic images of the liver showed irregular hepatocyte degeneration associated with Kupffer cell hyperplasia, lymphocytic infiltration, sinusoidal dilatation, and increased fibrotic structures around the portal tract. (Dhurandhar *et al.* 2018). Effects of sucralose on the gene expression of pro-inflammatory factors were observed by Bian *et al.* in the liver of male mice. Administration of 15 mg sucralose /kg bw per day via the drinking water for 24 weeks increased the hepatic mRNA levels of inducible nitric oxide synthase (iNOS) and matrix metalloproteinase 2 (MMP-2) (Bian *et al.* 2017a).

Changes in parameters in the spleen and thymus

Gaunt *et al.* observed a reduction in relative spleen weights including reduced leukocyte counts in male rats after a 2-year administration of 0.6% **CHA** via the diet (Gaunt *et al.* 1976). A decrease in spleen and thymus weights was also observed in male rats that were administered **sucralose** (5%, >2500 mg/kg bw per day), in the diet for four weeks. Histological examination of the thymus tissue indicated a reduced proportion of lymphocytes in the thymus cortex with increased lymphocytolysis and hypoplasia of the thymus cortex (Goldsmith 2000; SCF 2000c). Saad et al. observed degenerative changes in the white pulp and a reduction in the number of lymphocytes in the lymph follicles of the spleen of male rats which were treated with 15 mg sucralose /kg bw per day via the drinking water for three months. Comparable effects in relation to the reduced number of lymphocytes in the red and white pulp and a general loss of structure of the spleen tissue were also observed after administration of 40 mg **aspartame** /kg bw per day for three months (Saad 2017).

Table 7: Effects on biochemical parameters following administration of sweeteners with respective study details.

Sweeteners	Effect	NOEL* in mg/kg bw per day	LOEL* in mg/kg bw per day	Species	Duration in weeks	Route	Reference
Sucralose (ADI 15 mg/kg bw per day)	Decrease rel. weight of spleen and thymus; Decrease in the number of leukocytes and lymphocytes; Lymphocytolysis, cortical hypoplasia in the thymus	3,000	5% in the diet[a]	Rat, m	4-8	Diet	SCF 2000 (Cummins *et al.* 1983)[#]
	Decrease in serum ALT, leukocyte and lymphocyte counts; Decrease rel. weight of spleen and lymphocyte count thymus cortex	1%, 2.5% in the diet	5% in the diet[a]	Rat, m (n=15)	4	Diet	Goldsmith *et al.* 2000[#]
	Degeneration of white marrow in spleen; Depletion of lymphocytes in lymph follicles		15[b]	Rat, m (n=10)	12	Gavage	Saad *et al.* 2017
	Lymphocytic infiltration liver, degeneration liver cells, fibrosis, hyperplasia Kupffer cells		3,000	Rat, m (n=6)	4	Gavage	Dhurandhar *et al.* 2018
	Increase in inflammatory factors in the liver		14[c]	Mouse, m (n=10)	24	Drinking water	Bian *et al.* 2017c
Cyclamate (ADI 7 mg/kg bw per day)	Decrease in the total number of leukocytes in the serum (at 0.6% decrease in neutrophils, increase in lymphocytes (also in w))		0.06 % CHA[d]**	Rat, m (n=10)	104	Diet	Gaunt *et al.* 1976[#]
	Increase in relative weight of spleen	0.2 % CHA in the diet**	0.6 % CHA**				

Saccharin (ADI 5 mg/kg bw per day)	Increase in serum ALT, AST, ALP			10[e]	Rat, m (n=8)	4	Gavage	Amin *et al.* 2016
	Increase in MDA, decrease in SOD, CAT, GSH in liver	10		500	Rat, m (n=10)	4	Gavage	Amin *et al.* 2016
	Decrease plasma CAT, increase ALT, ALP; Fibrosis, mononuclear cell infiltration in liver			25[f]	Rat, m (n=5)	8	Oral uptake	Alkafafy et al. *2015*
	Increase in inflammatory factors in the liver			42[g]	Mouse, m (n=10)	24	Drinking water	Bian *et al.* 2017c
Aspartame (ADI 40 mg/kg bw per day)	Changes GSH, GST, SOD, LPO etc. in kidney and liver; Necrosis, periportal infiltration in liver; Hyperplasia of cuboidal cells in kidney		15	35[h]	Rat, m (n=5)	9	Gavage	Adaramoye et al. 2016
	Leukocyte infiltration in the liver, decrease in GSH, etc.			80	Rat, m (n=6)	13	Gavage	Finamor *et al.* 2017
	Increase in superoxide anion, H2O2, LPO, peroxynitrite, CAT, GSH in erythrocytes			40	Rat, m (n=5)	6	Drinking water	Prokic *et al.* 2014
	Increase in plasma ALT, ALP, fibrosis, mononucl. cell infiltration in liver			250	Rat, m (n=5)	8	Oral uptake	Alkafafy *et al.* 2015
	Depletion of lymphocytes in the white, red spleen marrow			40[i]	Rat, m (n=10)	12	Gavage	Saad *et al.* 2017

* Specified as NOEL and LOEL (and not as NOAEL or LOAEL), because the evaluation panels did not consider the effects described to be relevant for the establishment of the respective ADI; ** Doses dependent on week 1 -> 104: 0.06% in the diet (corresponding to 71->19 mg/kg bw per day); 0.2% in the diet (corresponding to 201->77 mg/kg bw per day); 0.6% in the diet (corresponding to 545->257 mg/kg bw per day); m male; w female; ALT alanine aminotransferase, AST aspartic aminotransferase, ASP alkaline phosphatase, CAT catalase, LPO lipid peroxides, GST glutathione transferase, GSH glutathione, MDA malondialdehyde; [a] Corresponds to 2794-6596 mg/kg bw per day; [b] Corresponds to 3 mg/ml; [c] Corresponds to 0.1 mg/ml; [d] Further effect doses: 0.2%, 0.6%; [e] Further effect dose: 500 mg/kg bw per day; [f] Further effect dose: 100 mg/kg bw per day; [g] Corresponds to 0.3 mg/ml; [h] Further effect dose: 70 mg/kg bw per day; [i] Corresponds to 8 mg/ml

3.3 Effects Relevant for the Evaluation of Possible Combined Effects

The evaluation of the studies, including the assessment reports conducted by the SCF, JECFA and EFSA, as well as other relevant studies on the identified effects of the selected sweeteners, showed that several of the examined sweeteners have comparable effects on the targets *kidney and urinary tract*. In order to be able to assess the relevance of a possible combined effect of different sweeteners on the same target organ, an assessment approach with two different exposure scenarios has been selected (see 3.3.1).

In addition, the literature review provided indications that various sweeteners also have comparable effects on the testicular tissue and on the gastrointestinal tract, as well as on reproduction and on biochemical parameters in the blood, liver and lymphatic organs. However, with regard to the effects considered, the current data is not considered to be sufficiently reliable in order to assess possible combed effects of different sweeteners (see 3.3.2).

In order to assess the relevance to health of the same or similar effects that different substances could potentially have on a target organ/system when used in combination (exposure), it was initially assumed that the exposure to the single sweeteners corresponded to the respective ADI value. In this scenario, the possible total exposure (combined exposure) to the sweeteners under consideration would correspond to the sum of these exposure values.

Different LOELs were identified with different sweeteners in the same animal species for certain observed effects. This indicates that the sweeteners have different toxic potencies with regard to the effects considered. Therefore, for each effect considered, weighting factors (also known as “potency factors” or “toxic equivalency factors”) were calculated for the sweeteners. The weighting factors were multiplied with the respective exposure values (which correspond to the respective ADI value in the assumed scenario) to calculate a weighted exposure. To determine the weighted combined exposure, the weighted exposure values of the sweeteners under consideration were added presuming purely additive effects. For the risk assessment, the weighted combined exposure was compared with the lowest dose of a sweetener at which the observed effect was observed. For this purpose, the Margin of Safety (MoS) was calculated for each effect considered as the quotient of the lowest LOEL and the weighted combined exposure.

3.3.1 Effects on Kidney and Urinary Bladder

In rats, treatment with the sweeteners sucralose, Na saccharin and aspartame led to an increased incidence of hyperplasia of the renal pelvic epithelium and increased mineralisation of the renal pelvis. In addition, an increased incidence of hyperplasia of the urinary bladder epithelium was observed after administration of Na saccharin. Comparable effects on the urinary bladder were not observed with the other sweeteners tested. In addition, altered levels of creatinine, urea and albumin in the blood of rats treated with Na saccharin, CHA or aspartame were reported. In addition, changes in the pH value and the mineral concentration in the urine after the administration of sweeteners were occasionally be observed.

No such effects on the kidney have been described for acesulfame K and steviol glycosides. With regard to the observations in the kidney after CHA administration, no dose-response relationship was established.

The data was only considered to be sufficiently reliable for the assessment approach used here with regard to hyperplasia and mineralisation of the renal pelvis (Table 2). Other renal and urological effects that were observed after treatment with sweeteners (Table 3) are not included in the assessment.

The lowest effect dose leading to mineralisation of the renal pelvic epithelium was observed with 500 mg Na saccharin /kg bw per day. A comparable effect was observed when the same

animal model was treated with 2,000 mg aspartame or 900 mg sucralose/kg bw per day. Assuming a combination of sucralose, Na saccharin and aspartame and an ADI that is fully exhausted, the unweighted total exposure is 60 mg/kg bw per day. Due to the different potencies of the sweeteners sucralose, Na saccharin and aspartame in relation to the *mineralisation* effect observed *in the renal pelvic epithelium*, the weighted combined exposure is 23.25 mg/kg bw per day. Assuming the lowest effect dose of 500 mg Na saccharin/kg bw per day , the range between this dose and the possible weighted combined exposure in this exposure scenario is smaller (factor 21.5) than in the case of considering the effect dose and the respective derived ADI of each single sweetener (Table 8).

With regard to increased hyperplasia of the renal pelvis, 200 mg/kg bw per day of sucralose was identified as the lowest effect dose. Increased hyperplasia of the renal pelvis was also observed in the same animal model when 8,000 mg/kg bw per day of aspartame were administered. If the ADI is fully exhausted, an unweighted combined exposure of 55 mg/kg bw per day can be assumed due to the combined intake of sucralose and aspartame. Assuming the lowest effect dose of 200 mg/kg bw per day for sucralose, the range between this dose and the theoretically possible weighted combined exposure of 16 mg/kg bw per and day is much smaller (factor 12.5) than in the case of considering the effect dose and the respective derived ADI of each single sweetener (Table 9).

Table 8: Assessment approach regarding the effect on "mineralisation of the renal pelvic epithelium" following administration of sweeteners.

Sweeteners (ADI in mg/kg bw per day)	**LOEL*** in mg/kg bw per day	**Weighting factor**[a]	**Weighted exposure**[b] in mg/kg bw per day	**Range between ADI and LOEL**	**Animal model**	**Reference**
Sucralose (15)	900	500/900=0,55	8.25	60	Rat	Mann *et al.* 2000
Na saccharin (5)	500	500/500= 1	5	100	Rat	Schoenig *et al.* 1985[c]
Aspartame (40)	2,000	500/2,000=0,25	10	50	Rat	EFSA 2013 (Ishii *et al.* 1981)

Exposure scenario: Assumption of a combined effect of sweeteners

Unweighted combined exposure	**Lowest LOEL***	Weighted combined exposure	**Range between weighted combined exposure and lowest LOEL**
60 mg/kg bw per day	500 mg/kg bw per day	23.25 mg/kg bw per day	21.5

* Given as LOEL (and not as LOAEL) because the described effect was not considered relevant for the establishment of the respective ADI by the evaluation panels; [a] Estimation of exposure assuming that the exposure to the single sweeteners corresponds to the relevant ADI value, the weighting factor takes into account the different potencies of the single sweetener with regard to the identified effect; [b] Calculation from weighting factor x ADI value of the corresponding sweetener; [c] i.a. key study for the ADI establishment: LOAEL ≥ 1,500 mg/kg bw per day for Na saccharin (rat): Disturbance of general homoeostasis

Table 9: Assessment approach regarding the effect on “hyperplasia of the renal pelvic epithelium” following administration of sweeteners.

Sweeteners (ADI in mg/kg bw per day)	**LOEL*** in mg/kg bw per day	**Weighting factor**[a]	**Weighted exposure**[b] in mg/kg bw per day	**Range between ADI and LOEL**	**Animal model**	**Reference**
Sucralose (15)	200	200/200=1	15	13	Rat	Mann *et al.* 2000
Aspartame (40)	8000	200/8,000=0,025	1	200	Rat	EFSA 2013 (E33-34)[c]

Exposure scenario: Assumption of a combined effect of sweeteners

Unweighted combined exposure	**Lowest LOEL***	Weighted combined exposure	**Range between weighted combined exposure and lowest LOEL**
55 mg/kg bw per day	200 mg/kg bw per day	16 mg/kg bw per day	12.5

* Given as LOEL (and not as LOAEL) because the described effect was not considered relevant for the establishment of the respective ADI by the evaluation panels; [a] Estimation of exposure assuming that the exposure to the single sweeteners corresponds to the relevant ADI value, the weighting factor takes into account the different potencies of the single sweetener with regard to the identified effect; [b] Calculated from weighting factor x ADI value of the corresponding sweetener; [c] i.a. key study for the ADI establishment: LOAEL 8,000 mg/kg bw per day for aspartame (rat)

3.3.2 Further Effects

3.1.3.1 Effects on Testicular Tissue

Studies were identified in which adverse health effects on testicular tissue were observed following administration of cyclohexylamine (CHA), a metabolite of cyclamate, aspartame and Na saccharin (**Table 4**).

In rats, treatment with CHA led to reduced testicular weight and disturbances in sperm development and function at doses ≥ 150 mg/kg bw per day. In mice and dogs, the effects were only observed at higher CHA doses. Compared to mice, rats have a greater ability to metabolise CHA to its ring-hydroxylated derivatives (3- or 4-aminocyclohexanol). However, CHA metabolites have been found not to be responsible for the testicular effects in rats and a direct effect of CHA, probably on Sertoli cells, in rats is assumed (Roberts *et al.* 1989; Creasy *et al.* 1990). Rats are more sensitive to the CHA-induced testicular effects than mice, in which the changes in the testicular tissue only were observed at doses of around 300 mg/kg bw per day, which may be due to the different pharmacokinetics of CHA in the above-mentioned species (Hardy *et al.* 1976; Roberts *et al.* 1989).

In male rats, administration of aspartame via the diet increased the incidence of vesicle atrophy, although no clear dose-dependence could be observed. No other effects of aspartame on testicular tissue or sperm function were described in the EFSA report (EFSA 2013).

Gong *et al.* observed adverse health effects on testicular tissue in mice treated with Na saccharin, as well as sucrose (Gong *et al.* 2016). In the opinions conducted by the SCF and JECFA, which served as the basis for the sweetener assessment, no effects in terms of impairment of sperm quality and function or change in testicular morphology were described after the intake of Na saccharin (JECFA 1993; SCF 1995). In contrast to the studies that were taken into account for the assessment and for the establishment of the ADI by the SCF and JECFA, and which did not show any adverse health effects on the testicular tissue, the study by Gong *et al.* was not performed according to OECD test guidelines.

The data with respect to the adverse health effects on the testicular tissue following the administration of sweeteners is therefore only reliable for CHA (the studies were mostly carried out according to OECD test guidelines, a dose-response relationship is available).

Therefore, no assessment was made with regard to a possible combined effect of different sweeteners on testicular tissue.

3.1.3.2 *Effects on the Reproductive System*

As part of the review of the SCF, JECFA and EFSA opinions on the single sweeteners, adverse health effects in relation to reproduction and postnatal development were observed, especially after administration of high doses of cyclohexylamine (CHA) or sodium cyclamate, Na saccharin and aspartame (Table 5).

Bopp *et al.* concluded that the adverse health effects on offspring body weight after maternal intake of CHA result from reduced body weight gain and lower food consumption of the dams (Bopp *et al.* 1986).

Changes in the nutritional status of the dams following intake of high doses of aspartame has been mentioned by EFSA as possible cause of the lower birth weight and increased offspring mortality. In addition, maternal gastrointestinal disturbances and a potential effect of phenylalanine are mentioned as factors (EFSA 2013).

Up to 10% of aspartame ingested is converted to methanol in the body. Stepwise oxidation converts methanol to formate via formaldehyde. Formate is then converted to carbon dioxide via folate- and catalase-dependent enzymes, in humans only via folate-dependent signalling pathways (Dikalova *et al.* 2001; Hanzlik *et al.* 2005). EFSA assumes that exposure to methanol through the intake of aspartame within the range of the ADI (40 mg/kg bw per day) has no adverse health effects. This is due to the low concentration of methanol in the blood when the ADI of 40 mg/kg bw per day of aspartame is exhausted compared to the methanol concentration that is endogenously produced. EFSA assumes that the exposure to methanol from aspartame intake is similar to the amount ingested from food or natural sources. Adverse health effects from dietary exposure to methanol, e.g. from aspartame, are not to be expected according to the EFSA (EFSA 2013).

The toxic effects on reproduction in mice following administration of 5% (corresponding to 2500 mg/kg bw per day) Na saccharin are discussed as a consequence of the increased mortality and reduced water consumption of the parent animals. In the animals that were treated with lower doses of Na saccharin (1.25% and 2.5%), no toxic effects on reproduction was observed, while the water consumption was increased (NTP 1997).

The observed effects on reproduction and (post)natal development of the offspring after administration of high doses of the sweeteners Na saccharin, cyclamate (CHA) and aspartame can be regarded as indirect effects caused by the change in the physiological status of the parent animals (e.g. lower feed consumption of the dams, increased mortality and reduced water intake). Flamm *et al.* justified the changes in body weight or body weight gain as a secondary response to reduced palatability of the feed due to the use of high levels of sweeteners in the diet (Flamm *et al.* 2003).

The observed effects on reproduction and (post)natal development were therefore not assessed with regard to a possible combined effect of sweeteners.

3.1.3.2 Gastrointestinal Effects

Effects on caecal weight and on the intestinal microbiome in animal models, especially in rodents, were observed for the investigated sweeteners.

It is difficult to transfer the relevant animal experimental findings on effects on the caecum from rodent models to humans. In comparison to rodents, the caecum in humans is only a small section of the large intestine and has little or no fermentation capacity (Nguyen *et al.* 2015). In general, there are numerous anatomical and morphological differences between the human, mouse and rat gastrointestinal tract (Kararli 1995). Enlargement of the caecum in rodent species may be a physiological response following administration of high amounts of various substances (e.g. polyols, dietary fibre, lactose, modified starch) that are poorly absorbed and osmotically active (Wallig 2018). An assessment of the effects on the gastrointestinal tract (changes in the caecum; the intestinal microbiome) with regard to a possible combined effect of sweeteners is not feasible due to the complexity of the physiological processes involved and the heterogeneous data.

3.1.3.4 Effects on Biochemical Parameters

Effects on haematological parameters were found for the sweeteners acesulfame K, cyclohexylamine (CHA), saccharin and aspartame (Table 6). However, the data on the identified parameters is very heterogeneous and no consistent picture was noted, e.g. with regard to changes in haemoglobin levels or erythrocyte volume.

In addition, animal studies were identified in which the administered sweeteners sucralose, CHA, saccharin, and aspartame altered the levels of various biochemical parameters (e.g. inflammatory parameters) in the blood, liver, and the lymphoid organs spleen and thymus (Table 7).

Due to the low significance and reliability of the results in terms of clinical relevance and heterogeneous data and study quality (most of the studies were not performed according to OECD test guidelines), these findings were not assessed with regard to a possible combined effect of sweeteners.

3.4 Discussion on the Transferability of the Effects on Kidney Tissue from Animal Experiments to Humans

With the assessment approach applied here, the BfR has examined whether the available data, especially from animal studies, provide indications of health risks associated with the combined use of relevant sweeteners. The consideration focused on the combined use of sweeteners in non-alcoholic soft drinks. For this purpose, three main questions were addressed:

(1) Do the scientific opinions on single substances conducted by the SCF and EFSA, which were carried out as part of the authorisation of sweeteners as food additives in the EU, provide indications of combined effects?

(2) Are the identified effect doses relevant for the assessment of health risks due to the effects upon combined exposure to humans?

(3) Do any identified combined effects pose health concerns?

The animal experimental data from the assessments of food additives conducted by the SCF, EFSA and JECFA served as basis for the toxicological evaluation. Based on the effects identified, a further literature review was carried out (status as of 2020), without achieving completeness due to the large number of publications. On one hand, toxicity studies were included in the analysis most of which were carried out in accordance with OECD test guidelines. On the other hand, studies were also included in the assessment even if their criteria do not correspond to OECD test guidelines, but which still appeared relevant for the identified effect.

As part of the assessment approach presented here, the kidney and the lower urinary tract were identified as a possible common target organ for *the effects hyperplasia and mineralisation of the renal pelvis* in rat studies after administration of the sweeteners sucralose, saccharin and aspartame. The data from the animal studies was considered to be sufficiently reliable for the comparative assessment performed here. With regard to the other effects considered, the data available was not considered to be sufficiently reliable in order to assess possible combined effects of different sweeteners. Therefore, the effects *hyperplasia* and *mineralisation of the renal pelvis* were considered further as examples.

3.4.1 Discussion of the Results from Animal Experiments in the Kidney and Urinary Tract

The effects on the urinary tract and with regard to renal parameters were particularly observed in animal models. In general, there is a high incidence of spontaneous lesions in the urinary tract in rat studies on chronic effects (Magnusson & Ramsay 1971; Robertson 1980). Tomonari *et al.* mentions the age-dependent mineral deposits in the fornices of the kidney, for example, as a frequent finding in the control group, which occurs in 27% of the male and 82% of the female rats after two study years (Tomonari *et al.* 2016).

The rat seems to be more sensitive than other species to adverse health effects on the kidney and urinary tract in general and also to the administration of high doses of sweeteners. For example, the male rat has an increased risk of stone retention compared to other species due to the anatomy of the bladder neck (Chowaniec & Hicks 1979). In the case of Na saccharin, Fukushima *et al.* (1983) identified differences between species as well as between individual rat strains. In rats, but not in mice, hamsters and guinea pigs, saccharin doses ≥ 2500 mg/kg bw per day led to an increased incidence of lesions in the urinary bladder and changes in the urothelium (Fukushima *et al.* 1983).

The high levels of α2u-globulins in the urine are discussed as one reason for the observed adverse health effects on the urinary bladder of male rats. Garland *et al.* refers to the potential role of α2u-globulin in relation to increased precipitation of Na saccharin crystals in urine and changes in urinary parameters and bladder morphology following Na saccharin administration in male rats (JECFA 1993; Garland *et al.* 1994). However, the SCF stated in its opinion that α2u-globulins are not the only cause of the observed effects (SCF 1995).

In the case of Na saccharin, high sodium intake can also change the mineral balance. Schoenig *et al.* observed neoplasia in the urinary bladder after administration of sodium hippurate compared to Na saccharin (Schoenig *et al.* 1985).

Hyperplasia of the renal pelvis can also be explained by the fact that the renal pelvic epithelium often response to mineral crystals by physiological hyperplasia (Lord & Newberne 1990). Mann *et al.* assumes that mineralisation, visualised in histological picture, is the primary effect when crystalline structures are trapped in the renal pelvic epithelium. According to the authors, the mineral deposits in the renal pelvis (e.g. as nephrocalcinosis) are not directly related to mineral deposits in the renal parenchyma or to mineralisation of the renal basement membrane and are accordingly not associated with an increased risk of developing nephropathy (Mann *et al.* 2000).

However, increased occurrence of crystalline structures can irritate the bladder epithelium, lead to cell death, stimulate mitotic activity of the epithelium, and induce chronic proliferation (Lord & Newberne 1990). The SCF stated in its opinion on saccharin that high concentration of sodium ions, increased urinary pH, bladder distension, decrease in urinary osmolality and crystalluria possibly cause increased cell proliferation (SCF 1995).

An association has been observed between the occurrence of hyperplasia and mineralisation of the renal pelvic epithelium and hypertrophy of the caecum in rats following administration of sweeteners (Lord & Newberne 1990; Mann *et al.* 2000). Leegwater *et al.* referred to substances that are not- or only partially absorbed as the cause of the caecum enlargement (Leegwater *et al.* 1974). The occurrence of hyperplasia and mineralisation of the renal pelvic epithelium after administration of high doses of sucralose and other substances (e.g. lactose, xylitol) is discussed in relation to the enlargement of the caecum as possible response to the reduced absorption and increased dwell time of these substances in the intestine. The effects

on the kidney and urinary tract could therefore also be a secondary response to the change in the physiological status of the animals (e.g. change in urinary pH and electrolyte balance, microbiome composition and intestinal enzyme profile, changes in water transport and calcium phosphate metabolism, amount of osmotically active substances) (Chowaniec & Hicks 1979; Lord & Newberne 1990).

As part of the literature review, studies were identified that describe effects of sweeteners on the gastrointestinal tract. With the exception of aspartame and steviol glycosides, the selected sweeteners have in common that the largest amount is excreted unchanged via the faeces or urine after ingestion. The osmotic activity of sweeteners in the intestinal tract can lead to increased intraluminal pressure and compensatory distension of the caecum. In addition, substances that are not absorbed reach the large intestine via the small intestine and can be metabolised by intestinal bacteria. The resulting low-molecular metabolites also change the osmolality (Leegwater *et al.* 1974). Symptoms such as polydipsia, polyuria and diarrhoea occurred multiple times in animals that were treated with sweeteners (Chowaniec & Hicks 1979; Schoenig & Anderson 1985; Anderson *et al.* 1988; JECFA 1991b; Goldsmith 2000; Palmnäs *et al.* 2014).

It has not yet been conclusively clarified, whether there is a mechanistic relationship between the effects on the renal pelvic epithelium and the enlargement of the caecum. However, it can be assumed that a change in the water and electrolyte balance after sweetener intake could have an effect on the renal system. Administration of Na saccharin, cyclohexylamine (CHA) and aspartame resulted in changes in creatinine, urea and serum albumin levels (Gaunt *et al.* 1976; Palmnäs *et al.* 2014; Saleh 2014; Adaramoye & Akanni 2016; Amin *et al.* 2016). Anderson *et al.* observed increased urea levels and reduced urinary ammonia levels following saccharin administration in rats and discussed the inhibition of urease activity of *Proteus vulgaris* by saccharin as a possible mechanistic explanation (Anderson 1979; Anderson & Kirkland 1980). Furthermore, changes in the pH value and in the excretion of calcium and phosphate were observed after sweetener intake (Ishii *et al.* 1981; Anderson *et al.* 1988).

With regard to the current data, the changes in renal parameters (e.g. urea or albumin) in relation to the renal function are considered to be of minimal toxicological significance due to the numerous dependent factors. Nevertheless, they can provide an indication of the presence of renal dysfunction (Kluwe 1981).

3.4.2 Results in Kidney Tissue from Human Data

The relationship between the intake of sweeteners and the development of kidney dysfunction and bladder cancer was investigated in epidemiological studies. A positive association between increased intake of sweeteners (artificial and non-nutritive) and the development of cancer of the bladder or urinary tract was observed in eleven case-control studies. In contrast, 20 epidemiological studies showed no association (Lohner *et al.* 2017). In particular, the human relevance of the effects of saccharin in relation to the development of bladder cancer in rats, as discussed by Bell *et al.* and Cohen *et al.* for example, remains a controversial topic (Bell *et al.* 2002; Cohen 2018).

The observed effects (*mineralisation and hyperplasia of the renal pelvis in rats*) were considered to be specific to the rat animal model by the international evaluation panels and therefore less relevant for the assessment of health risks. However, changes in the mineral and water balance can cause an imbalance in the renal system and urinary tract. In particular, crystal formation and retention can be key factors in the development of kidney stones in humans (Aihara *et al.* 2003; Baumann *et al.* 2010). Wong *et al.* have pointed out the high incidence and prevalence of bladder cancer and renal dysfunction, to some extent with unknown aetiology particularly in sensitive population groups (the elderly, people with diabetes mellitus, etc.) (Wong et al. 2018).

3.4.3 Conclusion and Recommendations

The ADI value of each single sweetener is usually based on the highest dose at which no adverse health effects were observed in animal models (no observed adverse effect level, NOAEL). Uncertainties regarding the transfer of the study results from animals to humans and individual differences are considered by factor. The quotient of NOAEL and ADI is usually 100.

Supposing that

- the effects considered are cumulative,
- the exposure corresponds to the respective acceptable daily intake (ADI) and
- weighting factors for the different toxicological potencies of the three sweeteners are applied,

the combined intake of the three sweeteners sucralose, saccharin and aspartame would only result in a factor (or “range”) of 21.5 between the dose at which mineralisation of the renal pelvis was observed in rat studies and the weighted total exposure (Table 8). In comparison, considering the sweeteners separately, the “range” between the dose exerting effects and the respective ADI would be 60 for sucralose, 100 for saccharin and 50 for aspartame. The “range” is smaller when considering the combined intake compared to the single substances. If one regards the effect of hyperplasia of the renal pelvic epithelium in rats, a similar picture emerges regarding the combined intake of the sweeteners sucralose and aspartame (Table 9).

The examples show that, in principle, combined effects can be observed. If the effects are considered as toxicologically relevant and the assumed exposure corresponds to the respective ADI, the long-term daily intake of sucralose, saccharin and aspartame could possibly no longer be regarded as harmless to health.

The observed effects (mineralisation and hyperplasia of the renal pelvis in rats) were considered as less relevant for the risk assessment of the three sweeteners by the international evaluation panels. However, the BfR points out that changes in the mineral and water balance can

cause an imbalance in the renal system and urinary tract. In particular, crystal formation and retention can be key factors in the development of kidney stones in humans. Hence, from the BfR's point of view, potential effects on the kidney and urinary tract due to the combined use of the three sweeteners cannot be finally assessed on the basis of the available data. As already described, the data on the other observed effects (testicular tissue, reproductive system, gastrointestinal tract, changes in biochemical parameters in the blood, in the liver and in the lymphatic organs) were not considered to be sufficiently robust for comparative assessment of combined effects.

An animal study in which several sweeteners are combined, and also administered separately at the same time, would be very helpful to assess potential combined effects.

Current assessments of exposure to sweeteners through non-alcoholic flavoured drinks and other product groups are currently not available for Germany. It is therefore presently unknown to what extent the values for the acceptable daily intake (ADI) of the single sweeteners are exhausted or even exceeded in Germany. It is however expected that increased use of sweeteners as part of the NRI strategy will lead to an increase in exposure to these sweeteners. In this respect, it seems advisable to determine the current exposure in consumers in Germany prior to a possible expansion of use of sweeteners.

Further information about sweetening agents is available on the BfR website:

https://www.bfr.bund.de/en/a-z_index/sweetening agents-130251.htmll

BfR 'Opinions app'

4 References

Adaramoye O. A. and Akanni O. O. (2016). Effects of long-term administration of aspartame on biochemical indices, lipid profile and redox status of cellular system of male rats. *Journal of Basic and Clinical Physiology and Pharmacology* **27:** 29-37.

Aihara K., Byer K. J., Khan S. R. (2003). Calcium phosphate-induced renal epithelial injury and stone formation: involvement of reactive oxygen species. *Kidney International* **64:** 1283-1291.

Alkafafy Mel S., Ibrahim Z. S., Ahmed M. M., El-Shazly S. A. (2015). Impact of aspartame and saccharin on the rat liver: Biochemical, molecular, and histological approach. *International Journal of Immunopathology and Pharmacology* **28:** 247-255.

Amin K. A., Al-muzafar H. M., Abd Elsttar A. H. (2016). Effect of sweetener and flavoring agent on oxidative indices, liver and kidney function levels in rats. *Indian Journal of Experimental Biology* **54:** 56-63.

Anderson R. L. (1979). Response of male rate to sodium saccharin ingestion: urine composition and mineral balance. *Food and Cosmetics Toxicology* **17:** 195-200.

Anderson R. L. and Kirkland J. J. (1980). The effect of sodium saccharin in the diet on caecal microflora. *Food and Cosmetics Toxicology* **18:** 353-355.

Anderson R. L., Lefever F. R., Maurer J. K. (1988). The effect of various saccharin forms on gastro-intestinal tract, urine and bladder of male rats. *Food Chemistry and Toxicology* **26:** 665-669.

Baumann J. M., Affolter B., Meyer R. (2010). Crystal sedimentation and stone formation. *Urological Research* **38:** 21-27.

Bell W., Clapp R., Davis D., Epstein S., Farber E., Fox D. A., Holub B., Jacobson M. F., Lijinsky W., Millstone E., Reuber M. D., Suzuki D., Temple N. J. (2002). Carcinogenicity of saccharin in laboratory animals and humans: letter to Dr. Harry Conacher of Health Canada. *Int J Occup Environ Health* **8:** 387-393.

Bian X., Chi L., Gao B., Tu P., Ru H., Lu K. (2017a). Gut Microbiome Response to Sucralose and Its Potential Role in Inducing Liver Inflammation in Mice. *Frontiers in Physiology* **8:** 487.

Bian X., Tu P., Chi L., Gao B., Ru H., Lu K. (2017b). Saccharin induced liver inflammation in mice by altering the gut microbiota and its metabolic functions. *Food Chemistry and Toxicology* **107:** 530-539.

Bopp B. A., Sonders R. C., Kesterson J. W., Renwick A. G. (1986). Toxicological aspects of cyclamate and cyclohexylamine. *Critical Reviews in Toxicology* **16:** 213-306.

Brune H. and Mohr U. (1978). Establishment of the no-effect dosage of cyclohexylamine hydrochloride in male Sprague-Dawley rats with respect to growth and testicular atrophy. Unpublished report from Abbott Laboratories, Ltd (cited in Bopp et al. 1986 and in Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives, 1982).

Chowaniec J. and Hicks R. M. (1979). Response of the rat to saccharin with particular reference to the urinary bladder. *British Journal of Cancer* **39:** 355-375.

Cohen S. M. (2018). Screening for human urinary bladder carcinogens: two-year bioassay is unnecessary. *Toxicology Research* **7:** 565-575.

Creasy D. M., Ford G. R., Gray T. J. (1990). The morphogenesis of cyclohexylamine-induced testicular atrophy in the rat: in vivo and in vitro studies. *Experimental and Molecular Pathology* **52:** 155-169.

Dhurandhar D., Bharihoke V., Kalra S. (2018). A histological assessment of effects of sucralose on liver of albino rats. *Morphologie* **102:** 197-204.

Dikalova A. E., Kadiiska M. B., Mason R. P. (2001). An in vivo ESR spin-trapping study: free radical generation in rats from formate intoxication--role of the Fenton reaction. *Proceedings of the National Academy of Sciences of the United States of America* **98:** 13549-13553.

EFSA (European Food Safety Authority: Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS)) (2010). Scientific Opinion on the safety of steviol glycosides for the proposed uses as a food additive. *EFSA Journal* **8(4):** 1537.

EFSA (European Food Safety Authority: Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS)) (2013). Scientific Opinion on the re-evaluation of aspartame (E 951) as a food additive. *EFSA Journal* **11(12):** 3496.

Finamor I., Perez S., Bressan C. A., Brenner C. E., Rius-Perez S., Brittes P. C., Cheiran G., Rocha M. I., da Veiga M., Sastre J., Pavanato M. A. (2017). Chronic aspartame intake causes changes in the trans-sulphuration pathway, glutathione depletion and liver damage in mice. *Redox Biology* **11:** 701-707.

Flamm W. G., Blackburn G. L., Comer C. P., Mayhew D. A., Stargel W. W. (2003). Long-term food consumption and body weight changes in neotame safety studies are consistent with the allometric relationship observed for other sweeteners and during dietary restrictions. *Regulatory Toxicology and Pharmacology* **38:** 144-156.

Friedman L., Richardson H. L., Richardson M. E., Lethco E. J., Wallace W. C., Sauro F. M. (1972). Toxic response of rats to cyclamates in chow and semisynthetic diets. *Journal of the National Cancer Institute* **49:** 751-764.

Fukushima S., Arai M., Nakanowatari J., Hibino T., Okuda M., Ito N. (1983). Differences in susceptibility to sodium saccharin among various strains of rats and other animal species. *Gan* **74:** 8-20.

Garland E. M., Shapiro R., Kraft P. L., Mattson B. J., Parr J. M., Cohen S. M. (1991). Effects of in utero and postnatal sodium saccharin exposure on the nutritional status of the young rat. II. Dose response and reversibility. *Food Chemistry and Toxicology* **29:** 669-679.

Garland E. M., St John M., Asamoto M., Eklund S. H., Mattson B. J., Johnson L. S., Cano M., Cohen S. M. (1994). A comparison of the effects of sodium saccharin in NBR rats and in intact and castrated male F344 rats. *Cancer Letters* **78:** 99-107.

Gaunt I. F., Sharratt M., Grasso P., Lansdown A. B., Gangolli S. D. (1974). Short-term toxicity of cyclohexylamine hydrochloride in the rat. *Food and Cosmetics Toxicology* **12:** 609-624.

Gaunt I. F., Hardy J., Grasso P., Gangolli S. D., Butterworth K. R. (1976). Long-term toxicity of cyclohexylamine hydrochloride in the rat. *Food and Cosmetics Toxicology* **14:** 255-267.

Goldsmith L. A. (2000). Acute and subchronic toxicity of sucralose. *Food and Chemical Toxicology* **38:** S53-S69.

Gong T., Wei Q. W., Mao D. G., Nagaoka K., Watanabe G., Taya K., Shi F. X. (2016). Effects of Daily Exposure to Saccharin and Sucrose on Testicular Biologic Functions in Mice. *Biology of Reproduction* **95:** 116.

Grice H. C. and Goldsmith L. A. (2000). Sucralose - An overview of the toxicity data. *Food and Chemical Toxicology* **38:** S1-S6.

Hanzlik R. P., Fowler S. C., Eells J. T. (2005). Absorption and elimination of formate following oral administration of calcium formate in female human subjects. *Drug Metabolism and Disposition* **33:** 282-286.

Hardy J., Gaunt I. F., Hooson J., Hendy R. J., Butterworth K. R. (1976). Long-term toxicity of cyclohexylamine hydrochloride in mice. *Food and Cosmetics Toxicology* **14:** 269-276.

Ishii H., Koshimizu T., Usami S., Fujimoto T. (1981). Toxicity of aspartame and its diketopiperazine for Wistar rats by dietary administration for 104 weeks. *Toxicology* **21:** 91-94.

James R. W., Heywood R., Crook D. (1981). Testicular responses of rats and dogs to cyclohexylamine overdosage. *Food and Cosmetics Toxicology* **19:** 291-296.

JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1982). Toxicological evaluation of certain food additives. Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Food Additives Series No. 17*.

JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1991a). Toxicological evaluation of certain food additives and contaminants. The thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Food Additives Series No. 28*.

JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1991b). Evaluation of certain food additives and contaminants. Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series No. 806*.

JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1993). Evaluation of certain food additives and contaminants. Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series No. 837*.

Johnsen S. G. (1970). Testicular biopsy score count--a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones* **1:** 2-25.

Kararli T. T. (1995). Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharmaceutics & Drug Disposition* **16:** 351-380.

Kluwe W. M. (1981). Renal function tests as indicators of kidney injury in subacute toxicity studies. *Toxicology and Applied Pharmacology* **57:** 414-424.

Kroes R., Peters P. W., Berkvens J. M., Verschuuren H. G., de Vries T., van Esch G. J. (1977). Long term toxicity and reproduction study (including a teratogenicity study) with cyclamate, saccharin and cyclohexylamine. *Toxicology* **8:** 285-300.

Leegwater D. C., de Groot A. P., van Kalmthout-Kuyper M. (1974). The aetiology of caecal enlargement in the rat. *Food and Cosmetics Toxicology* **12:** 687-697.

Lohner S., Toews I., Meerpohl J. J. (2017). Health outcomes of non-nutritive sweeteners: analysis of the research landscape. *Nutrition Journal* **16:** 55.

Lord G. H. and Newberne P. M. (1990). Renal mineralisation--a ubiquitous lesion in chronic rat studies. *Food Chemistry and Toxicology* **28:** 449-455.

Magnuson B. A., Burdock G. A., Doull J., Kroes R. M., Marsh G. M., Pariza M. W., Spencer P. S., Waddell W. J., Walker R., Williams G. M. (2007). Aspartame: a safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies. *Critical Reviews in Toxicology* **37:** 629-727.

Magnusson G. and Ramsay C. H. (1971). Urolithiasis in the rat. *Laboratory Animals* **5:** 153-162.

Mann S. W., Yuschak M. M., Amyes S. J. G., Aughton P., Finn J. P. (2000). A combined chronic toxicity/ carcinogenicity study of sucralose in sprague-dawley rats. *Food and Chemical Toxicology* **38:** S71-S89.

Murasaki G. and Cohen S. M. (1981). Effect of dose of sodium saccharin on the induction of rat urinary bladder proliferation. *Cancer Research* **41:** 942-944.

Nguyen T. L., Vieira-Silva S., Liston A., Raes J. (2015). How informative is the mouse for human gut microbiota research? *Disease Models & Mechanisms* **8:** 1-16.

NTP (U.S. National Toxicology Program) (1997). Reproductive toxicology. Sodium saccharin. *Environmental Health Perspectives* **105 Suppl 1:** 347-348.

Oser B. L., Carson S., Cox G. E., Vogin E. E., Sternberg S. S. (1976). Long-term and multigeneration toxicity studies with cyclohexylamine hydrochloride. *Toxicology* **6:** 47-65.

Palmnäs M. S., Cowan T. E., Bomhof M. R., Su J., Reimer R. A., Vogel H. J., Hittel D. S., Shearer J. (2014). Low-dose aspartame consumption differentially affects gut microbiota-host metabolic interactions in the diet-induced obese rat. *PLoS One* **9:** e109841.

Prasad O. and Rai G. (1987). Haematological abnormalities induced by feeding a common artificial sweetener, saccharin, in ICR Swiss mice. *Toxicology Letters* **36:** 81-88.

Prokic M. D., Paunovic M. G., Matic M. M., Djordjevic N. Z., Ognjanovic B. I., Stajn A. S., Saicic Z. S. (2014). Prooxidative effects of aspartame on antioxidant defense status in erythrocytes of rats. *J Biosci* **39:** 859-866.

Renwick A. G. (1986). The metabolism of intense sweeteners. *Xenobiotica* **16:** 1057-1071.

Roberts A., Renwick A. G., Ford G., Creasy D. M., Gaunt I. (1989). The metabolism and testicular toxicity of cyclohexylamine in rats and mice during chronic dietary administration. *Toxicology and Applied Pharmacology* **98:** 216-229.

Robertson J. L. (1980). Spontaneous Bladder and Kidney Lesions in Young Rats. *Toxicologic Pathology* **8:** 9-13.

Saad S. (2017). A Histological Study of the Effect of Aspartame Versus Sucralose on the Spleen of Adult Male Albino Rats. *The Egyptian Journal of Anatomy* **40:** 107-119.

Saleh A. A. S. (2014). Synergistic effect of N-acetylcysteine and folic acid against aspartame-induced nephrotoxicity in rats. **2:** 363-373.

SCF (European Commission: Scientific Committee for Food) (1995). Opinion on saccharin and its sodium, potassium and calcium salts (expressed on 2 June 1995). *ANNEX III TO DOCUMENT III/5157/97 CS/ADD/EDUL/148-FINAL. February 1997*. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_7_out26_en.pdf.

SCF (European Commission: Scientific Committee on Food) (2000a). Revised opinion on cyclamic acid and its sodium and calcium salts (Expressed on 9 March 2000). *SCF/CS/EDUL/192 final. 13 March 2000.* https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out53_en.pdf.

SCF (European Commission: Scientific Committee on Food) (2000b). Opinion Re-evaluation of acesulfame K with reference to the previous SCF opinion of 1991 (Expressed on 9 March 2000). *SCF/CS/ADD/EDUL/194 final. 13 March 2000*. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out52_en.pdf.

SCF (European Commission: Scientific Committee on Food) (2000c). Opinion of the Scientific Committee on Food on sucralose (Adopted by the SCF on 7 September 2000). *SCF/CS/ADDS/EDUL/190 Final. 12/9/2000*. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out68_en.pdf.

Schoenig G. P. and Anderson R. L. (1985). The effects of high dietary levels of sodium saccharin on mineral and water balance and related parameters in rats. *Food Chemistry and Toxicology* **23:** 465-474.

Schoenig G. P., Goldenthal E. I., Geil R. G., Frith C. H., Richter W. R., Carlborg F. W. (1985). Evaluation of the dose response and in utero exposure to saccharin in the rat. *Food Chemistry and Toxicology* **23:** 475-490.

Tomonari Y., Kurotaki T., Sato J., Doi T., Kokoshima H., Kanno T., Tsuchitani M., Seely J. C. (2016). Spontaneous Age-related Lesions of the Kidney Fornices in Sprague-Dawley Rats. *Toxicologic Pathology* **44:** 226-232.

wafg (Wirtschaftsvereinigung Alkoholfreie Getränke e.V.) (2018). wafg-Branchenbeitrag: Kalorienreduktion um 15 Prozent bei Erfrischungsgetränken. *Presseinformation*

Wallig Matthew A. (2018). Chapter 15 - Digestive System. In *Fundamentals of Toxicologic Pathology (Third Edition)*, Wallig M. A., Haschek W. M., Rousseaux C. G., Bolon B. (eds), pp 395-442. Academic Press,

Wong M. C. S., Fung F. D. H., Leung C., Cheung W. W. L., Goggins W. B., Ng C. F. (2018). The global epidemiology of bladder cancer: a joinpoint regression analysis of its incidence and mortality trends and projection. *Scientific Reports* **8:** 1129.

About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. The BfR advises the Federal Government and the States (‘Laender’) on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.

This text version is a translation of the original German text which is the only legally binding version.